State of the knowledge

about the side effects of Roaccutane (isotretinoin):

review of the scientific literature

Authors : many contributors, past, present, and future,

Opensource

Year : since 2005, to complete, correct, and enrich...

Thanks :

A big thank-you,

to all those who, from near or far, directly or indirectly, past, present and future,

participate in the research and / or dissemination of information to all,

on a problem of public health, still too neglected in 2018,

which deserves, at least, that independent researchers be commissioned,

and ideally, that a new nosological entity be created:

for example, **"exogenous isotretinoin (AR 13-cis) intolerance syndrome "**,

with all subclasses and nosological precisions (ranging from acute syndrome to

absence of syndrome) that implies.

A special thanks to the author Max, from the forum http://max001.proboards.com/, who has initiated this information movement in 2005 ...
He said (annexe 9) :
“**Please bring any information to a person with biochemical/specialist competence in the field where your symptoms are most pronounced for clarification and correct interpretation.”**

Everyone is free to enrich, complete, and correct this PDF with the keyword "to complete" inserted in each empty part (control + F, key word, and entry) :

In annex 5, you can find the list of abbreviations in biochemistry for an easier reading.

Thank you for sharing your contributions.

In memory of all past and present dramas, and to come if nothing changes on the part

health authorities (c.f. **Annexe 8 : declaration of mutagenesis** and **Annexe 10 : Pharmaco-épidémiologic studies**).

For the HEALTH, and for Hippocrate.

Table des matières

Abstract :

To complete

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US National Library of Medicine – National Institutes of Health

Introduction : Structure of the PDF and vision

Welcome to the Roaccutane Science PDF!

1. Structure of the PDF :

The Roaccutane Science PDF is divided in 14 sections. *Section number one* gives an overview of the suggested major hormonal effects of a (Ro)accutane exposure at receptor level and enzymes involved. *Section two* gives an overview of the neurotoxic effects on different parts of the brain, and suggested effects on receptors and metabolism of neurotransmitters. *Section three* discusses suggested pathways to (Ro)accutane induced apoptosis (programmed cell death). *Section four* gives an overview on the suggested immunomodulatory/ immunosuppressive effects of exposure. *Section five* gives an alphabitical overview of suggested (side)-effects that could be expected in subjects exposed to (Ro)accutane and their suggested chemical causes. *Section six* discusses how a (Ro)accutane exposure may affect the exposed subject long-term. [url=http://www.max001.proboards42.com/index.cgi?board=rewfsdfsd]*Section seven*[/url] gives a systematic overview classified by transcription factors that either are upregulated or inhibited by (Ro)accutane and their role in gene-expression. *Section eight* is currently under construction and is dedicated to explaining the (Ro)accutane induced effects with a simplified language.

- Roaccutane is a global concern :

The language selection is found in the header of the forum. *Section nine* is summary for spanish and portuguese speaking readers. *Section ten* is under development and a summary for swedish and norwegian speaking readers. *Section eleven* is a summary in french. *Section twelve* is a summary in german, *section thirteen* is a summary in italian and *section fourteen* is a summary in dutch. The language sections do not contain a perfect language nor a full translation, due to lack of resources and lingual competence. The forum thus remains with the goal of facilitating the information process for non-english speaking readers until an adequate status of public awareness about the substance is reached.

2. References :

References are provided in the end of each topic. All references used in this forum are available to the public and searchable in public databases. Links to studies from the reference lists are being updated.

3. Advertising :

This is a message board free of charge. Provided advertising has no relation with provided research, nor is any of the marketed product by any means recommended.

4. Scientifical advance :

More information about (Ro)accutane induced effects is continously coming in line with publicly available scientifical progress.

5. Visions :

The 21th century involves the possiblity of a technologically and ideologically driven diminishing information-gap between:

1) baseline biochemical research and clinically active specialists in a scientifical field

2) institutions, other commercial and non-commercial parties harbouring potential interest conflicts and on the other hand the individual citizen

It is a vision of (Ro)accutane Science to open up for public awareness and to diminish these above mentioned information-gaps for a juste and overall beneficial scientifical advance.

With an ideal flow of information, the practical consquences of held scientifical knowledge should always ensure the best possible health decisions for each individual. Whenever that regards the adaptation and modificaction of current medical practise, adequate changes should be encouraged.
Welcome to « Roaccutane Science », a scientifical non-commercial forum for dermatologists and the publics right of access to (Ro)accutane induced effects and the consequences of exposure

Section 1 : (Ro)accutane and the nuclear hormone receptors

1. Introduction :

- General overview of section 1 - (Ro)accutane and the nuclear hormone receptors :

(Ro)accutane is a form of the fat soluble vitamin A that is termed a vitamin, but is classified as a steroid, and is administered to human acne-subjects in a 40-100 times overdose*, and this during several months. Metabolites of (Ro)accutane, massive doses of retinoids, that are more active forms of vitamin A, affect hormonal receptors. The retinoid metabolites of (Ro)accutane are found to affect retinoid receptors, by the high binding-affinity for vitamin A isoforms, but also a large number of other hormonal and non-hormonal receptors are found to be affected [1]. These affected hormonal nuclear receptors and other non-hormonal affected receptors are widely expressed in different cell types all over the body, in nearly every single organ, including in several parts of the human brain [10]. This is likely the reason why retinoids (Roaccutane) are used for severe diseases such as prostate cancer, leukaemia [9], glioblastoma [8] (a form of brain cancer) and other areas.

Today (as of July 2005), (Ro)accutane exposure, often in very young adolescent human subjects, is part of current dermatological practise. The skin is one of many organs that have cells that express retinoid and other nuclear hormone receptors [7]. The effects are many and one effect is that the metabolism or the cell division and proliferation is significantly reduced by significant hormonal suppression (simplified, the rate by which cells use energy is reduced). (Ro)accutane is also known to induce apoptosis, or programmed cell death [9]. This occurs in several parts of the body. In one part of the brain (the orbitofrontal cortex) the metabolism is found to be reduced by a mean of more than 20% in human subjects after four months of (Ro)accutane exposure [5].

Several deficiencies occur after exposure. Some which are measured in human subjects exposed to (Ro)accutane, including thyroid deficiency [2], androgen deficiency [3] , vitamin D deficiency [4] and vitamin A deficiency. The fat metabolism is altered. In rats exposed to isotretinoin the insulin sensitivity in peripheral tissue was found to be significantly decreased [11]. Insulin production and release are with highest certainity significantly affected, due to the structure of the insulin receptor and the retinoid interaction with pancreatic beta cells. Even the growth hormone axis is linked to activity of the nuclear receptors and vitamin A, because the retinoid receptors are found to be expressed in somatropes, the cell type that produces growth hormone [6]. The hormonal effects are wide, and even more effects that are difficult to measure, or haven´t been clinically measured in public studies are suggested. Almost all hormones have receptors in cells in the brain, where they insert actions that are not fully discovered. please see section 2. It is not speculative to say, that these receptors are not designed for a 40 to 100 times overdose of any hormone, as the doses of (Ro)accutane exert.

The retinoid receptors (RXRs, RARs), the thyroid receptors (TRs), the androgen receptors

(AR), and the fat metabolism regulating PPAR receptors belong to the large superfamily of nuclear hormone receptors (NHRs), found in the nucleus of the cell, that regulate gene transcription [1]. It is not fully known what happens with these receptors, after a four month exposure of massive doses of (Ro)accutane.

- (Ro)accutane induced hypothyroidism :

In human acne-subjects exposed to (Ro)accutane, levels of thyroxine and triiodothyronine were significantly lower after exposure (p less than 0.05), indicating a (Ro)accutane induced clinical thyroid deficiency (hypothyroidism) [2].

- (Ro)accutane is strongly antiandrogen :

Roaccutane is strongly antiandrogen. A 50% suppression of androgen conversion rates have been found in related doses to what is seen in acne-subjects. The 5-alpha reductase is genetic and dependant on androgen receptor polymorphism. Androgen receptors (AR) as well as thyroid receptors (TR) belong to the superfamily of nuclear hormone receptors where also the retinoid receptors belong. The androgen receptor (AR) is a ligand-activated transcription factor that recognises and binds to specific DNA response elements upon activation by the steroids testosterone or dihydrotestosterone [3] .

- Clinical observations of 1,25-dihydroxyvitamin D in human subjects exposed to (Ro)accutane :

A significant fall in the level of 1,25-dihydroxyvitamin D, and a significant increase in the molar ratio of 24, 25-dihydroxyvitamin D to 25-hydroxyvitamin D was found in human subjects after exposure to (Ro)accutane, indicating a (Ro)accutane induced significant 1,25-dihydroxyvitamin D deficiency [4].

- (Ro)accutane induced decreased insulin sensitivity :

In all exposed rats, in 15 days, isotretinoin increased glycerol concentrations and decreased the insulin sensitivity of peripheral tissues [11].

- (Ro)accutane and decreased cellular hormonal uptake :

Also the hormonal uptake is suggested to be significantly inhibited, due to a significant downregulation of the megalin receptor. Androgens and estrogens are transported bound to the sex hormone binding globulin (SHBG). SHBG is believed to keep sex steroids inactive and to control the amount of free hormones that enter cells by passive diffusion. Contrary to the free hormone hypothesis, it is reaffirmed that megalin, an endocytic receptor in reproductive and other tissues, acts as a pathway for cellular uptake of biologically active androgens and estrogens bound to SHBG [12]. In mice, megalin has also shown to have an important function in the uptake of retinol [13], vitamin D [14] as well as other hormones.

(*) *:Official recommended US and European dose interval of Roaccutane by manufacturer* Hoffman la Roche compared to the daily recommended intake of 800 mikrograms/day of vitamin A by the European Food and Nutrition Labeling Division

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1.1 Steroidal genesis, nuclear hormone receptors :

- The superfamily of nuclear hormone receptors (NHRs) :

The thyroid receptors (TRs) and retinoid receptors (RXRs, RARs) belong to the large superfamily of nuclear hormone receptors (NHRs) that regulate gene transcription. These proteins control a diverse set of target genes in response to specific physiological signals. Family members include the endocrine receptors, such as the estrogen (ER) and androgen (AR) receptors; the adopted orphan receptors, such as the retinoid X receptor (RXR) and peroxisome proliferator-activated receptor (PPAR); and the orphan receptors, receptors that do not require an endogenous ligand or for which a ligand has yet to be identified, such as steroidogenic factor 1 (SF-1) and liver receptor homolog 1 (LRH-1) [1]. The receptor family also includes receptors for progesterone (PR) glucocorticoids (GR) and mineralocorticoids (MR) [8].

Each receptor forms hetero/homo-dimers with the compounds that they are sensitive to, and binds to DNA nuclear response elements. These can be described as hormone or steroid response elements (HREs or SREs). The HREs can then be subdivided into smaller sequences [8]. The response elements inserts transcription through either binding directly to the DNA or acting as a cofactor [9].

Smaller sequences of HREs include TREs and AREs. In the case of the thyroid receptor, it binds to mainly to thyroid response elements (TREs) [9], in the case of androgen receptor (AR) it binds to mainly to androgen response elements (AREs) [8].

- Significant androgen receptor inhibition and inhibition of 5-alpha-r :

The 5-alpha-reductase (5-alpha-r), an enzyme known to convert testosterone to the more potent and active compound dihydrotestosterone (DHT), was found to be inhibited by 50% in human prostate cells exposed to (Ro)accutane in doses comparable to associated plasma concentrations in (Ro)accutane exposed subjects with acne [2]. The 5-alpha-r has been found to be significantly inhibited by (Ro)accutane in acne-subjects in several repeated studies [5, [url=http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=1827343&query_hl=52] 6, [/url] 7 and more]. The androgen receptor (AR) gene promoter does not contain the TATA or CAAT box, but it contains a long (approximately 90-bp) homopurine/homopyrimidine (pur/ pyr) stretch immediately upstream of the Sp1-binding GC box site. This pur-pyr stretch is conserved at the same proximal position in the rat, mouse, and human AR gene promoters. TATA-less AR gene promoter, multiple weak Sp1 sites at the pur/pyr region adjacent to the GC box can provide a readily available source of this transcription factor to the functional GC box, thereby facilitating the assembly of the initiation complex [13].

It is here suggested that a similiar 50% suppression of the 5-alpha-r through suppression

of androgen receptors occurs after (Ro)accutane exposure in all human cells carrying retinoid receptors, including related cells found in various parts of the brain.

The 5-alpha reductase is genetic and dependant on androgen receptor polymorphism [3]. Androgen receptors (AR) as well as thyroid receptors (TR) belong to the superfamily of nuclear hormone receptors where also the retinoid receptors belong [1]. The androgen receptor (AR) is a ligand-activated transcription factor that recognises and binds to specific DNA response elements upon activation by the steroids testosterone or dihydrotestosterone [8].

- Significant thyroid receptor inhibition :

In rat GH1 cells, a cell type located in the pituitary gland, a 50-70 % inhibition of thyroid receptors was found, exposed to doses that are comparable to those seen in (Ro)accutane exposure in acne-subjects [4]. It is here suggested that all cell lines carrying retinoid and thyroid receptors in humans are suppressed in a similiar manner.

Since a 50 % inhibition of the 5-alpha reductase is found in human cells in related doses to those seen in (Ro)accutane exposure of acne-subjects, and a 50-70% inhibition of thyroid receptors belonging to the same receptor complex, such as TRbeta2 is observed in related doses to those seen in (Ro)accutane exposure in acne-patients, it is here suggested that a general 50% inhibition of the androgen (AR) and thyroid (TR) receptors occurs in various parts of the body and brain, in subjects exposed to (Ro)accutane in doses associated with what is received in acne-subjects.

Subdividing the hormonal response elements further, C´delta2 is a fragment suggested to be of importance for androgen function in the HREs. In mice C'delta2 expression was found not to be driven by glucocorticoids, as adrenalectomy had little effect, but are suggested to be dependent on the NF-kappaB-like element absent from the C'delta9 fragment [10]. The same conclusion was drawn in another study, NF-kappa B bound the region of C' delta 2 absent from C' delta 9. Expression of I kappa B decreased response of C' delta 2, but not C' delta 9, confirming a permissive role of NF-kappa B in steroid activation [11]. A nearly identical effect is suggested in humans.

Transcription factor AP-1 has been found to be modulated by thyroid receptors and TREs [12].

To further confirm the drastic anti-androgen and hypothyroid response in subjects exposed to (Ro)accutane, both AP-1, AP-2 and NFkappaB was found to be significantly altered in subjects exposed to retinoic acid in repeated studies.

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1.2 Thyroid hormone metabol. and cellular uptake

- Found clinical hypothyroidism in acne-subjects exposed to (Ro)accutane :

In human acne-subjects exposed to (Ro)accutane, levels of thyroxine and triiodothyronine were significantly lower after exposure (p less than 0.05), indicating a (Ro)accutane induced clinical thyroid deficiency (hypothyroidism) [5].

Decreased binding affinity of Sp1 to GC boxes here suggested to be one pathway to (Ro)accutane induced inhibition of transcriptional megalin expression

Hoffman la Roche itself has admitted to the findings of significantly raised homocysteine

(Hcy) levels in human subjects exposed to (Ro)accutane [0]. These findings of statistically significant elevation of Hcy, have in acne-subjects been repeatedly verified by independent studies [13 and more]. Elevated homocysteine levels have frequently been associated with clinical hypothyroidism [12 and more]. This frequent association is likely due to the elevated homocysteine being a sign of lessened activity of the cystathionine beta-synthase (CBS) that catalyzes the condensation of serine and homocysteine to form cystathionine. CBS is promoted by Sp1 and Sp3 [17], and a dephorphorylation of Sp proteins reduces the promotion significantly by decreased binding of the Sp protein to the GC boxes (GC boxes are binding sites for Sp induced gene-promotion) [18]. These same Sp proteins are in rats found to promote megalin expression, and a dephosporylation of Sp proteins is highly likely to reduce megalin expression by a lessened gene promotion [19]. In small doses RARs/RXRs physically interact with Sp1, potentiate Sp1 binding to the GC box motifs [20]. It is here suggested that a supraphysiological dose of (Ro)accutane inhibits Sp1 binding partly through inhibited phoshorylation, and thereby reduces promoter activity of megalin.

The cellular retinoic acid binding protein I gene is induced by thyroid hormone (T3) through a T3 response element (TRE) approximately 1 kb upstream of the basal promoter. The upstream region is organized into a positioned nucleosomal array with the N1 nucleosome spanning the GC box region [26].

High-affinity agonists for the retinoic acid X receptors (RXR) and retinoic acid receptor (RAR) have pleotropic effects when administered to humans. These include induction of hypertriglyceridemia and hypothyroidism [6 and 11]. The RAR/RXR pan-agonist 9-cis-retinoic acid induce 2- to 3-fold higher levels of serum triglycerides than the RAR-selective agonists alone [11].

- Inhibition of thyroxine and triiodthyronine release, likely partially through reduction of thyroglobulin due to downregulation of megalin mediated endocytosis :

When thyroglobulin (Tg) is endocytosed by thyrocytes and transported to lysosomes, thyroid hormones (T4 and T3) are released. Megalin (gp330), a Tg receptor on thyroid cells, is found to be a mediator in Tg transcytosis [7, 14 and more]. Following incubation with exogenous rat Tg at 37 degrees C, Fisher rat thyroid (FRTL-5) cells, a differentiated thyroid cell line, released T3 into the medium [7]. A significant reduction of thyroglobulin was noted in isotretinoin exposed human subjects with thyroid carcinoma at doses comparable to what acne-subjects are exposed to (from 1,0 mg/kg/day), indicating that a significant fall in thyroglobulin may partially explain the significant fall in thyroxine and triiodthyronine seen in acne-subjects exposed to the toxin [8]. In F9 cells, small plasma concentrations of both retnoic acid (10^-6 M) and vitamin D, alone or in combination, have been found to upregulate thyroglubulin receptor megalin (gp330) during differentiation [9]. The gp330/Megalin/LRP-2 protein belongs to the low-density lipoprotein receptor gene family and is believed to function as an endocytic receptor for the uptake of lipoproteins and many other ligands. Other functions of this protein may include a role in calcium sensing in the parathyroid glands and other tissues [10].

Unfortunately no publicly available study shows how megalin (gp330) expression is affected in toxic (Ro)accutane exposure in human subjects. However, serum Tg levels were significantly reduced in homozygous (megalin(-/-)) mice, which, more importantly,

were found to be hypothyroid, as demonstrated by significantly reduced serum free thyroxine and significantly increased serum thyroid stimulating hormone (TSH) levels [14]. In proximal-tubule-derived opossum kidney cells, TGF-beta1 was found to induce downregulation of megalin-cubilin-mediated endocytosis, sensitive to inhibition of translation and transcription and was preceded by Smad2 and 3 phosphorylation [15]. Six weeks of isotretinoin exposure caused a statistically significant 19% increase in suction blister fluid TGF-beta1 [16]. Type 3 iodothyronine deiodinase (D3) is the major inactivating pathway, preventing activation of the prohormone thyroxine (T4) and terminating the action of T3. TGF-beta stimulates transcription of the hDio3 gene via a Smad-dependent pathway [24].

The RAR/RXR pan-agonist 9-cis-retinoic acid effect included >50% inhibition of total heparin-releasable lipase activity in serum, consistent with inhibition of lipase-mediated triglyceride disposal [11].

- Significant supression of thyroid receptors, likely through inhibition of Sp1 formation and binding :

Ro(accutane) was found to suppress thyroid receptors in pituitary GH1 cells. Retinoic acid produced a time and dose-dependent depletion of thyroid hormone receptors in GH1 cells without modifying their affinity for triiodothyronine (T3) [3]. This is likely due to the thyroid receptor promoters contain a GC-box highly responsive to the transcription factor Sp1 [21]. In rats, TR beta1 promoter was regulated negatively by the proteins bound to the silencer sequence and the GC box, and positively by Oct-1. Adult brain extracts appear to contain more Oct-1 protein than do fetal extracts [25]. The effect on Oct-1 by a massive dose of retinoic acid, as seen in acne-subjects, is not known.

A maximal decrease of thyroid receptor expression (50-70%) was obtained after 24-48 h incubation with 5-10 microM retinoic acid [3]. The result is suggested to be hypothyroidism, or partial thyroid resistance.

The thyroid hormone receptor (TR) directly regulates the transcription of thyroid hormone-responsive genes in response to changing levels of thyroid hormone. Mechanistically TR utilizes a complex set of binding interactions, with hormone, response elements, and coregulatory proteins, to provide specific local control of patterns of transcriptional response that are partially responsible for inducing the tissue-selective responses to the circulating hormone. One of the apparently dominant phenomena in the regulation of thyroid hormone responses is the protein interactions between TR and its coregulators [2] .

Thyroid hormone receptors (TRs) often modulate transcriptional activity of target genes by heterodimerization with the 9-cis retinoic acid receptor (RXR). On positive thyroid response elements (TREs), RXR favors binding of the TR-RXR complex to DNA and stimulates transcription [1].

- Expression of TR isoforms in several tissue including high expression in the adult brain :

Thyroid hormone isoforms are derived from two separate genes to yield four major T3-

binding isoforms: alpha1, beta1, beta2, and beta3 [4]. TRbeta is the predominant isoform in liver, whereas T3 effects on heart rate are mediated mostly by TRalpha [22]. TR alpha 1, alpha 2, beta 1, and beta 2 was studied in human consecutive sections of six hypothalami and pituitaries. Staining intensity showed strong interindividual variation but was consistently present in the infundibular nucleus, paraventricular nucleus, and supraoptic nucleus. In addition, strong TR immunoreactivity was observed in the anterior pituitary [23].

For further information about thyroid hormone receptor expression:

Thyroid receptor alpha isoforms

Genecards - THR A

Thyroid receptor beta isoforms

Genecards - THR B

(Genecards database)

- Symptoms in mice deficient in TR-receptor expression :

Thyroid receptor knockout mice that show resistance to thyroid hormone show abnormalties. TRalpha1PV mice show no abnormalities in the pituitary-thyroid axis and other discernable RTH phenotypes. In addition, TRalpha1PV mice are dwarfs with high mortality, reduced fertility and survival, reduced glucose utilization in the brain and marked delay in bone development. TRbetaPV mice faithfully reproduce human RTH with dysfunction of the pituitary-thyroid axis, impairment in weight gain and accelerated bone development, hearing defects, abnormal regulation of serum cholesterol and increased physical activity reminiscent of attention deficit-hyperactivity disorder [4].

- Conclusions :

In human acne-subjects exposed to (Ro)accutane, levels of thyroxine and triiodothyronine were significantly lower after exposure (p less than 0.05), indicating a (Ro)accutane induced clinical thyroid deficiency (hypothyroidism) [5]. It is here suggested that the (Ro)accutane induced circulating hypothyroid findings is a result of renal failure, mainly of megalin inhibition through decreased formation and binding of megalin promoters Sp1 and Sp3. Ro(accutane) was found to suppress thyroid receptors in pituitary GH1 cells. Retinoic acid produced a time and dose-dependent depletion of thyroid hormone receptors in GH1 cells without modifying their affinity for triiodothyronine (T3) [3]. This is likely due to the thyroid receptor promoters contain a GC-box highly responsive to the transcription factor Sp1, which binding affinity and formation is suppressed. It is hightly likely that the suppression of thyroid receptors, in a similiar manner is general over all cell-lines.

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1.3 Growth hormone / IGF-1 axis :

- Significant interaction with the GH-IGF-axis :

In humans, vitamin A and retinoic acid are substances extensively known to be involved in the release of growth hormone [11 and more]. A clear correlation between nocturnal growth hormone (GH) secretion and Vitamin A status was was found in short slowly growing children [12]. However, except for the clear relation between growth hormone and growth, in adults growth hormone is known to mediate a wide variety of metabolic functions. Too low levels of growth hormone in adults results in adult growth hormone deficiency.

Data indicates that (Ro)accutane interacts with the GH/IGF-1 axis through several

pathways, and among other things highly likely result in:

x a significant endocrine alteration of circulating GH/IGF-1 through decreased pituitary GH production and release, kidney function and liver function

x decreased receptor function/expression of several to the GH/IGF axis related receptors (partial GH resistance and IGF resistance)

x alteration of IGF binding protein function through alterations in retinoid receptor function and alterations of GC-box binding motifs

An extensive amount of data shows clear interaction between retinoids and the GH/IGF-1 axis. Unfortunately no publicly available study of human acne-subjects exposed to (Ro)accutane clarifies and summarizes well the clinical effects on the GH/IGF-axis.

- Vitamin A-deficiency, growth hormone secretion and the GH-gene :

In human pituitary cells, Vitamin A and retinoic acid is found to stimulate cAMP release and growth hormone secretion [11]. A nearly identical correlation has been found in rats. In rat pituitary GH3 cells, Retinoic acid <1 microM stimulated growth hormone secretion by 220%. 50 nM HCT stimulated GH secretion 3,5 times and in synergy GH secretion was stimulated seven times [7]. The RXR-specific ligand induced rat growth hormone mRNA in GH3 pituitary cells, indicating that the effects of RA on growth hormone gene expression at least in part involve ligand binding to endogenous RXRs in vivo [13].

- Renal failure and alterations of the GH/IGF-1 axis :

Inhibition of megalin receptors may induce failure in the circulation of IGF-1, and instead increased urinary levels. In (cy/+) mice, the expression of proteins implicated in endocytosis, such as the chloride channel ClC-5 and the albumin receptor megalin, correlated well with the presence and absence of FITC-dextran in cysts. As an example of growth factor systems possibly being affected by this endocytosis defect, we could detect increased urinary levels of insulin-like growth factor-I protein in (cy/+) animals [8].

- Found significant interaction between Insulin like growth factor binding protein 3 (IGFBP-3) and RXR-alpha - suggested further significant interaction IGFBP-1 and 3 with retinoids through alterations of binding affinity to GX-box motifs and Sp1 transcription :

IGFBP-3 is particulary widely expressed during puberty [Genecards database]. An interaction between nuclear IGFBP-3 and 9-cis retinoic acid receptor-alpha (RXRalpha) exists. Insulin-like growth factor (IGF) binding protein (IGFBP)-3 has been shown to be a growth inhibitory, apoptosis-inducing molecule by virtue of its ability to bind IGFs [2].

Human genomic clones encompassing the tissue specific expressed gene IBP-1/IGFBP-1, an insulin-like growth factor binding protein were isolated and characterized. The gene is

organized in four exons and spans 5.9 kb. S1 nuclease analysis determined a single transcription start site. The first exon and 5' flanking region are highly GC rich and located in a CpG island. The CpG island enclose the CAAT box, the TATA box, the transcription start site and a potential SP1 transcription factor binding site. The presumptive promoter region is characteristic for genes expressed in a tissue specific fashion. All signals required for cleavage/polyadenylation are located within exon IV, predicting a mRNA of 1.5 kb which is consistent with the size seen on RNA blots [17].

- Significantly decreased receptor expression of Insulin receptor substrate 1 (IRS-1) induced by massive doses of retinoic acid :

Insulin receptor substrate-1 (IRS-1) mediates signaling from the insulin-like growth factor type-I receptor. All-trans retinoic acid (RA) is found to decrease IRS-1 protein levels in certain breast cancer cells, which are growth arrested by RA [9]. It is here suggested that more cell lines than certain breast cancer cells decrease IRS-1 protein levels and therefore constitute one pathway for inhibition of IGF-1 signaling. IRS-1, an intracellular substrate of the insulin receptor tyrosine kinase, also is heavily phosphorylated on serine and threonine residues; several serine phosphorylation sites alter the function of IRS-1 [10].

In Mv1Lu cells, insulin partially reverses transforming growth factor-beta1 (TGF-beta1) growth inhibition in the presence of alpha5beta1 integrin antagonists. TGF-beta1 appears to induce phosphorylation of IRS-2 in these cells; this is inhibited by a TGF-beta antagonist known to reverse TGF-beta growth inhibition. Stable transfection of 32D myeloid cells (which lack endogenous IRS proteins and are insensitive to growth inhibition by TGF-beta1) with IRS-1 or IRS-2 cDNA confers sensitivity to growth inhibition by TGF-beta1; this IRS-mediated growth inhibition can be partially reversed by insulin in 32D cells stably expressing IRS-2 and the insulin receptor (IR). These results suggest that growth inhibition by TGF-beta1 involves IRS proteins [1].

- Interaction with GH-secretion also through hydrocortisone (HCT) receptor modulation :

The retinoic acid receptor cDNA bears a 15% homology to the hydrocortisone (HCT) receptor, which thus here is suggested to be one additive pathway of (Ro)accutane induced significant interference with the GH/IGF-axis. In rat pituitary GH3 cells, hydrocortisone is known to stimulate GH secretion. Retinoic acid selectively stimulates basal and HCT-induced GH secretion and mRNA levels in these cells in a dose- and time-dependent manner [7].

- Thyroid hormone deficiency and the GH/IGF-axis :

A direct suppressive effect of thyroid function has been found in (Ro)accutane exposed subjects. Levels of thyroxine and triiodothyronine were found to be lower after (Ro)accutane exposure in acne-subjects ($p < 0.05$) [4]. Thyroid function is interrelated with the GH/IGF-1 axis. Thyroid hormone exerts profound effects on the insulin-like growth factors (IGFs)/IGF factor I receptor (IGF-IR) system through its action on the production of IGF-I peptide and IGF-binding proteins. Most of these actions are mediated by the direct control of pituitary GH gene by thyroid hormone. The IGFE/ IGF-IR system is depressed in hypothyroid animals [14]. Mouse GH- encoding gene (mGH)

analysis of 1767 bp of the 5' flanking region, with respect to putative regulatory elements, revealed a TATA box, two binding sites for growth hormone factor (GHF1), a GC box (SP1), a thyroid-response element (TRE) and a silencer (SiL) sequence motif. A similiar promoter actitivity is suggested in humans [16].

- **IL-1 expressed in rat pituitary cells** :

The mouse anterior pituitary contains both types of interleukin (IL)-1 receptors, IL-1 receptor type I (IL-1RI) and IL-1 receptor type II (IL-1RII). These receptors are expressed mainly on somatotroph cells. In the present study, the ability of the mouse pituitary to respond in vivo to IL-1 or to lipopolysaccharide (LPS) was demonstrated by measuring, with an electrophoretic mobility shift assay, the presence of an active NF kappa B complex in cell nuclei from pituitaries of mice injected intraperitoneally with recombinant rat-IL-1 beta or LPS. Using immunohistochemistry with an antibody directed against the p65 NF kappa B subunit, a rapid and transient NF kappa B response to LPS was observed [15].

- **IGF receptor expression - significant interaction with both the IGF-I and IGF-II receptors during heavy exposure. The significant effects on the IGF-receptor subtypes may result in mutation or partial loss of receptor function :**

Retinoic acid is also found to interact with the mannose-6-phosphate receptor (M6P) in a similiar manner. The M6P/IGF-II receptor binds RA with high affinity. The binding of RA to the M6P/IGF-II receptor alters the primary functions of this receptor. It is suggested that the M6P/IGF-II receptor mediates a RA response pathway that is important in cell growth regulation.

The mannose 6-phosphate (M6P)/insulin-like growth factor II receptor (IGF-II) receptor is a multifunctional transmembrane glycoprotein that consists of a 300-kDa single polypeptide chain, with a large extracellular domain, containing 15 repeat regions, and a small cytoplasmic domain. The expression of this receptor is developmentally regulated, with the receptor being highly expressed in fetal and neonatal tissues (including plasma and heart) and the expression declining postnatally, but however, remains expressed. This receptor is known to bind both M6P and IGF-II at distinct sites. A major function of the receptor is to bind and transport M6P-bearing glycoproteins (e.g., lysosomal enzymes) from the trans-Golgi network or the cell surface to lysosomes. The cell surface M6P/IGF-II receptor also binds and internalizes IGF-II, resulting in the lysosomal degradation of this ligand. In this manner, the receptor may serve as a suppressor of IGF-II proliferative actions. In addition, the M6P/IGF-II receptor binds the latent transforming growth factor-beta (TGF-beta), permitting cleavage into its active form, which is a potent growth inhibitor for most cell types. Thus, the M6P/IGF-II receptor plays a critical role in the regulation of cell growth.

Studies of human subjects show that loss or mutation of M6P/IGF-II receptor gene is associated with the etiology of both human breast and liver cancer. [18].

For further information about significantly affected receptors/genes:

Growth hormone receptor
Genecards - GHR
Insulin like growth factor I receptor

Genecards - IGF1R
Insulin like growth factor II receptor
Genecards - IGF2R
Insulin receptor substrate I
Genecards - IRS1
Insulin like growth factor binding protein 1
Genecards - IGFBP1
Insulin like growth factor binding protein 3
Genecards - IGFBP3
Interleukin 1 receptor type 1
Genecards - IL1R1
Signal transducer and activator of transcription 5A
Genecards - STAT5A
Signal transducer and activator of transcription 5B
Genecards - STAT5B

- Conclusions :

A (Ro)accutane exposure in human acne-subjects is due to a numer of known interactions suggested to result in a partial adult GH-deficiency - clearly measurable endocrine alterations. Levels of circulating hormones with induced partial GH/IGF-1 resistance are not predictable due to a known feedback loop of the GH/IGF-1 axis. However, a test of GH-release is here suggested to show a clear result, a decrease in the curve in all exposed subjects.

Vitamin A is found to be important for GH-release. Retinoids interact with promoter acitivity of the GH-gene, IGFBP-1 and 3. Thyroid deficiency is suggested to contribute to a suggested (Ro)accutane induced GH-deficiency. A possible loss of pituitary GH-producing cells (somatropes) due to apoptosis is likely, but studies of the effects on the pituitary gland are not yet publicly available.

Decreased receptor function of related receptors to the GH/IGF-1 axis is highly likely due to mutation, DNA damage, or the loss of functioning receptor promoter sites. A clear significant interaction with multiple receptors is found, however, the exact result after exposure is not known. These receptors play a role in the hormonal sensitivity, whereas a partial resistance is likely in subjects exposed to (Ro)accutane.

Decreased kidney function is suggested due to inhibition of the function of the megalin receptor in renal tubular cells, which is an important receptor for hormonal uptake and recirculation. Failure in the STAT-5 signaling system that fills a function in by the growth hormone receptor induced IGF-1 production can not be excluded.

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Influence of the crosstalk between growth hormone and insulin signalling on the

modulation of insulin sensitivity.

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Growth hormone (GH) is an important modulator of insulin sensitivity. Multiple mechanisms appear to be involved in this modulatory effect. GH does not interact directly with the insulin receptor (IR), but conditions of GH excess are associated in general with hyperinsulinemia that induces a reduction of IR levels and impairment of its kinase activity. Several post-receptor events are shared between GH and insulin. This signaling crosstalk could be involved in the diabetogenic effects of GH. The utilization of animal models of GH excess, deficiency or resistance provided evidence that the signaling pathway leading to stimulation of the phosphatidylinositol 3-kinase (PI3K)/Akt cascade is an important site of regulation, and pointed to the liver as the major site of GH-induced insulin resistance. In skeletal muscle, GH-induced insulin resistance might involve an increase in the amount of the p85 subunit of PI3K that plays a negative role in insulin signalling. GH also reduces insulin sensitivity by enhancing events that negatively modulate insulin signaling such as stimulation of serine phosphorylation of IRS-1, which prevents its recruitment to the IR and induction of the suppressor of cytokine signalling (SOCS)-1 and SOCS-3 which modulate the signalling potential of the IRS proteins. In addition, GH has been shown to decrease the expression of the insulin-sensitizing adipo-cytokines adiponectin and visfatin. Finally, genetic manipulation of mice indicated that whereas GH plays a major role in reducing insulin sensitivity, circulating IGF-I also participates in the control of insulin sensitivity and plays an important role in the hormonal balance between GH and insulin.

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J Biol Chem. 2005 Mar 25;280(12):10955-63. Epub 2005 Jan 27. Related Articles, Links

Identification of a distal STAT5-binding DNA region that may mediate growth hormone regulation of insulin-like growth factor-I gene expression.

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Growth hormone (GH) regulates insulin-like growth factor-I (IGF-I) gene expression through signal transducer and activator of transcription 5b (STAT5b) and STAT5a. The objective of this study was to identify the cis-regulatory DNA region involved in this process. By cotransfection analyses of shotgun DNA fragments of a bacterial artificial chromosome sequence containing the entire human IGF-I gene and a large 5'-flanking region, a approximately 700-bp DNA region approximately 75 kb 5' to the IGF-I gene was found to have the ability to enhance gene expression from both heterologous and homologous promoters in the presence of constitutively active STAT5a or STAT5b. This 700-bp DNA region contains two closely located consensus STAT5-binding sites, and its sequence appears to be evolutionarily conserved. Electrophoretic mobility shift assays verified the ability of the two putative STAT5-binding sites to bind to STAT5a and STAT5b. Cotransfection analyses confirmed that both STAT5-binding sites were necessary for the

700-bp DNA region to mediate STAT5a or STAT5b activation of gene transcription. Chromatin immunoprecipitation assays demonstrated that the chromosomal region containing these two STAT5-binding sites was bound by constitutively active STAT5b protein in HepG2 cells and that the binding was accompanied by increased expression of IGF-I mRNA. In reconstituted GH-responsive cells, this 700-bp DNA region was able to mediate GH-induced STAT5a or STAT5b activation of gene expression. These results together suggest that this STAT5-binding site-containing distal 5'-flanking region of IGF-I gene may be an enhancer mediating GH-induced STAT5 activation of IGF-I gene transcription.

PMID: 15677453 [PubMed - indexed for MEDLINE]

1.4 Insulin sensitivity, production and release :

- Decreased peripheral insulin sensitivity, major alterations in insulin release and secretion, insulin production and liver responses :

Various studies are pointing out clear interactions between (Ro)accutane and insulin function. The insulin patway is a *major* pathway in how (Ro)accutane mediates its effects, thus it should be considered an effect, and not a side-effect. In rats, isotretinoin increased glycerol concentrations and decreased the insulin sensitivity of peripheral tissues, allready after two weeks of (Ro)accutane exposure [22].

- Significantly altered insulin release/secretion in response to glucose, and antiproliferative, possibly apoptotic effects on beta cells :

Whereas it remains completely clear that insulin secretion is affected during exposure, it is uncertain how insulin release and secretion is affected after a (Ro)accutane exposure. Experiments in rats show that insulin secretion could be expected to be efficiently reduced , due to decreased lessened promoter activity of Sp1 and disruptions in the GC-box [11]. Another study of an INS-1 cell line points out the clear relation between the retinoid receptors and insulin release and suggests a significantly higher insulin release during exposure, but found cells to reaggregate to a single clump in massive doses [1]. The most probable is that insulin secretion, in massive doses of retinoic acid as seen in acne-subjects, is significantly impaired during exposure.

Retinoic acid in was found to induce the pyruvate decarboxylating activity in modest doses [19]. In rats the pyruvate decarboxylase is found to be dependent on Sp1 and NF-Y promoter activity [11]. It is therefore possible that a massive dose of retinoic acid, as seen in acne-subjects, inhibits the pyruvate decarboxylase through decreased Sp1 and NF-Y binding. The pyruvate carboxylase (PC) plays a crucial role in various metabolic pathways, including gluconeogenesis, lipogenesis, and glucose-induced insulin secretion. The pyruvate carboxylase gene is transcriptionally regulated by peroxisome proliferator-activated receptor-gamma (PPARgamma) [20].

In rats, pyruvate carboxylase plays diverse roles in different biosynthetic pathways, including glucose-induced insulin secretion in pancreatic beta-cells. Transient transfections of these constructs into INS-1 cells identified a CCAAT box and a GC box that are located at -65/-61 and -48/-41, respectively, as the important determinants. Disruption of the GC box resulted in a 4-fold reduction of the reporter activity. Electrophoretic mobility shift assays (EMSAs) and supershift EMSAs using nuclear extract from INS-1 cells demonstrated that Sp1 and Sp3 bind a GC box while the nuclear factor Y was shown to bind the proximal but not the distal CCAAT box [11].

In INS-1 cells retinoid receptors RAR and RXR were found to mediate insulin release [1]. In rat islets and in response to glucose (Ro)accutane (10(-4) M) stimulated insulin secretion at 9.7, 12.5, 16.7, and 27.7 mM glucose. Maximal effects of 13 cis-RA (174% of control) were evident during second phase release at 9.7 mM glucose. Thirteen cis-RA (10(-7) and 10(-6) M) caused cells to adhere to each other, and at higher concentrations, 13 cis-RA caused dispersed cells to reaggregate into a single clump. These retinoid-induced clumps were perifused in a Bio-Gel P-2 gel column. Secretion from the clump was twofold greater than from an equal number of perifused dispersed cells [7]. Both 9cRA and ATRA inhibit INS-1 cell proliferation [1].

Synthetic ligands of peroxisomal proliferator-activated receptor-gamma (PPAR-gamma), improve peripheral insulin sensitivity and glucose-stimulated insulin secretion in pancreatic beta-cells. The beta-cell-specific glucokinase (betaGK) promoter, which constitutes glucose-sensing apparatus in pancreatic beta-cells, contains a peroxisomal proliferator response element (PPRE) in the promoter. The betaGK-PPRE is located in the region between +47 and +68 bp. PPAR-gamma/retinoid X receptor-alpha heterodimer binds to the element and activates the betaGK promoter [6].

- **Significant inhibition of response to insulin by SREBP-1c in liver** :

Transcription of the gene encoding sterol regulatory element-binding protein 1c (SREBP-1c) is known to be activated by insulin in the liver [2]. Synthesis of fatty acids in the liver and adipose tissue in response to insulin is critically dependent on the transcription factor SREBP-1c [3]. The rat SREBP-1c promoter contains binding sites for: 1) LXR (liver X receptor), which binds to liver X response elements (LXREs) 2) transcription factor Sp1, that has a binding site on SREs 3) transcription factor NF-Y (nuclear factor-Y) that also has a binding site on SREs and 4) SREBP itself, which also binds to sterol response elements (SREs) [3].

Each of these sites is required for the full stimulatory response of the SREBP-1c promoter to insulin [3]. Complete insulin response (increase of 6- to 11-fold) requires two binding sites for liver X receptors (LXRs), which are nuclear receptors that are activated by oxygenated sterols. Disruption of these binding sites did not lower basal transcription but severely reduced the response to insulin. In contrast, disruption of the closely linked binding sites for SREBPs and nuclear factor Y lowered basal transcription drastically but still permitted a 4- to 7-fold increase in response to insulin. Arachidonic acid, an inhibitor of LXR activation, blocked the response to insulin [2].

- Significant inhibition of the insulin receptor, suggested inhibition of response to insulin due to inhibition of insulin receptor

promoter :

The human insulin receptor promoter region (HINSR) contains six GGGCGG sequences [4]. Sp1 binds to canonical GGGCGG or its atypical hexanucleotide sequence, called "GC box" motif, of several cellular and viral genes and activates transcription of these genes by RNA polymerase II. Retinoid receptors RARs/RXRs physically interact with Sp1, potentiate Sp1 binding to the GC box motifs [5]. However, in massive doses of retinoic acid as seen in acne-subjects, Sp1 binding affinity to the GC box may be decreased, thus resulting in downregulation of the insulin receptor.

- Altered renal clearance of insulin :

Renal clearance is a major pathway for regulating the levels of insulin and other low molecular weight polypeptide hormones in the systemic circulation.
Reabsorption of insulin from the glomerular filtrate occurs by binding to megalin on the luminal surface of proximal tubule cells followed by endocytosis and degradation in lysosomes. An insulin binding site, megalin, was identified in renal microvillar membranes. Megalin is a large multiligand binding endocytic receptor that is abundantly expressed in clathrin-coated pits on the apical surface of proximal tubule cells. Megalin is able to internalize insulin into endocytic vesicles. In ligand blotting assays, megalin also bound several other low molecular weight polypeptides, including beta2-microglobulin, epidermal growth factor, prolactin, lysozyme, and cytochrome c, suggesting that megalin may play a significant role as a renal reabsorption receptor for the uptake of insulin and other low molecular weight polypeptides from the glomerular filtrate [6].

- Suggested alteration of insulin production :

Well-orchestrated transcriptional regulation of pancreatic beta cells is essential for insulin production and glucose homeostasis. Pancreas duodenum homeobox-1 (PDX-1) is a key regulator of glucose-dependent insulin production and glucose metabolism. We find that PDX-1 interacts with the PDZ-domain coactivator Bridge-1 in yeast interaction trap assays. Rat Bridge-1 and PDX-1 interact directly in GST pull-down assays via Bridge-1 interactions with the amino-terminal transactivation domain of PDX-1. Bridge-1 also interacts with wild-type and mutant human PDX-1 (IPF-1) proteins and strongly interacts with the amino-terminal PDX-1 P63fsdelC (MODY4) mutant protein. Transcriptional activation by PDX-1 is increased by addition of Bridge-1 in multiple contexts, including synergistic activation of a Gal4 reporter by Gal4-Bridge-1 and Gal4-PDX-1 fusion proteins, activation of the somatostatin promoter TAAT1 enhancer, and synergistic activation of the rat insulin I promoter FarFlat enhancer by PDX-1, E12, and E47. We propose that the coactivator Bridge-1 modulates PDX-1 functions in the regulation of its target genes [8].

- Reduced resistin levels :

In small doses, Retinoic acid (RA), the acid form of vitamin A, exerts funtions a signal that inhibits the expression of resistin, an adipocyte-secreted protein previously proposed to act as an inhibitor of adipocyte differentiation and as a systemic insulin resistance factor. Both 9-cis and all-trans RA reduced resistin mRNA levels in white and brown adipocyte cell model systems; the effect was time- and dose-dependent, was followed by a reduced secretion of resistin, and was reproduced by selective agonists of both RA receptors and

retinoid receptors. Association of CCAAT/enhancer-binding protein alpha (a positive regulator of the resistin gene) and its coactivators p300, cAMP response element-binding protein binding protein, and retinoblastoma protein with the resistin gene promoter was reduced in RA-treated adipocytes. RA administration to normal mice resulted in reduced resistin mRNA levels in brown and white adipose tissues, reduced circulating resistin levels, reduced body weight, and improved glucose tolerance. Resistin expression was also downregulated after dietary vitamin A supplementation in mice. The results raise the possibility that vitamin A status may contribute to modulate systemic functions through effects on the production of adipocyte-derived protein signals [9].

It is unknown how the levels of resistin are affected during a severe retinoic acid exposure and how resisitin levels are affected in a chronical vitamin A deficient condition.

- Significant interaction with the pancreatic secretin receptor :

The human secretin receptor (hSR) is an important glycoprotein receptor for regulating the secretion of pancreatic bicarbonate, water, and electrolytes. The secretin receptor is in humans suggested to be regulated by Sp1 and Sp3 binding to GC-box in the promoter [10]. A significant downregulation of the secretin receptor in association with (Ro)accutane exposure is therefore here suggested.

- Alterations of glucose transport :

GLUT-1

In the development of diabetic nephropathy, angiotensin (Ang) II is thought to exert numerous actions on the glomerulus, and especially on the mesangium. However, the role(s) played by Ang II in the glucose metabolism per se in mesangial cells remains unclear. Ang II, at least via its type 1 receptor (AT1-R)-mediated effect, phosphorylates extracellular signal regulated kinase (ERK) by transactivation of epidermal growth factor receptors (EGF-Rs) via the Ca2+ or protein kinase C (PKC) pathways. Ang II upregulated GLUT1 mRNA accumulation in a time- and dose-dependent manner (peaking at 12 h; approximately 3.8-fold vs. control), and this upregulation was completely inhibited by the PKC inhibitor calphostin-C. The Ang II-induced GLUT1 expression was significantly inhibited by the EGF-R inhibitor AG1478 (approximately 80% inhibition), by inactivation of ERK by PD98059, and by pretreatment with heparin and the metalloproteinase (MMP) inhibitor batimastat [13]. In Xenopus oocytes, all six native cysteine residues of GLUT1 were changed to either glycine or serine residues by site-directed mutagenesis, resulting in a functional Glut1 construct with Cys mutated to Gly/Ser (C-less). The GLUT1 reporter molecule was engineered from C-less GLUT1 by creating a unique cleavage site for factor Xa protease within the central cytoplasmic loop and by eliminating the site of N-linked glycosylation [14]. The data suggests that the clusering of GLUT-1 is of importance for function.

GLUT-2

ATRA ralsed the GLUT 2 mRNA in a bell-shaped concentration response curve after 48 h [1]. This bell shaped curve is here suggested to possibly result in inhibition of GLUT-2 in higher doses of retinoic acid. In mice, the expression of type 2 glucose transporter isoform (GLUT2) could be regulated by PPAR-gamma in the liver through binding of PPARgamma

to the GLUT-2 promoter [21].

Gene 33 is found to be regulated by both insulin and glucocorticoids. It is suggested that that the MEK-ERK, but not the phosphatidylinositol 3-kinase (PI3-K), pathway plays a direct role in insulin regulation of Gene33 transcription and protein expression. Gene33 is found to be involved in proliferation and differentiation of cells [16]. Inhibition of insulin signaling via the ERK pathway is thus suggested to contribute to apoptosis, and and pathway of (Ro)accutane induced hypometabolism.

- Significant downregulation of the farnesoid X receptor (FXR) :

Dyslipidemia and gallbladder diseases are two current anomalies observed in patients suffering from the metabolic syndrome and type 2 diabetes. The bile acid-activated nuclear receptor farnesoid X receptor (FXR) controls bile acid as well as lipid metabolism. Recent observations indicate a role for FXR also in carbohydrate metabolism. Hepatic FXR expression is altered in diabetic animal models in vivo and regulated by hormones and nutrients in vitro. At the molecular level, FXR activation modifies the transcriptional activity of different transcription factors controlling gluconeogenesis and lipogenesis, thus affecting in concert bile acid, lipid and carbohydrate metabolism [12].

- Calpain :

Calpains have shown to be altered during the caspases, and modulate NF-kappB, both mechanisms that are involved in (Ro)accutane exposure.

The mRNA expression of type 2 diabetes-related genes in white blood cells (WBC) was examined before and after onset in Otsuka Long-Evans Tokushima Fatty (OLETF) rat. The level of the calpain 10 (CAPN10) transcript was significantly decreased compared to control animals in WBC before and after onset. Significant decreases in this gene expression were also found in the major insulin-target tissues as well as WBC before onset. These results suggest that gene expression in WBC could be a useful screening system for predicting the incidence of type 2 diabetes before onset in OLETF rats, and that CAPN10 represents a potential candidate gene for predicting type 2 diabetes in human [15].

- Autoimmune contribution to diabetic complications :

An overproduction of cytokines are normally connected with a cell destruction in autoimmune diabetes. Indeed (Ro)accutane may trigger such an event, by for example a significant elevation of TGFbeta1. However, (Ro)accutane may exert an opposite effect, inhibit cytokine production, which also may affect several pathways that may severely affect the beta cells, production, release and response to insulin.

Cytokines are important humoral mediators of beta cell destruction in autoimmune diabetes. Exposure of RINm5F cells to IL-1beta or to a cytokine mixture (IL-1beta, TNF-alpha, IFN-gamma) for 6 h resulted in the differential expression of a functional gene cluster. Apart from the well-known up-regulation of the cytokine-responsive genes iNOS, NF-kappaB, MnSOD and Hsp70, several genes that belong to the functional cluster of the endocytotic pathway were identified. These endocytotic genes comprised: clathrin,

megalin, synaptotagmin and calcineurin, which were up-regulated by IL-1beta or the cytokine mixture [18].

- (Ro)accutane induced diabetes mellitus :

The insulin patway is a *major* pathway in how (Ro)accutane mediates its effects. (Ro)accutane exposure in human subjects may result in diabetes mellitus. The (Ro)accutane induced general effects share quite well the picture with a diabetic diagnosis, and depending on severity, diabetes mellitus may be adequate in some subjects.

The clinical picture of type 2 diabetes mellitus (T2DM) is formed by impairment in insulin secretion and resistance to insulin action. Sequence differences in a few genes have been associated, so far, with complex, polygenic forms of T2DM, for example, calpain 10, PPARgamma, KCJN11, and insulin. In addition, some evidence exists that genes, such as adiponectin, IRS-1, and some others may also influence the susceptibility to T2DM. It is expected that in the nearest future more T2DM susceptibility genes will be identified [17].

- Conclusions :

Insulin secretion and release are most definitely significantly affected during a (Ro)accutane exposure in human acne-subjects. A clear and significant interaction with several components of the insulin related system is found. The lasting effects after exposure on insulin secretion, recirculation and receptor function are not known. The insulin receptors are found to be heavily affected by retinoid receptors, and their GC-boxes in the insulin receptor promoter area, show exact binding sites for Sp1 that are inhibited. The insulin receptors are with highest certainity significantly suppressed during a (Ro)accutane exposure, resulting in reduced insulin sensitivity. In rats, isotretinoin increased glycerol concentrations and decreased the insulin sensitivity of peripheral tissues, allready after two weeks of exposure. This is suggested not to be considered a side-effect, but an actual effect, which among other things is significantly antiproliferative. In the liver, the insulin response through SREBP-1 is suggested to be significantly inhibited.

Both renal clearance of insulin and angiotensin II are suggested to heavily affected, thus contibuting to inhibition of glutamate transporters. Glut-1 and Glut-2 via PPARgamma are found to be affected, but it is likely that more glutamate transporters are involved. Insulin production may be affected due to interaction with PDX promoters. A possible significant loss of insulin producing beta-cells can not be excluded.

Both resistin and secretin are heavily affected. (Ro)accutane induces, in the normal response profile, a significantly downregulated cytokine production, which may contribute to an atypical immune-dependent diabetes. Insulin and insulin receptors are suggested to insert important functions in the brain, which not are fully clarified. The effects on the insulin-system are major, and may result in both type 1 and 2 diabetes, severe insulin resistance, or a combination.

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1.5 Vitamin A metabol. and cellular uptake :

To complete

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4449691/

It is shown here that bovine liver membranes, but not supernatant fractions, can isomerize (only trans-) retinoic acid into 9-cis-retinoic acid and 13-cis-retinoic acid.

https://www.ncbi.nlm.nih.gov/pubmed/8172607

"It has already been shown that S2 cells and COS-1 cells can isomerize (only trans-) retinoic acid to generate 9-cis-retinoic acid and 13-cis-retinoic acid [11]. -13]. "

https://www.researchgate.net/publication/15025868_Isomerization_of_All-trans-retinoic_acid_to_9-cis-Retinoic_acid

"However, the observed non-stereospecific isomerization process seems to mimic what is observed when (trans-) retinoic acid is added to COS-1 or S2 cells in culture [11-13]. Here, large amounts of 13-cis-retinoic acid have been generated in addition to 9-cis-retinoic acid. "

https://www.ncbi.nlm.nih.gov/pubmed/8172607

- "The human small intestine can isomerize (only trans-) retinoic acid. 13-cis-retinoic acid is the predominant cis isomer after incubation of intestinal mucosal receptors for (trans) retinoic acid. "

https://www.ncbi.nlm.nih.gov/pubmed/2324641

- "It has recently been found that all trans-and 13-cis-RA are metabolized in hamsters from 13-cis-4-oxo-RA to sufficient vitamin A (5). Studies have shown that all trans-and 13-cis-RA in a ratio of 2 to 1 are present in the intestinal mucosa of canine bile duct rats shortly after the administration of acid (only trans) retinoic (6, 7). "
http://www.jlr.org/content/31/2/175.full.pdf

https://www.ncbi.nlm.nih.gov/pubmed/2324641

Thus, after subtraction of 13-cis-RA formed from the controls (1.0 ng), incubation of the intestinal mucosal homogenates affiliated with all-trans-RA (26 ng) resulted in the formation of 8 to 8.3 ng of 13-cis-RA which corresponds to the formation of 0.1 to 0.2 ng of 13-cis-RAlh per mg of protein "

https://www.ncbi.nlm.nih.gov/pubmed/2324641

- "The appearance of 13-cis-RA depended linearly on the protein concentration of the intestinal mucosa in the same subject (in a typical experiment, the equation of the regression line was Y = 0.012 + 1.226X, r * 0.99996) but not between individual subjects who had different protein concentrations. "

https://www.ncbi.nlm.nih.gov/pubmed/2324641

- "The presence of 13-cis-RA as the predominant form of retinoic acid in human urine under normal physiological conditions has recently been reported (18). An earlier study demonstrated that 13-cis-RA is an in vivo metabolite of all-trans-RA in the intestinal mucosa of rats (17). In our experiments, homogenates of human intestinal mucosa were used, and the data obtained presented unequivocal evidence for the in vitro formation of 13-cis-RA from all-trans-RA. Although it is reasonable to assume that all-trans-RA can be converted to isomers other than 13-cis-RA (such as 13-cis-, 11-cis-, 9,13-di-cis-, etc.). Our studies using the human small intestine and our analysis of human sera have shown that 13-cis-RA is the predominant cis isomer. This is in agreement with the isomerization models of all-transRA that we obtained in photo-catalytic isomerization experiments of all-trans-RA (G. Tang and R. Russell, unpublished results). "
[...] Although 13-cis-RA in human serum is most likely an all-trans-RA isomerization metabolite, it is possible that 13-cis-RA is also an oxidation metabolite of 13-cis-retinol. "

https://www.ncbi.nlm.nih.gov/pubmed/2324641

The rapid isomerization of 13-cis retinoic acid at high levels of all-trans retinoic acid was a sebocyte-specific event, as no significant isomerization of 13-cis retinoic acid to total retinoic acid occurred. in HaCaT keratinocytes.

https://www.ncbi.nlm.nih.gov/pubmed/10951254

The two possible metabolites of 13-cis-RA are isomerization to the all-trans isomer or oxidation followed by glucuronidation and secretion (19). 13-cis-RA will occur in equilibrium with all-trans-RA, which may activate the acid-retinoic-dependent transcriptional pathway.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1149451/

Part of this efficiency probably stems from the ability of 13-cis-retinoic acid to undergo isomerization for the significantly higher all-trans and 9-cis isomers; however, this does not take into account all the pharmacological effects observed when using this retinoid.

https://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+3929

13-cis-RA can be isomerized to all-trans RA and then 9-cis-RA, two strong RA receptor ligands (RAR)

https://www.ncbi.nlm.nih.gov/pubmed/11606944

Another study (which talks about the inhibiting effect):

http://www.nature.com/jid/journal/v115/n2/full/5600791a.html

- Clinical observations of (Ro)accutane induced significant vitamin A deficiency :

13-cis-retinoic acid, and all-trans-retinoic acid are vitamin A derivatives used in among other things the treatment of cancer and currently also severe acne. Patients taking these drugs often show side effects resembling the symptoms of hypovitaminosis A (significant vitamin A deficiency) [12 and more]. Vitamin A status plays an important role in reducing infectious disease morbidity and mortality by enhancing immunity, an effect that is partly mediated by macrophages [15]. Other revealed functions in studies of vitamin A include cellular differentiation of epithelial tissues, growth, reproduction and vision [17]. Retinoid metabolism has also been found to take place in several parts of the human brain [18 and more].

- Suggested (Ro)accutane induced inhibition of kidney function, significantly decreased uptake of RBP and retinol :

Plasma retinol-binding protein (RBP) combined with vitamin A (retinol) is partially filtered through the glomerulus and then absorbed by proximal tubule cells, leading to recycling of retinol to the circulation. Reabsorption of RBP-retinol complexes by proximal tubule cells is mediated by megalin (gp 330), an apical endocytic receptor [4]. Analysis of mice with target disruption of the gene for a major endocytic receptor of proximal tubules, megalin, revealed no RBP in proximal tubules of these mice. Western blotting and HPLC of the urine of the megalin-deficient mice instead revealed a highly increased urinary excretion of RBP and retinol, demonstrating that glomerular filtered RBP-retinol of megalin-deficient mice escapes uptake by proximal tubules [1].

It is well established that the kidney plays an essential role in regulating the homeostasis of body fluids. Evidence shows a significant role of the kidney to be an important organ for the regulation in the metabolism of both fat (vitamin A, D) and water soluble vitamins (e.g. vitamin B12). This regulation is mediated by glomerular filtration as well as reabsorption and secretion processes of protein-bound vitamins. Vitamin transport proteins such as

retinol-binding protein, vitamin D-binding protein and transcobalamin II are filtered in renal glomeruli and subsequently reabsorbed in the proximal tubules by endocytosis from the tubular fluid. Megalin, a scavenger receptor belonging to the LDL receptor family, is probably the most important receptor in this process in the proximal tubule cells [2]. A significant deficiency of vitamin A after a (Ro)accutane exposure in human subjects is here suggested, partly due to difficulties with absorbtion of retinol binding protein (RBP).

- (Ro)accutane induced inhibition of vitamin A metabolism :

Retinol dehydrogenase-4 (RoDH-4) converts retinol and 13-cis-retinol to corresponding aldehydes in human liver and skin in the presence of NAD(+). RoDH-4 also converts 3 alpha-androstanediol and androsterone into dihydrotestosterone and androstanedione. 13-cis-retinoic acid (isotretinoin), 3,4-didehydroretinoic acid, and 3,4-didehydroretinol, but not all-trans-retinoic acid or the synthetic retinoids acitretin and adapalene, are competitive inhibitors of the oxidative 3 alpha-HSD activity of RoDH-4 [3]. At micromolar concentrations, 13-cis retinoic acid also was found to inhibit intestinal lecithin-retinol acyltransferase (LRAT) and to a lesser extent liver LRAT and intestinal retinal reductase [12].

Retinal dehydrogenase type 1 (RALDH1) catalyzes the oxidation of retinal to retinoic acid (RA). In the RALDH1 promoter region of rat kidney cells, TATA and CCAAT cis-acting elements as well as SP1, AP1 and octamer (Oct)-binding sites were present. The CCAAT box and Oct-binding site, located between positions -72 and -68 and -56 and -49, respectively, were shown by deletion analysis and site-directed mutation to be critical for promoter activity. Nuclear extracts from kidney cells contain proteins specifically binding the Oct and CCAAT sequences, resulting in the formation of six complexes, while different patterns of complexes were observed with non-kidney cell extracts [16].

- Inhibition of formation of Pyridine diNucleotides through inhibition of Sp1, Sp3, and Egr-1 mediated transcription of PRS - vitamin A metabolism is a downstream target :

One of the metabolic pathways that has an absolute requirement for pyridine dinucleotides is the conversion of retinol (vitamin A) to its physiologically active derivative retinoic acid (RA1). Pyridine dinucleotides are found in mitochondria, in the intracellular membranes, associated with both faces of the plasma membrane, in the cytosol, and in the nucleus. Biosynthesis of RA involves reversible dehydrogenation of retinol to retinaldehyde followed by irreversible oxidation of retinaldehyde to retinoic acid. All of the enzymes that can potentially contribute to the production of RA require at least one form of pyridine dinucleotides as a co-substrate, some can utilize all four dinucleotides, albeit with different efficiencies. Because the pyridine dinucleotide pools are not uniformly distributed across organelles, the activities of RA synthesizing enzymes can be substantially influenced by the availability of pyridine dinucleotides within the relevant sub-cellular compartments. In keratinocyte microsomes the conversion of retinol to retinaldehyde depends on NADP and is regulated by the ratio between its oxidized and reduced forms [9].

Phosphoribosylpyrophosphate(PRPP) synthetase(PRS) catalyzes the formation of PRPP from ATP and ribose-5-phosphate. PRPP is an important substrate for the synthesis of purine, pyrimidine, and pyridine dinucleotides. Human PRS exists as complex aggregates

composed of the 34 kDa catalytic subunits(PRS1 and PRS2) and other 39 kDa component designated PRPP synthetase-associated protein (PAP39) [11]. Three C(2)H(2) zinc finger proteins, namely Sp1, Sp3, and Egr-1, bind to PRS [10].

- Cellular retinoic acid binding protein (Crabp) I and II expression, during and after (Ro)accutane exposure :

Seven binding proteins have been discovered and well characterized: retinol binding protein (RBP); cellular retinol-binding protein (CRBP); cellular retinol-binding protein type two (CRBP-II); cellular retinoic acid binding protein (CRABP); cellular retinoic acid binding protein type two (CRABP-II); cellular retinal-binding protein (CRALBP) and interphotoreceptor retinol-binding protein (IRBP) [17].

(Ro)accutane exposure is found to significantly upregulate CRABP during exposure [6, 7 and more]. CRABP II expression was induced in a dose-dependent manner in SH SY 5Y cells treated with both ATRA and 13-cis RA. Incubation with 10 M ATRA for 6 hr resulted in a 7-fold increase in CRABP II expression relative to the control as compared to a 4.5-fold increase after a comparable 13-cis RA treatment (Table 3). A greater induction of CRABP II expression was observed at 24 hr, with approximately 15- and 11-fold increases relative to the control after incubation with 10 M ATRA and 13-cis RA, respectively [6]. ATRA bound to CRABP I is a more active substrate for metabolism compared to ‘free’ ATRA, indicating a direct role for CRABP I in ATRA metabolism, and CRABP I overexpression in F9 and HNSCC cells reduces cellular response to RA. On this basis, CRABP I appears a mediator of retinoid degradation [8].

- Retinoid receptor expression - induced during heavy exposure, here suggested to be significantly decreased after exposure :

In SH SY 5Y cells, RAR- was induced in a dose-dependent manner after incubation with ATRA. An approximate 5-fold increase in RAR- expression relative to the control was observed at 6 hr with an ATRA concentration of 10 M. Incubation with 13-cis RA resulted in a weaker and delayed induction of RAR-, with a maximum 2.5-fold increase in expression at 24 hr after incubation with 10 M 13-cis RA [6]. The upregulation of RAR during exposure is suggested to be a general response in all retinoid responsive cell lines.

A significantly decreased retinoid receptor expression in human subjects after exposure to (Ro)accutane is here suggested. In hypothyroid subjects, the concentration of TSH was elevated, and dramatically low T3 and T4 concentrations were associated with a decrease in the expression of TR beta. Expression of RAR alpha and RAR gamma significantly decreased in hypothyroid versus control subjects [5].

- Suggested (Ro)accutane induced alterations in transthyretin function :

In primary hepatocytes, RBP complexed with TTR led to a 70% decrease of TTR internalization, whereas TTR bound to thyroxine led to a 20% increase. In the kidney, megalin, a member of the low-density lipoprotein receptor family (LDLr) internalizes TTR. Competition studies showed that lipoproteins inhibit TTR internalization [14]. Transthyretin (TTR) is a beta-sheet rich protein whose plasma half life is 1.9 days. It behaves as a

tetramer and binds to retinol binding protein (RBP) and thyroxin in plasma. TTR is also an anti-acute phase protein, and the concentration is influenced by various conditions, such as inflammation and infection, Mutated forms of TTR are the precursor protein of familial amyloidotic polyneuropathy (FAP). Since plasma TTR is predominantly synthesized by the liver, liver transplantation has been performed as an effective therapy for FAP. Recent research revealed that TTR plays important roles in various central nervous system disorders, such as Alzheimer disease, depression, and lead intoxication. To elucidate the pathogenesis of those disorders, an accurate measurement of TTR concentrations in plasma and cerebrospinal fluids is of vital importance.
[13].

- Conclusions :

(Ro)accutane is likely to result in a renal failure and failure of uptake of vitamin A due to significant inhibition of the megalin receptor complex. The normal vitamin A metabolism is likely to be disrupted due to inhibition of vitamin A metabolizing enzymes such as Rodh-4 and Raldh-1. Inhibition of formation of Pyridine dinucleotides through inhibition of Sp1, Sp3, and Egr-1 mediated transcription of PRS are likely to be of major importance in (Ro)accutane mediated significant disturbance of retinoid metabolism, since pyridine dinucleotides have shown to be highly involved.

Taken together, (Ro)accutane exposure is likely to result in a pronounced clinical vitamin A deficiency bound to disruption in several metabolizing pathways, inhibition of receptor mediated uptake and internalization and altered receptor expression.

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1.6 Vitamin D metabol. and cellular uptake :

To complete

http://www.ncbi.nlm.nih.gov/pubmed/1357865

- Clinical observations of 1,25-dihydroxyvitamin D in human subjects exposed to (Ro)accutane :

A significant fall in the level of 1,25-dihydroxyvitamin D, and a significant increase in the molar ratio of 24, 25-dihydroxyvitamin D to 25-hydroxyvitamin D was found in human subjects after exposure to (Ro)accutane, indicating a (Ro)accutane induced significant 1,25-dihydroxyvitamin D deficiency [3].

- Interaction with insulin-like growth factor binding proteins (IGFBPs) :

Recently, insulin-like growth factor binding proteins (IGFBPs) have been found to be primary mediators of the anti-proliferative actions of the nuclear hormone 1alpha,25-dihydroxyvitamin D3 [1alpha,25(OH)2D3], but dependent on cellular context IGFBPs can also have a mitogenic effect. Expression profiling of all six human IGFBP genes in prostate and bone cancer cells and demonstrated that IGFBP1, 3 and 5 are primary 1alpha,25(OH)2D3 target genes [18].

- Vitamin D metabolism :

The enzyme 1alpha-hydroxylase, which converts vitamin D to its active form, has been found to be coordinated by Nuclear Factor Kappa B (NFkappaB) [5]. All-trans-retinoic-acid (ATRA) was found to significantly reduce nuclear levels of both subunits (p50 and p65) of NF-kappaB [6]. HB-EGF dramatically suppressed NF-kappaB activity and IL-8 release and decreased NO production in cells pretreated with HB-EGF. HB-EGF blocked NF-kappaB activation by inhibiting IkappaB kinase activation and IkappaB phosphorylation and degradation, thus interfering with NF-kappaB nuclear translocation, DNA-binding activity, and NF-kappaB-dependent transcriptional activity [7]. In cultured keratinocytes, real-time PCR analyses revealed that HB-EGF mRNA expression was elevated dose-dependently with atRA, peaking at 12 h. All-trans retinal and all-trans retinol were found to upregulate HB-EGF when used at 0,1-1,0 microM/l to a similiar extent of all-trans retinoic acid at 1,0-10,0 microM [8]. Cubilin is a membrane-associated protein colocalizing with megalin, facilitates the endocytic process by sequestering steroid-carrier complexes on the cellular surface before megalin-mediated internalization of the cubilin-bound ligand [13]. In rats, cubilin is a 460-kDa multipurpose, multidomain receptor that contains an NH(2)-terminal 110-residue segment followed by 8 epidermal growth factor (EGF)-like repeats and a contiguous stretch (representing nearly 88% of its mass) [11]. This indicates that a significant upregulation of Heparin binding-EGF may strongly affect cubulin receptors, thus being one pathway of (Ro)accutane inhibition of endocytosis in exposed human subjects. This is further confirmed by the finding that heparin inhibits binding of thyroglobulin to the endocytotic receptor megalin [12] , which also, in a similiar manner binds the plasma carrier for vitamin D, vitamin D binding protein [13].

- Inhibition of kidney function of vitamin D-metabolism and endocytosis :

In endocytic receptor megalin deficient mice, it has been shown that the 1alpha-hydroxylase can also be coregulated by megalin, and a fall in kidney vitamin D metabolites seems to be accompanied by a raise in TGF-beta1 target genes [9]. Megalin is highly expressed in the proximal tubules of the kidney. This receptor is essential for the renal uptake and conversion of 25-OH vitamin D3 to 1,25-(OH)2 vitamin D3 [10, 17 and more].

- Receptor expression and function :

The Vitamin D Receptor (VDR) belongs to the superfamily of steroid/thyroid hormone receptors that is activated by 1alpha,25-dihydroxyvitamin D3 [1]. Receptors bind to hormone response elements (HREs) via their DNA-binding domains (DBDs) [2]. In small doses RARs/RXRs physically interact with Sp1, potentiate Sp1 binding to the GC box motifs [15]. The nucleotide sequence of the Sp1 region is well conserved between the

mouse, the human, and the chicken VDR genes, suggesting an important role for these Sp1 sites. Gel shift analysis of the four Sp1 sites of the mVDR promoter has confirmed specific binding complexes to Sp1-1, Sp1-2, and Sp1-4 (Sp1-3 rather binds an unknown complex that is unable to bind the canonical Sp1 GGGGCGGGGC). Deletion or mutation of all the Sp1 sites eliminates promoter activity [16]. It is here suggested that a high dose RA as opposed to a small dose, inhibits Sp1 formation and binding.

The Vitamin D receptor (VDR) is a ligand-responsive transcription factor that forms homo- or heterodimers on response elements composed of two hexameric half-sites separated by three base pairs of spacer DNA. Binding of 1alpha,25-dihydroxyvitamin D(3) to the full-length VDR causes destabilization of the VDR homodimer and formation of a heterodimeric complex with the 9-cis retinoic acid receptor (RXR) [2].

Traditional targets for 1alpha,25-dihydroxyvitamin D3 action include tissues involved in the maintenance of calcium homeostasis, bone development and remodeling including the bone, kidney, skeletal and cardiac muscle. PGC-1alpha, a transcriptional coactivator which plays a role in mitochondrial biogenesis and energy metabolism, is predominantly expressed in the kidney, heart, liver and skeletal muscle tissues [1]. The VDR also plays a role in cellular proliferation and differentiation [2]. The 1,25-dihydroxyvitamin D3 receptor (VDR), is also expressed in the human brain, where its function is not clarified, as well as 1alpha-hydroxylase (1alpha-OHase), the enzyme responsible for the formation of the active vitamin in the human brain. The receptor and the enzyme were found in both neurons and glial cells in a regional and layer-specific pattern. The VDR was restricted to the nucleus whilst 1alpha-OHase was distributed throughout the cytoplasm. The distribution of the VDR in human brain was strikingly similar to that reported in rodents [4]. Down-regulation of 1alpha-hydroxylase promoter through NFkappaB signaling may contribute to the pathogenesis of inflammation-associated osteopenia/osteoporosis [5].

1,25-dihydroxyvitamin D3 (D3) is found accelerate integrin beta 3 transcription [14], signifying that a clinical 1,25-dihydoxyvitamin D3 deficiency likely decreases integrin beta 3 transcription, this due to that the integrin beta 3 is suggested to contain a VDRE.

- For further information about VDR receptor expression :

Genecards - VDR
(Genecards database)

GenAtlas - VDR
(GenAtlas database)

For further information about megalin/gp330/LRP-2

Genecards - LRP-2
(Genecards database)

- Conclusions :

Vitamin D metabolism is found to be significantly altered in human subjects after exposure to (Ro)accutane, indicating a clinical 1,25 dihydroxyvitamin D deficiency. This is highly

likely partly due to TGF-beta1 induced renal failure, significant inhibition of the megalin/cubilin receptor complex, which is known to mediate uptake and recirculation of vitamin D. The vitamin D receptor (VDR) is highly affected by (Ro)accutane mainly through Sp1 sites in the VDR promoter. It is here suggested that by doses of retinoic acid beyond the physiological limit, the VDR receptor is suppressed (downregulated) in a similiar manner as thyroid receptors and androgen receptors.

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Nucleic Acids Res. 2005 Sep 26;33(17):5521-32. Print 2005. Related Articles, Links
Regulation of multiple insulin-like growth factor binding protein genes by 1alpha,25-dihydroxyvitamin D3.
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Recently, insulin-like growth factor binding proteins (IGFBPs) have been found to be primary mediators of the anti-proliferative actions of the nuclear hormone 1alpha,25-dihydroxyvitamin D3 [1alpha,25(OH)2D3], but dependent on cellular context IGFBPs can also have a mitogenic effect. In this study, we performed expression profiling of all six human IGFBP genes in prostate and bone cancer cells and demonstrated that IGFBP1, 3 and 5 are primary 1alpha,25(OH)2D3 target genes. In silico screening of the 174 kb of genomic sequence surrounding all six IGFBP genes identified 15 candidate vitamin D response elements (VDREs) close to or in IGFBP1, 2, 3 and 5 but not in the IGFBP4 and 6 genes. The putative VDREs were evaluated in vitro by gelshift assays and in living cells by reporter gene and chromatin immuno-precipitation (ChIP) assays. Of these 10 VDREs appear to be functional. ChIP assays demonstrated for each of these an individual, stimulation time-dependent association profile not only with the vitamin D receptor, but also with first heterodimeric partner the retinoid X receptor, other regulatory complex components and phosphorylated RNA polymerase II. Some of the VDREs are located distantly from the transcription start sites of IGFBP1, 3 and 5, but all 10 VDREs seem to contribute to the regulation of the genes by 1alpha,25(OH)2D3. In conclusion, IGFBP1, 3 and 5 are primary 1alpha,25(OH)2D3 target genes that in intact cells are each under the control of multiple VDREs.

PMID: 16186133 [PubMed - in process]

1.7 Fat metabolism and absorbtion :

- The PPAR isoforms :

The PPARs (Peroxisome proliferator-activated receptors) are forming a part of the steroidal superfamily of receptors [1]. PPARgamma, a receptor subtype, is accompanied

by a selective upregulation of several adipogenic and lipogenic genes including adipose differentiation-related protein (ADRP), adipocyte fatty acid-binding protein 4, sterol regulatory element-binding protein-1 (SREBP-1), fatty acid synthase (FAS), and acetyl-CoA carboxylase [2].

- Significant inhibition of the Fatty acid synthase :

In rats, fatty acid synthase (FAS), one of the main lipogenic enzymes, converts dietary calories into a storage form of energy. The transcription factors, stimulatory proteins 1 and 3 (Sp1 and Sp3), nuclear factor Y (NF-Y), upstream stimulatory factor (USF) and sterol regulatory element binding protein-1 (SREBP-1) have cognate binding sites on the promoter of the FAS gene [9].

- (Ro)accutane induced inhibition of sterol regulatory binding protein-1 (SREBP-1) function and inhibition of lipoprotein lipase activity :

Sterol regulatory element-binding protein-1 (SREBP-1) is a transcription factor which regulates genes involved in the synthesis of fatty acids and triglycerides [3]. Significantly elevated levels of triglycerides, low density lipoproteins and cholesterol have been found in human subjects exposed to retinoic acid, (Ro)accutane, in repeated studies [4, 5 and more]. SREBP-1c is also found to be coregulated by RXR-LXR receptors in mice [10]. Cholesterol metabolism is tightly controlled by members of the sterol regulatory element-binding protein (SREBP) family of transcription factors [14].

- Inhibition of the lipoprotein lipase (LPL) :

Isotretinoin significantly reduced the fat elimination rate as measured by IVFTT (p less than 0.001) and also decreased the muscle lipoprotein lipase activity (LPLA) (p less than 0.05) [15]. Some studies during the 1980s have denied (Ro)accutane induced significant inhibition of the lipoprotein lipase [16 and more]. The lipoprotein lipase contains promoter regions for Sp1 and SREBP and is therefore with highest certainity affected by (Ro)accutane. An evolutionarily conserved 5'-CCTCCCCCC-3' motif (from -91 to -83, CT element) in the human LPL gene promoter is found. Deletion or mutation caused approximately 70-80% decrease in promoter activity. In the lipoprotein lipase promoter, Sp1 and Sp3 in THP-1 nuclear protein extracts bind specifically to this element. Co-transfection with Sp1 and Sp3 expression plasmids transactivated the LPL promoter via the CT element in Drosophila SL2 cells devoid of Sp proteins. Sp3 moderately repressed Sp1-mediated LPL promoter activation when both were co-expressed in SL2 cells. Furthermore, co-expression of an active sterol regulatory element binding protein (SREBP-1), with Sp1, but not with Sp3, synergistically activated the LPL promoter in SL2 cells.

- Significant inhibition of lipoxygenases :

Peroxisome proliferator-activated receptor-gamma (PPARgamma) activators inhibit cytokine-stimulated LOX-1 expression [8]. The lipoxygenases catalyze the dioxygenation of polyenoic fatty acids such as linoleate and arachidonate. Lectin-like OxLDL receptor was found to be significantly inhibited in cells exposed to retinoic acid. Both LOX-1 and LOX-2 were found to be significantly inhibited by retinol, 13-cis-retinoic acid and all-trans-

retinoic acid. A 50% inhibition of conversion towards linoleic acid (omega 3 isoforms) was observed in doses of 10microM [6]. The expression of LOX-1 affects a variety of gene expression, including adhesion molecules, endothelial constitutive nitric oxide synthetase (eNOS), and monocyte chemoatractant protein-1 (MCP-1) [7].

Resolvins (Rvs) are oxygenated products derived from omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid that carry potent protective bioactions present in resolving inflammatory exudates. Resolvin E1 (RvE1) is biosynthesized in vivo from EPA via transcellular biosynthetic routes during cell-cell interactions, and thus RvE1 is formed in vivo during multicellular responses such as inflammation and microbial infections. RvE1 protects tissues from leukocyte-mediated injury and counterregulates proinflammatory gene expression. These newly identified Rvs may underlie the beneficial actions of omega-3 PUFAs especially in chronic disorders where unresolved inflammation is a key mechanism of pathogenesis [12].

- Inhibition of uptake of Liver-type fatty acid binding protein, L-FABP, and inhibition of cellular uptake of fat :

Liver-type fatty acid binding protein (L-FABP) binds with high affinity to hydrophobic molecules including free fatty acid, bile acid and bilirubin, which are potentially nephrotoxic, and is involved in their metabolism mainly in hepatocytes. Circulatory L-FABP was found to be filtered by glomeruli and internalized by PTC probably via megalin-mediated endocytosis. These results suggest a novel renal uptake pathway for L-FABP, a carrier of hydrophobic molecules, some of which may exert nephrotoxic effects [11].

Surfactant-like particles (SLP) are unilamellar secreted membranes associated with the process of lipid absorption and isolated previously only from the apical surface of enterocytes. In rats, cubilin, and its anchoring protein megalin have been identified as components of extracellular SLP, but only cubilin is present to any extent in intracellular SLP. During fat absorption, intestinal alkaline phosphatase (IAP) is modestly enriched in intracellular SLP, but full-length cubilin (migrating at 210 kDa in fat-fed mucosal fractions) falls by one-half, although fragments of cubilin are abundant in the intracellular SLP. Both IAP and cubilin colocalize to the same cells during corn oil absorption and colocalize around lipid droplets [13]. It is here suggested that (Ro)accutane induced downregulation of megalin and cubilin through inhbition of Sp1 and Sp3 promoter acitivity inhibits cellular uptake of fat and transport of lipid droplets through the cellular membrane.

- The role of fatty acids :

(Ro)accutane induced altered metabolism of fatty acids induce several alterations in various parts of the body, including the brain. It functions as a source of energy and structural components for cells; as a regulator of gene expression that impacts lipid, carbohydrate, and protein metabolism, as well as cell growth and differentiation. Fatty acids interact with the genome through several mechanisms. They regulate the activity or nuclear abundance of several transcription factors, including PPAR, LXR, HNF-4, NFkappaB, and SREBP. Fatty acids or their metabolites bind directly to specific transcription factors to regulate gene transcription. Further definition of these fatty acid-regulated pathways will provide insight into the role dietary fat plays in human health and the onset and progression of several chronic diseases, like coronary artery disease and atherosclerosis, dyslipidemia and inflammation, obesity and diabetes, cancer, major

depressive disorders [18].

- Conclusion :

The fat metabolism is here suggested to be significantly altered in subjects exposed to (Ro)accutane. The toxin is a non-selective PPAR-ligand, while other receptors in the same complex, such as the thyroid receptors (TR) and androgen receptors (AR) are suppressed, resulting in hypometabolism through among other things an inhibition of fat synthesis (through lessened promoter activity of SREBP-1), an alteration of fat storage (via inhibition of FAS) and conversion rates, and a deficiency of omega3 fatty acids (linoleic acids) through PPARgamma mediated inhibition of LOX.

Hoffman la Roche itself sells Xenical a lipoprotein lipase inhibitor, which in itself can be considered a substance filled with doubtful effects on health, such as inhibition of fat uptake and increased circulation of lipoproteins, and should be aware about the exact Sp1 and Sp3 binding sites of the lipoprotein lipase, and an induced significant inhibition by (Ro)accutane.

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Mol Cell Proteomics. 2004 Jul;3(7):692-703. Epub 2004 Apr 8. Related Articles, Links
Polyunsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor alpha ligand-binding domain.
Lengqvist J, Mata De Urquiza A, Bergman AC, Willson TM, Sjovall J, Perlmann T, Griffiths WJ.
Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-17177 Stockholm, Sweden.

Nuclear receptors (NRs) constitute a large and highly conserved family of ligand-activated transcription factors that regulate diverse biological processes such as development, metabolism, and reproduction. As such, NRs have become important drug targets, and the identification of novel NR ligands is a subject of much interest. The retinoid X receptor (RXR) belongs to a subfamily of NRs that bind vitamin A metabolites (i.e. retinoids), including 9-cis-retinoic acid (9-cis-RA). However, although 9-cis-RA has been described as the natural ligand for RXR, its endogenous occurrence has been difficult to confirm. Recently, evidence was provided for the existence of a different natural RXR ligand in mouse brain, the highly enriched polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) (Mata de Urquiza et al. (2000) Science 290, 2140-2144). However, the results suggested that supra-physiological levels of DHA were required for efficient RXR activation. Using a refined method for ligand addition to transfected cells, the current study shows that DHA is a more potent RXR ligand than previously observed, inducing robust RXR activation already at low micromolar concentrations. Furthermore, it is shown that other naturally occurring PUFAs can activate RXR with similar efficiency as DHA. In additional experiments, the binding of fatty acid ligands to RXRalpha is directly demonstrated by electrospray mass spectrometry of the noncovalent complex between the RXR ligand-binding domain (LBD) and its ligands. Data is presented that shows the

noncovalent interaction between the RXR LBD and a number of PUFAs including DHA and arachidonic acid, corroborating the results in transfected cells. Taken together, these results show that RXR binds PUFAs in solution and that these compounds induce receptor activation, suggesting that RXR could function as a fatty acid receptor in vivo.

PMID: 15073272 [PubMed - indexed for MEDLINE]

Biochem Biophys Res Commun. 2004 Oct 22;323(3):1116-23. Related Articles, Links
Peroxisome proliferator-activated receptor alpha ligands activate transcription of lectin-like oxidized low density lipoprotein receptor-1 gene through GC box motif.
Hayashida K, Kume N, Minami M, Inui-Hayashida A, Mukai E, Toyohara M, Kita T.
Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, Japan.

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a receptor for oxidized LDL. Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors regulating transcription of various genes. We examined effects of PPAR ligands on LOX-1 expression and their transcriptional regulation in vascular endothelial cells. PPARalpha-specific ligands, such as fenofibrate and WY-14643, but not PPARgamma-specific ligands induced LOX-1 expression. Reduced expression of PPARalpha by antisense oligonucleotides directed to PPARalpha blocked fenofibrate-induced LOX-1 expression. Luciferase reporter gene assays with deletion and point mutations in the LOX-1 promoter revealed that transcriptional activity of LOX-1 gene by fenofibrate was localized in the -114/-106 GC box. Electrophoretic mobility shift assays with the radiolabeled GC box sequence showed inducible bands by PPARalpha ligands, which is competitively suppressed by unlabeled GC box motif and by an antibody to PPARalpha. In conclusion, PPARalpha appears to be one of the key regulators that induce LOX-1 expression, utilizing the GC box as a promoter.

PMID: 15381115 [PubMed - indexed for MEDLINE]

Endocrinology. 2004 Apr;145(4):1527-38. Epub 2003 Dec 4. Related Articles, Links
Comment in:
Endocrinology. 2004 Apr;145(4):1525-6.
Tyrosine agonists reverse the molecular defects associated with dominant-negative mutations in human peroxisome proliferator-activated receptor gamma.
Agostini M, Gurnell M, Savage DB, Wood EM, Smith AG, Rajanayagam O, Garnes KT, Levinson SH, Xu HE, Schwabe JW, Willson TM, O'Rahilly S, Chatterjee VK.
Department of Medicine, University of Cambridge, Addenbrooke's Hospital, United Kingdom.

Loss-of-function mutations in the ligand-binding domain of human peroxisome proliferator-activated receptor gamma (PPARgamma) are associated with a novel syndrome characterized by partial lipodystrophy and severe insulin resistance. Here we have further characterized the properties of natural dominant-negative PPARgamma mutants (P467L, V290M) and evaluated the efficacy of putative natural ligands and synthetic thiazolidinedione (TZD) or tyrosine-based (TA) receptor agonists in rescuing mutant receptor function. A range of natural ligands failed to activate the PPARgamma mutants and their transcriptional responses to TZDs (e.g. pioglitazone, rosiglitazone) were

markedly attenuated, whereas TAs (e.g. farglitazar) corrected defects in ligand binding and coactivator recruitment by the PPARgamma mutants, restoring transcriptional function comparable with wild-type receptor. Transcriptional silencing via recruitment of corepressor contributes to dominant-negative inhibition of wild type by the P467L and V290M mutants and the introduction of an artificial mutation (L318A) disrupting corepressor interaction abrogated their dominant-negative activity. More complete ligand-dependent corepressor release and reversal of dominant-negative inhibition was achieved with TA than TZD agonists. Modeling suggests a structural basis for these observations: both mutations destabilize helix 12 to favor receptor-corepressor interaction; conversely, farglitazar makes more extensive contacts than rosiglitazone within the ligand-binding pocket, to stabilize helix 12, facilitating corepressor release and transcriptional activation. Farglitazar was a more potent inducer of PPARgamma target gene (aP2) expression in peripheral blood mononuclear cells with the P467L mutation. Having shown that rosiglitazone is of variable and limited efficacy in these subjects, we suggest that TAs may represent a more rational therapeutic approach.

PMID: 14657011 [PubMed - indexed for MEDLINE]

J Lipid Res. 1998 Oct;39(10):2054-64. Related Articles, Links
Sp1 and Sp3 transactivate the human lipoprotein lipase gene promoter through binding to a CT element: synergy with the sterol regulatory element binding protein and reduced transactivation of a naturally occurring promoter variant.
Yang WS, Deeb SS.
Department of Genetics, University of Washington, Seattle 98195, USA.

Lipoprotein lipase (LPL) is a key enzyme in lipoprotein and energy metabolism and, therefore, regulation of its expression could have an important bearing on these processes. We have identified an evolutionarily conserved 5'-CCTCCCCCC-3' motif (from -91 to -83, CT element) in the human LPL gene promoter, deletion or mutation of which caused approximately 70-80% decrease in promoter activity. We found that Sp1 and Sp3 in THP-1 nuclear protein extracts bind specifically to this element. Co-transfection with Sp1 and Sp3 expression plasmids transactivated the LPL promoter via the CT element in Drosophila SL2 cells devoid of Sp proteins. Sp3 moderately repressed Sp1-mediated LPL promoter activation when both were co-expressed in SL2 cells. Furthermore, co-expression of an active sterol regulatory element binding protein (SREBP-1), with Sp1, but not with Sp3, synergistically activated the LPL promoter in SL2 cells. We previously reported a naturally occurring T-->G substitution at position -93 of the human LPL promoter which reduces promoter activity by 40-50% in transient transfection assays. In this study, we showed that this substitution results in reduced binding affinity to Sp1/Sp3 and in diminished transactivation by Sp1/Sp3 alone and by the synergistic action of Spl and SREBP-1 In conclusion, recruitment of Sp1/Sp3 by the CT element may play an important role in expression of the human lipoprotein lipase gene. Synergistic transcriptional activation by Sp1 and SREBP-1 may provide a mechanism for cross-talk between cholesterol and triglyceride metabolic pathways.

PMID: 9788252 [PubMed - indexed for MEDLINE]

Nippon Ronen Igakkai Zasshi. 2002 May;39(3):264-7. Related Articles, Links
[New oxidized LDL receptors and their functions in atherogenesis]
[Article in Japanese]

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Department of Geriatric Medicine, Graduate School of Medicine, Kyoto University.

Oxidized low density lipoprotein (Ox-LDL) appears to play key roles in atherosclerotic progression and plaque rupture. Biological effects of Ox-LDL on vascular cells may, at least in part, be mediated by cell surface receptors for Ox-LDL. Lectin-like oxidized LDL receptor (LOX)-1 and scavenger receptor for phosphatidylserine and oxidized lipoprotein (SR-PSOX) are type II and I membrane glycoprtoeins, respectively, both of which can act as cell-surface endocytosis receptors for atherogenic oxidized LDL (Ox-LDL). LOX-1 expression can dynamically be induced by proinflammatory stimuli, and is detectable in cultured macrophages and activated vascular smooth muscle cells (VSMC), in addition to endothelial cells. LOX-1-dependent uptake of Ox-LDL induced apoptosis of cultured VSMC. In vivo, endothelial cells that cover early atherosclerotic lesions, and intimal macrophages and VSMC in advanced atherosclerotic plaques dominantly express LOX-1. LOX-1 expressed on the cellsurface can be cleaved, in part, and released as soluble molecules, suggesting the diagnostic significance of plasma soluble LOX-1 levels. SR-PSOX appeared to be identical to CXCL16, a novel membrane-anchored chemokine directed to CXCR6-positive lymphocytes, suggesting another role of SR-PSOX as T-cell chemoattractant. In contrast to LOX-1 expressed by a variety of cell types. SR-PSOX expression appeared relatively confined to macrophages in atherogenesis. Taken together, LOX-1 and SR-PSOX may play important roles in atherogenesis and athrosclerotic plaque rupture.

PMID: 12073583 [PubMed - indexed for MEDLINE]

1.8 Cortisol metabolism :

- Receptor expression and metabolism :

Cortisol binds to the human glucocorticoid receptor (hGR) [1]. In humans, hGR is found to exist in two isoforms, receptor protein a (94kDa) and b (91kDa) [4]. 11Beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) catalyzes the conversion of 11-dehydrocorticosterone to its active form corticosterone in rodents (or cortisone to cortisol in humans). The reductive reaction of the 11-keto to 11-hydroxyl is the pivotal switch in the activation of glucocorticoids [2, 3 and more].

C/EBPalpha is a potent activator of the 11beta-HSD1 gene in hepatoma cells and that mice deficient in C/EBPalpha have reduced hepatic 11beta-HSD1 expression. In contrast, C/EBPbeta is a relatively weak activator of 11beta-HSD1 transcription in hepatoma cells and attenuates C/EBPalpha induction, and mice that lack C/EBPbeta have increased hepatic 11beta-HSD1 mRNA. The 11beta-HSD1 promoter (between -812 and +76) contains 10 C/EBP binding sites, and mutation of the promoter proximal sites decreases the C/EBP inducibility of the promoter. One site encompasses the transcription start, and both C/EBPalpha and C/EBPbeta are present in complexes formed by liver nuclear proteins at this site. The regulation of 11beta-HSD1 expression, and hence intracellular glucocorticoid levels, by members of the C/EBP family provides a mechanism for cross-talk between the C/EBP family of transcription factors and the glucocorticoid signaling pathway [5].

11beta-HSD-1 is particularly expressed in adipocytes and liver and appears to be causally linked to the development of type 2 diabetes and the metabolic syndrome. Liver X receptor (LXR)-alpha and -beta are nuclear oxysterol receptors whose key role in lipid metabolic regulation has recently been established. Adipocytes derived from 3T3-L1 cells and mouse embryonic fibroblasts in vitro with synthetic or natural LXR agonists decreases mRNA expression of 11beta-HSD-1 by approximately 50%, paralleled by a significant decline in 11beta-HSD-1 enzyme activity [3].

A clinical vitamin A deficiency is here suggested to be a result after a (Ro)accutane exposure in human subjects, partly due to TGF-beta1 induced megalin/cubulin inhibition leading to severe failure in the uptake and recirculation of retinol binding protein (RBP) see sections about vitamin A and D.

- Hydrocortisone (HCT) :

The retinoic acid receptor cDNA bears a 15% homology to the hydrocortisone (HCT) receptor, which thus here is suggested to be one additive pathway of (Ro)accutane induced significant interference with the GH/IGF-axis. In rat pituitary GH3 cells, hydrocortisone is known to stimulate GH secretion. Retinoic acid <1 microM stimulated growth hormone secretion by 220%. 50 nM HCT stimulated GH secretion 3,5 times and in synergy GH secretion was stimulated seven times. Retinoic acid selectively stimulates basal and HCT-induced GH secretion and mRNA levels in these cells in a dose- and time-dependent manner [6].

- Conclusions :

to complete...

- References :

[1] **von Langen J, Fritzemeier KH, Diekmann S, Hillisch A.** *Molecular basis of the interaction specificity between the human glucocorticoid receptor and its endogenous steroid ligand cortisol.* (2005)Chembiochem. Jun;6(6):1110-8.
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Crystal structure of murine 11 beta-hydroxysteroid dehydrogenase 1: an important therapeutic target for diabetes. (2005) Biochemistry. May 10;44(18):6948-57.
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Stulnig TM, Oppermann U, Steffensen KR, Schuster GU, Gustafsson JA. (2002) Diabetes. Aug;51(8):2426-33.
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(2000) J Biol Chem. Sep 29;275(39):30232-9.

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Retinoic acid selectively stimulates growth hormone secretion and messenger ribonucleic acid levels in rat pituitary cells. (1989) Endocrinology. May;124(5):2052-6.

Acta Biochim Pol. 2004;51(4):907-17. Related Articles, Links
Inhibition of CYP17 expression by adrenal androgens and transforming growth factor beta in adrenocortical cells.
Biernacka-Lukanty JM, Lehmann TP, Trzeciak WH.
Department of Biochemistry and Molecular Biology, University of Medical Sciences, Poznan, Poland.

Cytochrome P450c17, encoded by the CYP17 gene, is a component of the 17alpha-hydroxylase/17,20-lyase enzyme complex essential for production of adrenal glucocorticoids and androgens as well as gonadal androgens. The expression of CYP17 in adrenocortical cells is stimulated by corticotropin (ACTH) via the signal transduction pathway involving cAMP and protein kinase A (PKA). Thus, in addition to glucocorticoids, ACTH stimulates formation of adrenal androgens, which are known to induce transforming growth factor beta (TGF-beta) secretion. TGF-b in turn inhibits steroid hormone output by attenuating both basal and ACTH-dependent expression of CYP17. The present study revealed that treatment of bovine and human H295R adrenocortical cells with androgens resulted in a decrease in the basal level of CYP17 transcript and cortisol secretion, without affecting forskolin-stimulated levels. We also demonstrated that in H295R cells TGF-beta inhibited both basal and forskolin-stimulated accumulation of CYP17 mRNA. Determination of promoter activity, directing luciferase reporter gene expression in H295R cells transfected with deletion fragments of bovine CYP17 promoter, indicated that the -483 to -433 bp fragment of the promoter was necessary for the inhibitory action of TGF-beta on CYP17 expression. It is concluded that in bovine and human adrenocortical cells, androgens inhibit basal CYP17 expression probably at the transcriptional level and independently of the effect of TGF-beta.

PMID: 15625562 [PubMed - in process]

Chembiochem. 2005 Jun;6(6):1110-8. Related Articles, Links
Molecular basis of the interaction specificity between the human glucocorticoid receptor and its endogenous steroid ligand cortisol.
von Langen J, Fritzemeier KH, Diekmann S, Hillisch A.
Institute for Molecular Biotechnology, Beutenbergstrasse 11, 07745 Jena, Germany.

We analyzed the binding of five steroids to the human glucocorticoid receptor (hGR) experimentally as well as theoretically. In vitro, we measured the binding affinity of aldosterone, cortisol, estradiol, progesterone, and testosterone to hGR in competition with the ligand dexamethasone. The binding affinity relative to the endogenous ligand cortisol (100%) is reduced for progesterone (22%) and aldosterone (20%) and is very weak for testosterone (1.5%) and estradiol (0.2%). In parallel, we constructed a homology model of the hGR ligand-binding domain (LBD) based on the crystal structure of the human progesterone receptor (hPR). After docking the five steroids into the hGR model ligand-binding pocket, we performed five separate 4-ns molecular dynamics (MD) simulations with these complexes in order to study the complex structures. We calculated the binding affinities with two different approaches (MM/PBSA, FlexX) and compared them with the

values of the experimentally determined relative binding affinities. Both theoretical methods allowed discrimination between strongly and weakly binding ligands and recognition of cortisol as the endogenous ligand of the hGR in silico. Cortisol binds most strongly due to a nearly perfect steric and electrostatic complementarity with the hGR binding pocket. Chemically similar ligands such as estradiol, testosterone, and progesterone also fit into the hGR binding pocket, but they are unable to form all those contacts with the amino acids of the protein that are necessary to yield a stable, transcriptionally active receptor conformation. Our analysis thus explains the selectivity of the human glucocorticoid receptor for its endogenous ligand cortisol at a molecular level.

PMID: 15883974 [PubMed - in process]

Metabolism. 2005 May;54(5):584-9. Related Articles, Links
Corticosteroid-binding globulin affects the relationship between circulating adiponectin and cortisol in men and women.
Fernandez-Real JM, Pugeat M, Lopez-Bermejo A, Bornet H, Ricart W.
Diabetes, Endocrinology and Nutrition Research Unit, Dr. Josep Trueta University Hospital of Girona, Carretera de Francia s/n, 17007 Girona, Spain.
uden.jmfernandezreal@htrueta.scs.es

Inflammatory pathways are increasingly recognized to be tightly associated with insulin resistance in humans. The promoter region of the adiponectin gene--Apm1--encompasses consensus sequences for glucocorticosteroid receptor responsive element. Dexamethasone induced downregulation of adiponectin secretion in vitro, whereas prednisolone administration increased circulating adiponectin concentrations. As previous studies have demonstrated an inverse relationship between corticosteroid-binding globulin (CBG), body mass index, and insulin resistance, we studied whether CBG could explain cortisol-to-adiponectin relationship. One hundred twenty-two healthy subjects were enrolled in a cross-sectional study. Plasma CBG and serum cortisol concentration were measured by radioimmunoassay. The cortisol-to-CBG ratio was used to calculate free cortisol. An RIA kit (Linco Research, St Louis, MO) was used to measure adiponectin levels. Insulin resistance was calculated using the homeostatis model of assessment (HOMA) value. Circulating adiponectin was associated with serum CBG (r = 0.38, P < . 00001), both in men (r = 0.26, P = .03, n = 79) and women (r = 0.48, P = .003, n = 43), and with insulin resistance (HOMA index) (r = -0.30, P < .0001) in both. Free cortisol correlated negatively with adiponectin only in women (r = -0.32, P = .04), but not in men (r = 0.01, P = .89). Serum CBG concentration was significantly lower among men in the lowest quartile of adiponectin when compared with the remaining subjects (37.3 +/- 5.7 vs 40.6 +/- 5.1, P = .016), whereas men in the highest quartile of adiponectin showed significantly increased free cortisol index (14.2 +/- 3.3 vs 12.2 +/- 3.1, P = .039). Women in the lowest quartile of adiponectin also displayed significantly lower CBG concentration than that present in the remaining subjects (38.6 +/- 6.9 vs 44.4 +/- 5.5, P = .016), whereas free cortisol index was not significantly different across adiponectin quartiles (P = .1). In a stepwise regression analysis, body mass index (P = .0011), CBG (P = .0047), and sex (P = .04) contributed to 15%, 8%, and 3%, respectively, of adiponectin variance. Using CBG as dependent variable, both adiponectin (P = .0002) and fasting cortisol (P = .019) contributed to 14% and 4%, respectively, of CBG variance. In summary, circulating adiponectin, CBG concentration, and fasting cortisol were significantly interrelated in healthy subjects. A significant sexual dimorphism exists in this association.

PMID: 15877287 [PubMed - indexed for MEDLINE]

Mol Endocrinol. 2005 Jan;19(1):52-64. Epub 2004 Sep 30. Related Articles, Links
The human glucocorticoid receptor (hGR) beta isoform suppresses the transcriptional activity of hGRalpha by interfering with formation of active coactivator complexes.
Charmandari E, Chrousos GP, Ichijo T, Bhattacharyya N, Vottero A, Souvatzoglou E, Kino T.
Pediatric and Reproductive Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, 10 Center Drive, Building 10, Room 9D42, Bethesda, Maryland 20892-1583, USA. charmane@mail.nih.gov

The human glucocorticoid receptor (hGR) beta, a splicing variant of the classic receptor hGRalpha, functions as a dominant-negative inhibitor of hGRalpha. We explored the mechanism(s) underlying this effect of hGRbeta by evaluating the interactions of this isoform with known steroid receptor coactivators. We found that hGRbeta suppressed the transcriptional activity of both activation function (AF)-1 and AF-2 of hGRalpha, indicating that hGRbeta may exert its dominant-negative effect by affecting the function of coactivators that are attracted to these transactivation domains. hGRbeta bound to one of the p160 coactivators, the glucocorticoid receptor-interacting protein 1 (GRIP1) via its preserved AF-1 but not via its defective AF-2 in vitro. In a chromatin immunoprecipitation assay, hGRbeta prevented coprecipitation of GRIP1 with hGRalpha tethered to glucocorticoid response elements of the endogenous tyrosine aminotransferase promoter, whereas deletion of the AF-1 of hGRbeta abolished this effect. In further experiments, overexpression of GRIP1 attenuated the suppressive effect of hGRbeta on hGRalpha-mediated transactivation of the mouse mammary tumor virus promoter. Competition for binding to glucocorticoid response elements or heterodimerization with hGRalpha via the D loop dimerization interface occurred, but they were not necessary for the suppressive effect of hGRbeta on the transcriptional activity of hGRalpha. Our findings suggest that hGRbeta suppresses the transcriptional activity of hGRalpha by competing with hGRalpha for binding to GRIP1, and possibly other p160 coactivators, through its preserved AF-1. These findings suggest that participation of hGRbeta in the formation of a coactivator complex renders this complex ineffective.

PMID: 15459252 [PubMed - indexed for MEDLINE]

Mol Cell Endocrinol. 2004 Jul 30;222(1-2):33-40. Related Articles, Links
Regulation of human glucocorticoid receptor gene transcription by Sp1 and p53.
Suehiro T, Kaneda T, Ikeda Y, Arii K, Kumon Y, Hashimoto K.
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The human glucocorticoid receptor (hGR) gene has several GC boxes in the promoter 1C region. We studied the effects of Sp1 and p53 on promoter 1C in HepG2 and HEK293 cells using luciferase (Lu) reporter assay. The results showed that the first GC box upstream of the transcription site activated the hGR promoter and over-expression of Sp1 obviously enhanced the activity. A mutant Lu-hGR vector, whose first GC box was defective, lost promoter activity nearly completely. Further, over-expression of p53 strongly suppressed the stimulating effect of Sp1 on hGR promoter activity. We concluded that Sp1 activates the hGR gene promoter, at least in part, by acting on the first GC box in promoter

1C, while p53 suppresses the transactivation by Sp1. These phenomena, demonstrated in cultured cells, may be important for the expression of hGR in vivo.

PMID: 15249123 [PubMed - indexed for MEDLINE]

1.9 Other gonads affected :

- Interaction with human Oxytocin gene promoter :

A retinoic acid response element (RARE) was identified in the human oxytocin (OT) gene promoter by DNA-mediated gene transfer techniques. RA elicited a marked stimulation of the transcriptional activity of the OT promoter in cells cotransfected with either the human RA receptor alpha, beta, or gamma. In cells cotransfected with the RA receptor alpha, the ED50 of this response was 5 x 10(-10) M. The RA response could also be conferred to a heterologous promoter independent of orientation. 5'-Deletions as well as site-directed mutations demonstrated that four TGACC motifs, located at -162, -156, -103, and -83 in the OT promoter, are necessary for optimal RA induction. Mutation or deletion of any of these elements reduces significantly the RA response. The first two TGACC motifs overlap with the estrogen response element that we have previously characterized in this gene. Furthermore, the TGACC motif located at -83 overlaps with the CCAAT box [1]. A significant suppression of the oxytocin gene in doses associated with exposure in human acne subjects can not be excluded.

GnRh

FSH/LH

- **References :**

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1.1.1 Endocrinal glands affected: Pituitary gl. :

To complete

Isotretinoin greatly reduces the functioning of the pituitary gland

http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1013&html=1

- Significant inhibition of thyroid receptors :

Retinoic acid is involved in important physiological processes such as the regulation of growth and differentiation of several tissues, including the pituitary gland. These biological effects are mediated by their binding to two specific intracellular receptors termed retinoic acid and retinoid X receptors, RARs, RXR, respectively). AlphaRXR mRNA expression was demonstrated using the reverse transcription coupled to polymerase chain reaction

(RT-PCR) in the human pituitary gland suggesting the possibility that RXR may regulate the human pituitary gene expression and hormone secretion [7]. In human nontumorous pituitaries. RXR alpha was expressed in the nuclei of almost all cells, while RXR gamma was only expressed in thyrotropin (TSH) cells and in some cells positive for growth hormone (GH) and glycoprotein alpha-subunit (alpha SU) [8].

In rat GH1 cells, a cell type located in the pituitary gland, a 50-70 % inhibition of thyroid receptors was found, exposed to doses that are comparable to those seen in (Ro)accutane exposure in acne-subjects [2].

- **Significant inhibition of growth hormone-releasing hormone receptor (GHRH-R) internalization and expression and downregulation of growth hormone secretagogue receptor (GHS-R) :**

In small doses retinoic acid is found to stimulate GH-secretion. In rat pituitary GH3 cells, hydrocortisone is also known to stimulate GH secretion. Retinoic acid <1 microM stimulated growth hormone secretion by 220%. 50 nM HCT stimulated GH secretion 3,5 times and in synergy GH secretion was stimulated seven times. Retinoic acid selectively stimulates basal and HCT-induced GH secretion and mRNA levels in these cells in a dose- and time-dependent manner [1]. However, in massive doses, as seen in (Ro)accutane exposed human subjects, evidence is pointing out that an opposite effect is present, a significant inhibition of GH secretion.

The neuropeptide growth hormone-releasing hormone (GHRH) exerts a crucial role in the anterior pituitary to stimulate growth hormone secretion and synthesis. GHRH is also involved in somatotroph proliferation and differentiation. These biological responses are initiated by the high affinity binding of GHRH to a G-protein-coupled receptor (GPCR). Activation of the pituitary GHRH receptor (GHRH-R) exclusively localized on somatotroph cells, predominantly induces the production of cAMP and Ca2+ influx [3]. Hoffman la Roche itself has admitted to a significantly increased alkaline phosphatase in human subjects exposed to (Ro)accutane [0]. In rat brain cell membranes, AP-2 á subunits are redistributed to the cytosol by exposure to alkaline phosphatase due to dephosphorylation of these proteins [4]. In rats, hGHRH-R internalization is suggested to be AP-2-clathrin-dependent, while fatty acid acylation of rGHRH-R appears to be a prerequisite to caveolin-dependent internalization. Both receptor primary structure and concentration at the plasma membrane play important roles in internalization and trafficking of specific G-protein-coupled receptors (GPCR) [3]. It is here suggested that the (Ro)accutane induced translocation of AP-2 subunits, inhibits the clathrin dependent internalization process of the growth hormone releasing receptor, thus leading to an inhibited release of growth hormone, and inhibition of somatroph proliferation and differentiation.

In human acne-subjects exposed to (Ro)accutane, levels of thyroxine and triiodothyronine were significantly lower after exposure (p less than 0.05), indicating a (Ro)accutane induced clinical thyroid deficiency (hypothyroidism) [5]. In adult rats, thyroid hormones regulate growth hormone (GH) secretion by actions both at the hypothalamus and at the pituitary gland. At the level of the pituitary, thyroid hormones increase GH and GH-releasing hormone receptor (GHRH-R) mRNA expression. T3 increased pituitary GHS-R mRNA levels in a dose- and time-dependent manner [6]. It is suggested that a (Ro)accutane exposure in human subjects also downregulates both GHRH-R and GHS-R as additional effects in its inhibition of growth hormone secretion.

- Conclusions :

to complete

- References :

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1.1.2 Endocrinal glands affected: Thyroid gl. :

"Homocysteine is an amino acid that can be generated in response to nutritionally deficient or nutritionally deficient diets. "

"When homocysteine levels increase in the blood, it is linked to massive inflammation and neurodegeneration!
In Alzheimer's disease and many other chronic diseases, we find a significant increase in homocysteine in blood tests. Since homocysteine is a potent excitotoxin and neurotoxin, high levels of homocysteine have been found to exacerbate the symptoms of Alzheimer's disease and other chronic diseases. Components of the metabolic degradation of homocysteine alter the NMDA (N-methyl-D-aspartate) receptor sites, resulting in multiple negative effects, including free radicals and a massive inflammatory cascade!
These free radicals and inflammation can trigger an autoimmune response in which the patient's immune system attacks the thyroid gland and / or other body systems. "

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5120102/

https://www.ncbi.nlm.nih.gov/pubmed/17852421

to complete

- References :

to complete

1.1.3 Endocrinal glands affected: Parathyroid gl. :

The gp330/Megalin/LRP-2 protein belongs to the low-density lipoprotein receptor gene family and is believed to function as an endocytic receptor for the uptake of lipoproteins and many other ligands. Other functions of this protein may include a role in calcium sensing in the parathyroid glands and other tissues [1]. In rat pituitary GH4C1 cells, binding sites for NF-Y reside in the near vicinity and were shown to be important for full activity of the PTH gene promoter [2].

The negative regulation of the human parathyroid hormone (PTH) gene by biologically active vitamin D3 (1,25-dihydroxyvitamin D3; 1,25(OH)2D3) was studied in rat pituitary GH4C1 cells, which express factors needed for the negative regulation. We report here that NF-Y binds to sequences downstream of the site previously reported to bind the vitamin D receptor (VDR). Additional binding sites for NF-Y reside in the near vicinity and were shown to be important for full activity of the PTH gene promoter. VDR and NF-Y were shown to exhibit mutually exclusive binding to the VDRE region. According to our results, sequestration of binding partners for NF-Y by VDR also affects transcription through a NF-Y consensus binding element in GH4C1 but not in ROS17/2.8 cells. These results indicate that 1,25(OH)2D3 may affect transcription of the human PTH gene both by competitive binding of VDR and NF-Y, and by modulating transcriptional activity of NF-Y [2].

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25-hydroxyvitamin D(3)-1alpha-hydroxylase expression in normal and pathological parathyroid glands.
Segersten U, Correa P, Hewison M, Hellman P, Dralle H, Carling T, Akerstrom G, Westin G.
Department of Surgical Sciences, Endocrine Unit, Uppsala University Hospital, SE-751 85 Uppsala, Sweden.

Active vitamin D, 1,25-dihydroxyvitamin D(3) [1,25(OH)(2)D(3)], plays a pivotal role in

calcium homeostasis and bone metabolism. Circulating levels of 1,25(OH)(2)D(3) are thought to be dependent mainly on the activity of the renal cytochrome P450 enzyme 25-hydroxyvitamin D(3)-1alpha-hydroxylase (1alpha-hydroxylase), which is potently induced by PTH. However, 1alpha-hydroxylase activity or expression has also been reported at several extrarenal sites, at which local synthesis of 1,25(OH)(2)D(3) appears to fulfill autocrine or paracrine functions. This includes tissues such as placenta and brain that also express LRP-2/megalin, an endocytic receptor for multiple ligands, which is involved in the renal uptake of the substrate for 1alpha -hydroxylase, 25-hydroxyvitamin D(3). We have previously demonstrated LRP-2/megalin in parathyroid cells, and here we present results from RT-PCR and immunohistochemical analyses showing coincident expression of 1alpha-hydroxylase in normal and pathological parathyroid tissue. With real-time quantitative RT-PCR analysis, the expression of 1alpha-hydroxylase mRNA was higher in the majority of parathyroid adenomas and secondary hyperplastic glands but lower in parathyroid carcinomas, compared with normal parathyroid tissue. The findings imply that in addition to feedback control by circulating 1,25(OH)(2)D(3) levels, parathyroid cells may also be influenced by local 1alpha -hydroxylase activity with possible growth regulatory and differentiating effects.

PMID: 12050281 [PubMed - indexed for MEDLINE]

1.1.4 Endocrinal glands affected: Adrenal gl. :

Differentially Expressed Nucleolar TGF-beta1 Target (DENTT) is a new member of the TSPY/TSPY-like/SET/NAP-1 (TTSN) superfamily whose mRNA is induced by TGF-beta1. Monkey DENTT mRNA contains a 2085-bp open reading frame that encodes a predicted polypeptide of 695 amino acids with five nuclear localization signals, two coiled-coil regions, and a domain that shows significant identity to a region that defines the TTSN superfamily. RT-PCR amplification and Western blot analyses showed DENTT mRNA and protein in adult monkey tissues, including the adrenal gland [1].

Section 2 : Affected neurotransmitters and parts of the brain

2. Introduction :

- Vitamin A extensively linked to adult CNS function :

Since retinoids readily enter the central nervous system, Vitamin A neurotoxicity in adults is possible from excessive consumption of supplements [2 and more]. In the cerebrum, cerebellum and meninges, rates of all-trans-retinoic-acid (ATRA) synthesis were comparable to, or exceeded, rates measured in rat liver [2]. In human acne-subjects, Roaccutane is taken in a 50-100 times overdose of the by consensus recommended daily intake of vitamin A, which is 0,8 micrograms*. (Ro)accutane is admininstrated to human acne-subjects during several months, which if less than one week of (Ro)accutane "therapy" results in a dose similiar to one years consumption, the cumulative dose represents the use of several decades of vitamin A consumption. In recent studies, it has been shown that all participating human subjects in studies of (Ro)accutane showed significant metabolic changes in the brain, whereas effects on the nervous system can be seen as a *predictable consequence, and not only a possibililty*.

Neural stem cells (NSCs) are the self-renewing, multipotent cells that generate neurons, astrocytes, and oligodendrocytes in the nervous system. Contrary to the long-held dogma, neurogenesis occurs in discrete areas of the adult brain, the hippocampus and the subventricular zone, and NSCs reside in the adult central nervous system. Recent studies have shown that neurogenesis is increased in the diseased brains, after strokes and traumatic brain injuries, and that new neuronal cells are generated at the sites of injury, where they replace some of the degenerated nerve cells [5]. It is not known to which degree the (Ro)accutane induced CNS effects are repaired through replacement by neuronal stem-cells. It is not known, whether a partial replacement by neuronal stem-cells after a suggested (Ro)accutane traumatic degenerative exposure in human acne-subjects has a diminishing effect on the remaining NSC:s, thus causing a more limited repair throughout life.

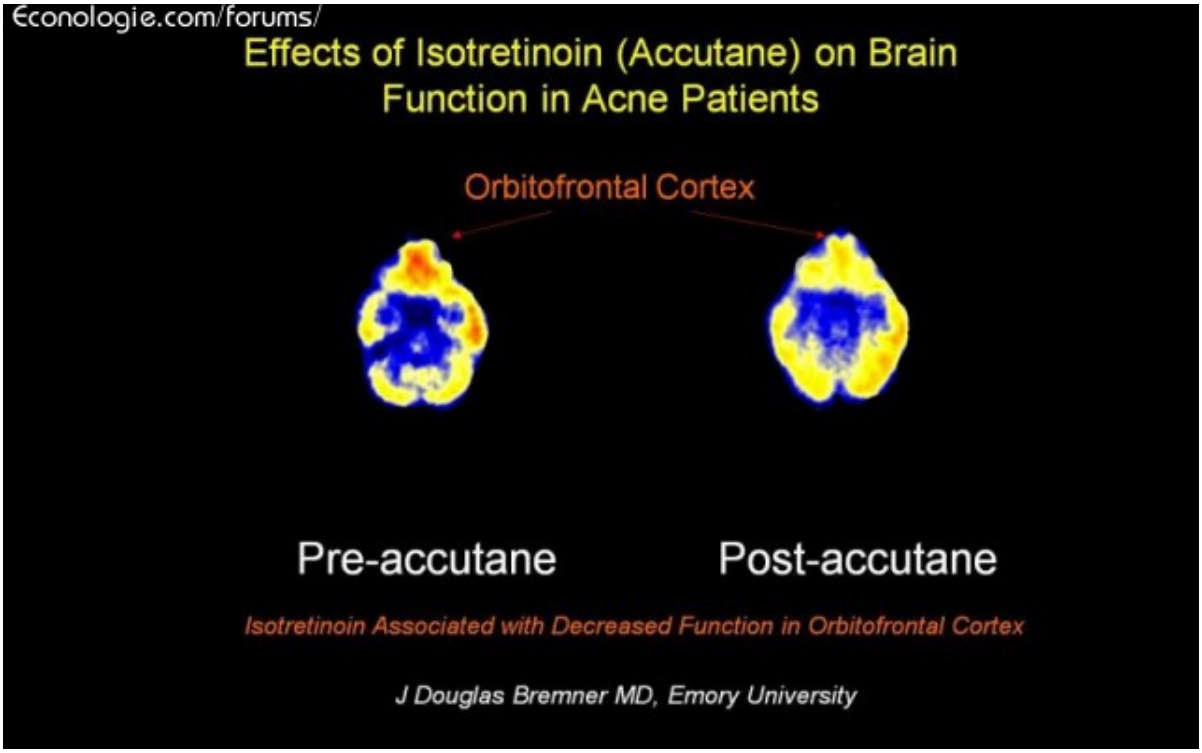
- Suggested inhibitory effects on several neurotransmitters, including serotonin, acetylcholine, melatonin, norephinephrine, and other neurosteroids :

Potential targets for retinoid signalling in depression include dopaminergic, serotonergic or noradrenergic pathways or a complex interaction between these neurotransmitter systems [7]. As seen in this chapter, there are even more singnaling systems in the human brain suggested to be involved in mood and well-being that with high certainity are affected in subjects exposed to (Ro)accutane (reviewed in section 2.1-2.6).

- Significant metabolic changes in human acne- subjects measured that were exposed to (Ro)accutane :

A mean -21% fall in metabolism the orbitofrontal cortex in human subjects exposed to (Ro)accutane was observed. Brain functioning in adults was measured with [(18)F]fluorodeoxyglucose positron emission tomography before and after 4 months of treatment with isotretinoin (N=13) or an antibiotic (N=15). Isotretinoin but not antibiotic

treatment was associated with decreased brain metabolism in the orbitofrontal cortex (-21% change versus 2% change for antibiotic), a brain area known to mediate symptoms of depression [1].



Picture 1. Bremner JD et al. *Functional brain imaging alterations in acne patients treated with isotretinoin.* (2005) Am J Psychiatry. May;162(5):983-91.

- Suggested significant cell losses in hippocampus in subjects exposed to (Ro)accutane, further losses in other areas can not be excluded :

In a mouse model, it was shown that endogenous RA generated by synthetic enzymes in the meninges acts on hippocampal granule neurons, and chronic (3-week) exposure to a clinical dose of 13-cis RA may result in hippocampal cell loss [3]. Similiar effects in the subventricular zone of rats have been experimentally observed. In adult rat brains, both retinoic acid and thyroid hormone have shown to regulate differentiation and proliferation of precursor cells in the subventricular zone (SVZ) [6]. Similiar effects in humans are highly likely. Further yet not described affected areas of the mammalian brain in association with (Ro)accutane exposure in a similiar manner are highly likely.
(the areas of the brain suggested to be affected are reviewed under section 2.1.1-2.1.5).

- Significantly elevated 8-hydroxy-2'-deoxyguanosine (8-OHdG) as an index of oxidative stress in the brain of rats exposed to arsenic :

To clarify the association between oxidative DNA damage and the neurotoxicity of arsenic, the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) as an index of oxidative DNA damage in the brain was examined in mice fed with drinking water containing 1 or 2 ppm arsenic, using an HPLC-electrochemical detector and immunohistochemical method. 8-OHdG levels were significantly increased in the brain of mice given arsenic and its immunoreactivity was distributed in the cerebral and cerebellar cortexes. Cerebral cortex neurons and Purkinje cells in the cerebellar cortex showed degenerative changes in accordance with the distribution of 8-OHdG immunoreactivity. The levels of arsenic in this study were lower than those reported in epidemiological studies. Thus, it is concluded that environmentally relevant levels of arsenic induce pathological changes through oxidative DNA damage in the brain tissues in vivo and that cerebral and cerebellar cortex neurons seem to be the major targets of arsenic neurotoxicity [9].

Most cells that are active in neurotransmission express action through g-protein-coupled receptors. Interaction bewtween retinoic acid and several types of g-proteins have been found, as well as inhibitory actions on enzymes that metabolize neurotranmittors. TGF-beta is found to be upregulated in subjects exposed to retinoic acid.

CYP2C6
CYP2D6
tryptofan hydroxylase - alkaline phosphatase
tyrosine hydroxylase
g-protein coupled receptors
TGF-beta

*Consensus by the Food and Nutritional labeling dep. of the european union

- References :

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2.1 Roaccutane and brain degeneration :

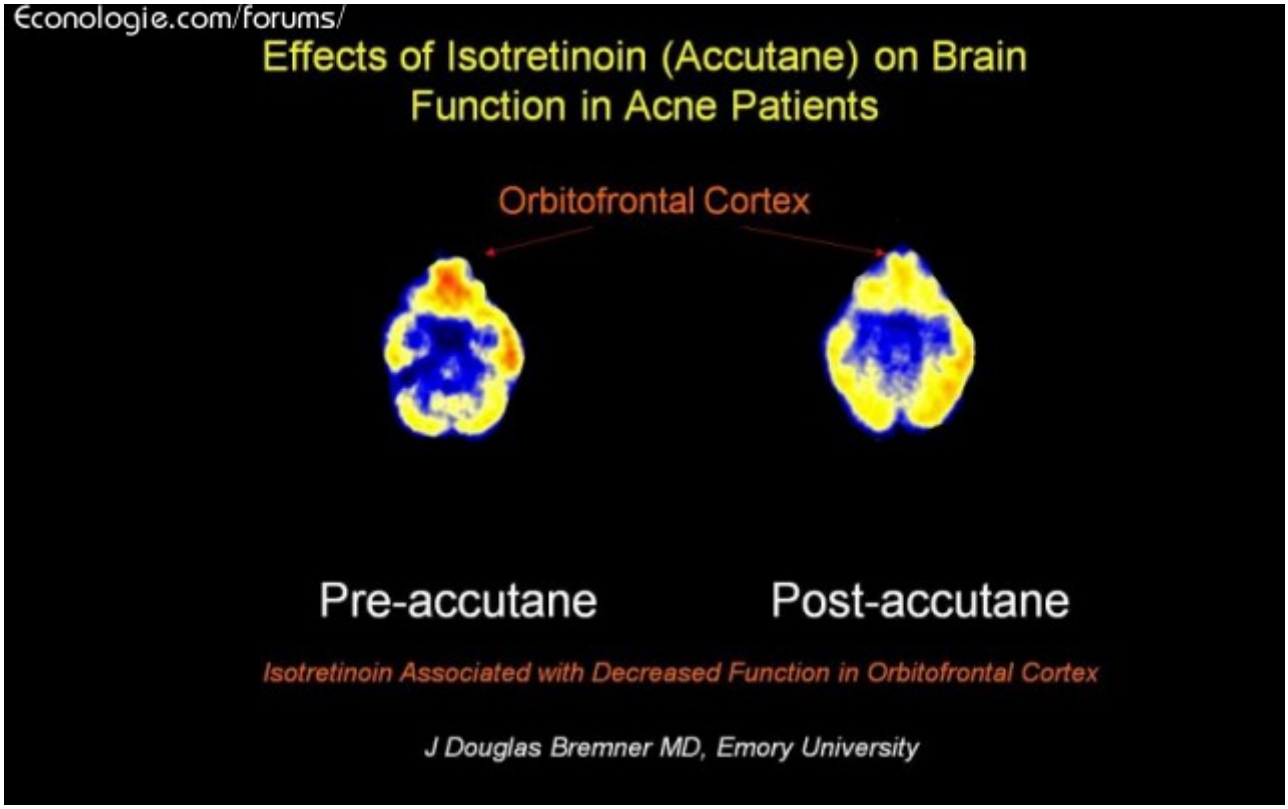
- Several factors link Accutane exposed subjects to factors causative for dementia and similiar degenerative diseases :

A significantly elevated plasma homocysteine level has by independent research been observed in human acne subjects exposed to (Ro)accutane, as well as been reported by Hoffman la Roche itself [0, 5 and more]. It is suggested that the elevated Hcy levels in patients after 45 days on Iso therapy could be due either to the 'inhibition' of cystathionine-beta-synthase by the drug and/or their liver dysfunction [1]. Increased concentration of plasmatic homocysteine (tHcy) and decreased vitamin B 12 (B12) and folate (FOL) are associated with Alzheimer's (AD) and vascular (VaD) dementias [2]. Retinoic acid is of importance for myelination in the adult CNS [9 and more], and a vitamin A deficiency is suggested to have negative short and long term consequences. (Ro)accutane induced significant inhibition of angiogenesis, the formation of new blood vessels, may also play a substantial role in degenerative pathologies.

- Inhibition of IP(3) receptors in various areas of the brain found in subjects exposed to (Ro)accutane most likely due to decreased Sp1 binding affinity to GC boxes and reduced Sp1 formation :

In rats IP(3) receptors have been found to be promoted via Sp1 proteins that bind to GC-boxes. The promoter had no TATA-box but was highly GC-rich and contained two putative Sp1-binding sites. There was no sequence similarity between promoter regions of IP(3)R3 and IP(3)R2, another ubiquitous gene, except for GC-boxes [4].

In rats, mRNA levels of the type 1 IP(3) receptors were decreased significantly in cerebellum and hypothalamus, but not in the brain stem of rats exposed to 13-cis-retinoic acid, (Ro)accutane, compared to untreated littermates. The mRNA levels of the type 2 IP(3) receptor were significantly decreased in all tested tissues, cerebellum, hypothalamus, and also in brain stem after the treatment with retinoic acid. These results show that gene expression of both type 1 and 2 IP(3) receptors is regulated by retinoic acid, although the effect of retinoic acid on mRNA levels of the type 1 IP(3) receptors is dependent on brain area [3].



Picture 1. Bremner JD et al. *Functional brain imaging alterations in acne patients treated with isotretinoin.* (2005) Am J Psychiatry. May;162(5):983-91.

- Retinoic acid dependent neurogenesis: Inhibition of neurogenesis - the proliferation and development of stem-cells to functioning cells - a significant suppression during exposure suggested due to growth arrest and apoptosis, and also an inhibition of replacement *post* exposure due to vitamin A-deficiency is suggested :

Neurogenesis persists throughout life in the rodent subventricular zone (SVZ)-olfactory bulb pathway. The molecular regulation of this neurogenic circuit is poorly understood. Components for retinoid signaling are present in this pathway. The influence of retinoic acid (RA) on postnatal SVZ-olfactory bulb neurogenesis was studied. Using both SVZ neurosphere stem cell and parasagittal brain slice cultures derived from postnatal mouse, a modest RA exposure increased neurogenesis by enhancing the proliferation and neuronal differentiation of forebrain SVZ neuroblasts. The RA precursor retinol had a similar effect, which was reversed by treating cultures with the RA synthesis inhibitor disulfiram. Electroporation of dominant-negative retinoid receptors into the SVZ of slice

cultures also blocked neuroblast migration to the olfactory bulb and altered the morphology of the progenitors. Moreover, the administration of disulfiram to neonatal mice decreased in vivo cell proliferation in the striatal SVZ. These results indicate that RA is a potent mitogen for SVZ neuroblasts and is required for their migration to the olfactory bulb. The regulation of multiple steps in the SVZ-olfactory bulb neurogenic pathway by RA suggests that manipulation of retinoid signaling is a potential therapeutic strategy to augment neurogenesis after brain injury [7].

- Roaccutane induced demyelination, block of myelination through multiple pathways in CNS and peripheral nervous system :

In small doses retinoic acid has shown to be positive for myelination through effects on the myelin basic protein (MBP) promoter [8]. However, it is here suggested that a toxic dose induces demyelination both during and post exposure. Also the myelination of the the peripheral nervous system is suggested to be significantly disrupted. Lipid binding activities of the P2 protein in peripheral nerve myelin were examined using retinoic acid, retinol and oleic acid as ligands. The P2 protein also showed the specific binding affinity to both of retinoic acid and retinol. The binding site of these ligands was suggested to be similar [11]. Also the Egr family is of importance for myelination. In the peripheral nervous system Egr proteins are critical modulators of transcription in myelinating Schwann cells. The Egr-family is found to be inhibited by retinoic acid in various human cell lines [16 and more].

- Thyroid hormone and myelination :

Thyroid hormone plays an important role in brain development, in part by regulating myelination. Previous studies have shown that the myelin basic protein (MBP) promoter is activated by thyroid hormone (T3) via a T3-response element (T3RE) at position -186 [8].

- Other factors of importance for myelination :

G21.3, a monoclonal antibody previously shown to block central nervous system (CNS) myelination is also found tp be upregulated by retinoic acid. The antigen is present on the surface of neurons but not oligodendrocytes and is highly abundant in the white matter of the adult rat brain; however, it is not found in isolated myelin [9]. The myelin basic protein (MBP) gene produces two families of proteins, the classic MBPs, important for myelination of the CNS, and the golli proteins, whose biological role in oligodendrocytes (OLs) is still unknown. Golli expression is regulated during OL development and can be modulated by growth factors such as basic fibroblast growth factor, neurotrophin-3, and retinoic acid [10].

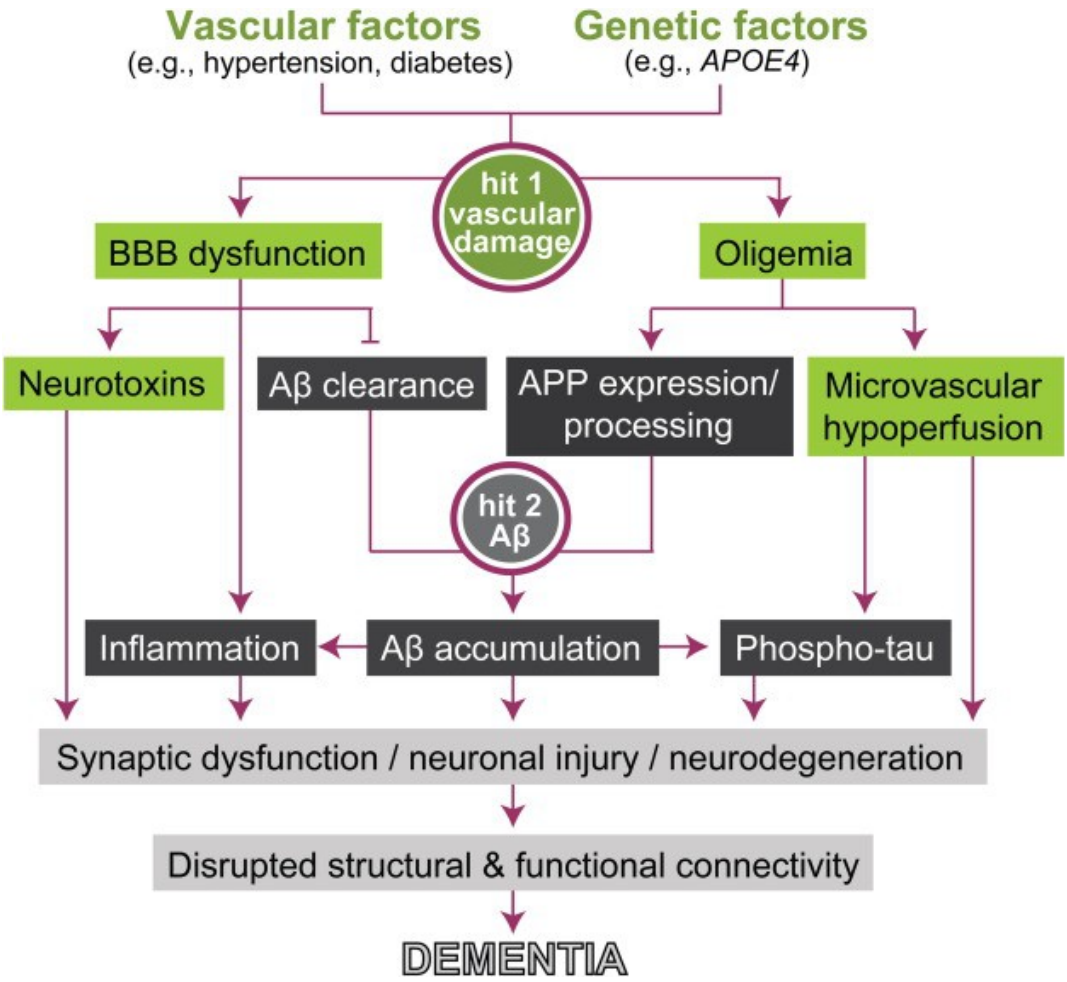
- (Ro)accutane induced disruption of recycling of synaptic vesicles in nerve terminals through disruption and desphosphyraltion of AP-2 (clathrin adaptor mediated endocytosis) :

Clathrin-mediated endocytosis plays a key role in the recycling of synaptic vesicles in nerve terminals, and several components of the molecular machinery involved in this process have been identified. These include, in addition to clathrin and the clathrin

adaptors, the guanosine triphosphatase dynamin 1, the amphiphysin dimer, and synaptojanin 1. Dynamin 1 oligomerizes into collar structures at the neck of deeply ingirl thingyted clathrin-coated pits, and its conformational change is thought to be an essential step leading to vesicle fission. Synaptojanin 1 is a presynaptic inositol 5-phosphatase enriched on endocytic intermediates (3). The amphiphysin dimer (4-6) binds to both dynamin 1 and synaptojanin 1 through the COOH-terminal SH3 domains of its two subunits. Disruption of SH3-mediated interactions of amphiphysin blocks clathrin-mediated endocytosis at the step of ingirl thingyted coated pits. The amphiphysin dimer also binds to clathrin and to the -adaptin subunit of the plasma membrane clathrin adaptor AP-2 and thus may mediate recruitment of dynamin 1 and synaptojanin 1 to the site of clathrin-mediated endocytosis [6].

- Disrupted angiogenesis in the adult brain, a possible role in degenerative diseases such as Alzheimers disease :

Retinoic acid is found to significantly inhibit angiogenesis in several types of cancer tissue [13 and more]. However, the neurovascular effects of a (Ro)accutane exposure in human subjects is not clarified. Angiogenesis is a term for the continous formation of new blood vessels and currently targeted in anti-cancer therapy of various types of cancer [12 and more], because an inhibition of angiogenesis deprives the tumour-cells of oxygen and energy. Recent Alzheimers theory is beginning to include the possiblility that alterations in angiogenesis is involved in the pathology of AD [14 and more]. BEC-mediated formation of capillaries is greatly reduced in the Tg2576 mouse model of AD, and high concentrations of AfÀ that is rich in fÀ-sheets is anti-angiogenic. In AD, reduced length of brain capillaries in the CA1 hippocampal region correlates well with increased clinical dementia rating scores. BEC-mediated capillary formation in AD is regulated by the Gax homebox gene, which controls differentiation of the vascular system and vascular remodeling in AD (Z. Wu et al., unpublished). Given a recent recognition that some molecular pathways mediating vasculogenesis might also regulate neurogenesis during brain development, insufficient angiogenesis and vascular regression in the AD brain could represent an important, yet to be fully defined, new pathogenic mechanism involved in the disease progression affecting repair of both vasculature and neurons [14].



Picture 2. Slokovich et al. (2005) Trends Neurosci. Apr;28(4):202-8.*Neurovascular mechanisms of Alzheimer's neurodegeneration.*

Neurovascular model of Alzheimer's disease. Multiple pathogenic cascades originating from altered cerebral arteries (green) or altered brain capillaries (red) can initiate disintegration of the neurovascular unit, including aberrant angiogenesis, cerebral amyloid angiopathy (CAA), senescence and faulty clearance of Aβ across the BBB, resulting in increased Aβ levels. Both sets of cascades can initiate neurovascular uncoupling and hypoperfusion, although only aberrant angiogenesis and accumulation of amyloid result in vessel regression and inflammation. This leads to the BBB compromise and reduced control of the chemical composition of brain interstitial fluid, which can result in, or amplify, synaptic and/or neuronal and oligodendroglial dysfunction, neuronal injury and loss.

In HNSCC tumour cells, owing to a decrease in the secretion of MCP-1 and transforming growth factor-beta 1 (TGF-beta 1), tumor cells treated with RA were unable to induce peripheral blood monocyte (PBM) chemotaxis. Also, as a result of the decrease in TGF-beta 1 secretion, RA-treated tumor cells were unable to activate macrophages for secretion of vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8). In addition to its affects on tumor cells, RA also directly altered the ability of monocytes to participate in the tumor angiogenesis process. PBM exposed to RA were unable to migrate toward inducers of PBM such as MCP-1 and TGF-beta 1. Finally, RA decreased the ability of tumor-activated macrophages to secrete IL-8 and VEGF [13].

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Cancer Res. 1989 Feb 15;49(4):1014-9. Related Articles, Links
Modulation of growth and epidermal growth factor receptor activity by retinoic acid in human glioma cells.
Yung WK, Lotan R, Lee P, Lotan D, Steck PA.
Department of Neuro-Oncology, University of Texas M. D. Anderson Cancer Center, Houston 77030.

The growth-inhibitory activity of beta-all-trans-retinoic acid (RA) was examined on seven cultured human gliomas and cells derived from one normal brain. Response in monolayer cultures was heterogenous: three cell lines were completely resistant whereas five cell lines were growth inhibited with 50% inhibitory dose ranging from greater than 10(-5) to 1 x 10(-8) M. Two glioma cell lines capable of forming colonies in soft agar exhibited dose-dependent sensitivity to RA-induced growth inhibition, whereas another cell line was not affected by RA under either growth condition. Cell cycle analysis of the glial-derived cells has shown that the RA-sensitive cells accumulated in the G0-G1 phase. The cell surface expression of epidermal growth factor (EGF) receptors displayed by the various cells was either slightly increased or not affected by RA. In addition, the affinity of binding was slightly decreased in some sensitive cells. The activity of EGF receptor as assessed by immunocomplex-kinase assays revealed a dose-dependent decrease in autophosphorylation activity that appeared to correlate with the growth inhibition. The decrease in phosphokinase activity represented a dose-dependent inhibition of phosphorylation on tyrosine residues on EGF receptor as well as several other substrates. Furthermore, the autophosphorylation of either RA-treated or untreated EGF receptors occurred on similar amino acid residues. These results demonstrate that RA exhibits a heterogeneous growth-inhibitory activity against human glioma cells and suggest that the effects of RA may be mediated, at least in part, by modulation of EGF receptor phosphotyrosine kinase activity.

PMID: 2912547 [PubMed - indexed for MEDLINE]

J Neurochem. 2002 Oct;83(1):67-79. Related Articles, Links
Analysis on the promoter region of exon IV brain-derived neurotrophic factor in NG108-15 cells.
Takeuchi Y, Miyamoto E, Fukunaga K.
Department of Pharmacology, Kumamoto University School of Medicine, 2-2-1 Honjo, Kumamoto 860-0811, Japan. yusuket@gpo.kumamoto-u.ac.jp

We have reported that the nuclear isoforms of Ca2+/calmodulin-dependent protein kinase II (CaM KII) are involved in the expression of the exon IV-containing brain-derived neurotrophic factor (BDNF) mRNA. We document here the cis-elements and transcription factors responsive to CaM KII in the activation of the promoter upstream of the exon IV (exon IV-BDNF promoter). Effects of constitutively active mutants of CaM KIV, MAPK kinase kinase (MEKK) and protein kinase A (PKA) on the exon IV-BDNF promoter activity were also evaluated by transfection and luciferase assay. The exon IV-BDNF promoter activity was increased by transfection with CaM KII, MEKK and PKA, but not by CaM KIV.

Deletion and mutational analysis of the promoter revealed that the region between nucleotides -56 and -27 was responsive to CaM KII, which contained a CCAAT-box in the region between nucleotides -56 and -43. Expression of C/EBPbeta increased the promoter activity and potentiated the effects of CaM KII. The region between nucleotides -79 and -56 was responsive to MEKK, in which both a GA-rich sequence and a GC-box were included. Expression of Sp1 increased the promoter activity, which was further enhanced by transfection with MEKK. The region between nucleotides -43 and -27 was responsive to both PKA and CaM KII, but the transcription factors involved in the region remained unclear. These results suggest that the promoter of the exon IV-BDNF is at least regulated by CaM KII, MEKK and PKA, and that C/EBP/beta and Sp1 are potential transcription factors activated by CaM KII and MEKK, respectively.

PMID: 12358730 [PubMed - indexed for MEDLINE]

Stroke. 2005 Jul;36(7):1533-7. Epub 2005 Jun 9. Related Articles, Links

Angiopoietin-2 facilitates vascular endothelial growth factor-induced angiogenesis in the mature mouse brain.
Zhu Y, Lee C, Shen F, Du R, Young WL, Yang GY.
Center for Cerebrovascular Research, Department of Anesthesia, University of California, San Francisco, CA, USA.

BACKGROUND AND PURPOSE: A better understanding of angiogenic factors and their effects on cerebral angiogenesis is necessary for the development of effective therapeutic strategies for ischemic brain injury. Vascular endothelial growth factor (VEGF) has been shown to induce angiogenesis in the adult mouse brain. However, the function of angiopoietin-2 (Ang-2) in cerebral angiogenesis has not been clarified. The goal of this study was to identify the combined effects of VEGF and Ang-2 on cerebral angiogenesis and the blood-brain barrier (BBB). METHODS: Six groups of 6 adult male CD-1 mice underwent AdlacZ (viral vector control), AdVEGF, AdAng2, VEGF protein, VEGF protein plus AdAng2, or saline (negative control) injection. Microvessels were counted using lectin staining on tissue sections after 2 weeks of treatment. Matrix metalloproteinase-9 (MMP-9) activity was determined by zymography. The presence of zonula occludens-1 (ZO-1) protein was determined by Western blot and immunohistochemistry. RESULTS: Mice treated with VEGF protein infusion plus AdAng-2 significantly increased microvessel counts relative to all other groups ($P<0.05$). The changes in MMP-9 activity paralleled the reduced ZO-1 expression in the VEGF plus Ang-2-treated group compared with the other 5 groups ($P<0.05$). Double-labeled immunostaining demonstrated that ZO-1-positive staining was significantly decreased on the microvessel wall in the VEGF plus Ang-2-treated group. CONCLUSIONS: Our study demonstrates that the combination of VEGF and Ang-2 promotes more angiogenesis compared with VEGF alone. Furthermore, the combination of VEGF and Ang-2 may lead to BBB disruption because it increases MMP-9 activity and inhibits ZO-1 expression.

PMID: 15947259 [PubMed - in process]
Dement Geriatr Cogn Disord. 2005;19(1):1-10. Epub 2004 Sep 21. Related Articles, Links
Decreased release of the angiogenic peptide vascular endothelial growth factor in Alzheimer's disease: recovering effect with insulin and DHEA sulfate.
Solerte SB, Ferrari E, Cuzzoni G, Locatelli E, Giustina A, Zamboni M, Schifino N,

Rondanelli M, Gazzaruso C, Fioravanti M.
Department of Internal Medicine and Geriatrics, University of Pavia, Pavia, Piazza Borromeo 2, IT-27100, Italy. brunos@unipv.it

Changes of vascular endothelial growth factor (VEGF) secretion have recently been demonstrated in patients with Alzheimer's disease (AD). Since VEGF has been involved in brain angiogenesis, neuroprotection and cerebromicrovascular exchange of substrates and nutrients, the study of VEGF could have important relapses into the pathogenesis and treatment of AD. Within this context, 35 healthy subjects (16 of young and 19 of old age), 18 patients with dementia of the vascular type (VAD) and 22 with dementia of the Alzheimer's type (AD) were included in the study. VEGF levels were determined in the supernates of circulating natural killer (NK) immune cells isolated by immunomagnetic separation (pure CD16 + CD56 + NK cells at a final density of 7.75 x 10(6) cells/ml). VEGF was measured in spontaneous conditions (without modulation) and after exposure of NK cells with IL-2, lipopolysaccharide (LPS), dehydroepiandrosterone sulfate (DHEAS), LPS + insulin, amyloid-beta (Abeta) fragment 1-42, the inactive sequence Abeta(40-1) and Abeta(1-42) + insulin. A significant decrease in VEGF released by NK cells was demonstrated in AD subjects compared to the other groups. No differences of VEGF levels were found between healthy subjects of old age and the VAD group. The incubation with LPS and DHEAS significantly increased, in a dose-dependent manner, VEGF levels in AD as well as in healthy subjects of young and old age and in VAD patients. The incubation of NK cells with Abeta(1-42) completely suppressed VEGF generation in AD subjects, also reducing VEGF release in the other groups. The co-incubation of NK with LPS + insulin, at different molar concentrations, significantly restored (4- and 6-fold increase from LPS alone) VEGF in AD, also enhancing VEGF secretion in healthy subjects and the VAD group, while the co-incubation of NK with Abeta(1-42) + insulin promptly abolished the negative effects of Abeta(1-42) on VEGF release. These data might suggest that the decreased VEGF secretion by peripheral immune cells of AD subjects could have a negative role for brain angiogenesis, neuroprotection and for brain microvascular permeability to nutrients, increasing brain frailty towards hypoxic injuries. On the contrary, insulin and DHEAS could have beneficial effects in AD, as well as in VAD and in physiological aging, by increasing, in a dose-dependent fashion, VEGF availability by peripheral and resident immune and endothelial cells, so contributing to increase its circulating pool.

PMID: 15383738 [PubMed - indexed for MEDLINE]
Physiol Res. 2005 Aug 5; [Epub ahead of print] Related Articles, Links

Inhibition of vascular endothelial growth factor-induced retinal neovascularization by retinoic acid in experimental retinopathy of prematurity.

Ozkan H, Duman N, Kumral A, Kasap B, Ozer EA, Lebe B, Yaman A, Berk T, Yilmaz O, Ozer E.
Department of Pediatrics, Dokuz Eylul University, School of Medicine, 35340 Izmir, Turkey. hasan.ozkan@deu.edu.tr.

Vascular endothelial growth factor (VEGF) has an important role in the pathogenesis of retinopathy of prematurity (ROP) and inhibition of VEGF expression at the neovascular phase might prevent destructive neovascularization in ROP. It is suggested that retinoids have highly potent antiangiogenic activity by inhibiting VEGF expression. The aim of this

study was to demonstrate the preventive effect of retinoic acid (RA) on the VEGF-induced retinal neovascularization in a rat model of ROP.Wistar albino rats were placed into incubators at birth and exposed to an atmosphere alternating between 50 % and 10 % O(2) every 24 hours. After 14 days, the animals were removed to room air and received either intraperitoneal injection of RA (5 mg/kg/day) (n=9) or saline (n=4) daily for six days, and sacrificed at 21 days. Additional rats (n=4) were raised in room air and served as age-matched controls. The globe of each eye was cut through the cornea and embedded in paraffin. Serial sections were stained with hematoxylin-eosin for quantification of neovascular nuclei. The avidin-biotin peroxidase method was performed for evaluation of VEGF expression. The average number of neovascular nuclei was significantly lower in the control group compared to that in the ROP groups. In addition, it significantly decreased in the RA-treated ROP group compared to that in the saline-administrated ROP group. VEGF immunostaining was overall negative in room air-exposed rats. VEGF immunostaining score significantly decreased in RA-treated ROP group compared to that in the saline-administrated ROP group. RA treatment might be beneficial in preventing neovascularization resulting from oxygen-induced retinopathy by downregulation of VEGF expression.

PMID: 16083310 [PubMed - as supplied by publisher]

2.1.1 Parts of the brain affected: Orbitof. Cortex :

- Significant fall in metabolism after 4 months (Ro)accutane exposure in all scanned human acne-subjects :

A mean -21% fall in metabolism the orbitofrontal cortex in human subjects exposed to (Ro)accutane was observed. Brain functioning in adults was measured with [(18)F]fluorodeoxyglucose positron emission tomography before and after 4 months of treatment with isotretinoin (N=13) or an antibiotic (N=15). Isotretinoin but not antibiotic treatment was associated with decreased brain metabolism in the orbitofrontal cortex (-21% change versus 2% change for antibiotic), a brain area known to mediate symptoms of depression [1].

Picture 1. Bremner JD et al. Functional brain imaging alterations in acne patients treated with isotretinoin. (2005) Am J Psychiatry. May;162(5):983-91.

- The function of the orbitofrontal cortex :

The function of the orbitofrontal cortex (OFC) is not fully known. However, alterations in behavior could with high certainity be expected in subjects with altered OFC function. By imaging techniques the orbitofrontal cortex has been associated with mood, but also with learning, response to rewards and decision-making [2]. These associations may except for effects on mood also to certain extent predict human behavior. Further yet not suggested behavioral functions may exist. Further yet not described physiological functions of the OFC are highly likely to exist.

https://www.ncbi.nlm.nih.gov/pubmed/15863802
https://www.ncbi.nlm.nih.gov/pubmed/17707566

- Review of the OFC in Elliott et al (2004):

"Several previous studies have suggested that the orbitofrontal cortex and amygdala interact closely in associative learning paradigms (Baxter et al., 2000; Kalivas and Nakamura, 1999 and Schoenbaum et al., 1998). It has been suggested (Baxter et al., 2000 and Schoenbaum et al., 1998) that the connection between OFC and amygdala is critical for response selection on the basis of incentive value. However, the present results suggest that the responses of these regions can be functionally dissociated. While amygdala responses to reward were significantly modulated by movement, OFC responses were not. Recent results from functional neuroimaging (Breiter et al., 2001; Elliott et al., 2003 and O'Doherty et al., 2001), as well as animal electrophysiology (Schultz, 2000) and lesion studies (Mobini et al., 2002), suggest that a critical function of the OFC is assigning relative reward value. The rewards in the movement and the no-movement conditions of the present study are of equal value. It has also been argued that the OFC plays a role in analytic reward detection and expectation of reward (Schultz, 2000). Again both the experience of rewards and the expectation of rewards are matched in the movement and no-movement conditions, and a lack of differential OFC activity is therefore plausible. It is important to note that both animal studies reviewed above, and neuropsychological studies of patients with OFC damage (Bechara et al., 1994; Bechara et al., 1996 and Rolls et al., 1994), suggest that OFC plays a role in behavioural modification in the face of changing reward values. The present study is not incompatible with this hypothesis. Reward values here remain constant and therefore no behavioural modification is required. It is plausible that amygdala and striatum mediate already established reward-eliciting behaviours, while medial OFC monitors ongoing reward experience, ready to modify behaviour (via top-down connections to the amygdala and striatum) if necessary" [2].

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Instrumental responding for rewards is associated with enhanced neuronal response in subcortical reward systems. (2004) Neuroimage. 2004 Mar;21(3):984-90. **[Abstract PubMed]**

Orbitofrontal cortex activity related to emotional processing changes across the menstrual cycle
Xenia Protopopescu *, Hong Pan *, Margaret Altemus , Oliver Tuescher *, Margaret Polanecsky , Bruce McEwen , David Silbersweig *, and Emily Stern *¶
*Functional Neuroimaging Laboratory, Department of Psychiatry, and Iris Cantor Women's Health Center, Department of Obstetrics and Gynecology, Weill Medical College of Cornell University, New York, NY 10021; and Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021

Edited by Marcus E. Raichle, Washington University School of Medicine, St. Louis, MO, and approved September 15, 2005 (received for review April 5, 2005)

The orbitofrontal cortex (OFC) has been implicated in the representation of emotional stimuli, assignment of emotional valence/salience to stimuli, stimulus-reinforcement association learning, motivation, and socio-emotional control. Using functional magnetic resonance imaging in female subjects without premenstrual mood symptoms, we found that OFC activity to emotional linguistic stimuli varies depending on the menstrual cycle phase. Specifically, anterior-medial OFC activity for negative vs. neutral stimuli was increased premenstrually and decreased postmenstrually. The inverse pattern was seen in the lateral OFC. These findings suggest that specific subregional OFC activity to emotional stimuli is modulated across the menstrual cycle. The data also demonstrate that menstrual cycle phase is an important consideration in further studies attempting to elucidate the neural substrates of affective representation.

Hormonal cycle modulates arousal circuitry in women using functional magnetic resonance imaging.

Goldstein JM, Jerram M, Poldrack R, Ahern T, Kennedy DN, Seidman LJ, Makris N.

J Neurosci. 2005 Oct 5;25(40):9309-16. Related Articles, Links

Department of Psychiatry, Harvard Medical School, Brigham and Women's Hospital, Connors Center for Women's Health and Gender Biology, Boston, Massachusetts 02115, USA. jill_goldstein@hms.harvard.edu

Sex-specific behaviors are in part based on hormonal regulation of brain physiology. This functional magnetic resonance imaging (fMRI) study demonstrated significant differences in activation of hypothalamic-pituitary-adrenal (HPA) circuitry in adult women with attenuation during ovulation and increased activation during early follicular phase. Twelve normal premenopausal women were scanned twice during the early follicular menstrual cycle phase compared with late follicular/midcycle, using negative valence/high arousal versus neutral visual stimuli, validated by concomitant electrodermal activity (EDA). Significantly greater magnitude of blood oxygenation level-dependent signal changes were found during early follicular compared with midcycle timing in central amygdala, paraventricular and ventromedial hypothalamic nuclei, hippocampus, orbitofrontal cortex (OFC), anterior cingulate gyrus (aCING), and peripeduncular nucleus of the brainstem, a network of regions implicated in the stress response. Arousal (EDA) correlated positively with brain activity in amygdala, OFC, and aCING during midcycle but not in early follicular, suggesting less cortical control of amygdala during early follicular, when arousal was increased. This is the first evidence suggesting that estrogen may likely attenuate arousal in women via cortical-subcortical control within HPA circuitry. Findings have important implications for normal sex-specific physiological functioning and may contribute to understanding higher rates of mood and anxiety disorders in women and differential sensitivity to trauma than men.

PMID: 16207891 [PubMed - in process]

2.1.2 Parts of the brain affected: Hippocampus :

- Introduction :

The (Ro)accutane induced degenerative process in the hippocampus are here suggested to be of two categories:

1) The suggested immediate continous degeneration during exposure, due to decreased cell survival and induced apoptosis or programmed cell death

2) The neuroendocrine condition after exposure, characterized by hormonal decline (including severe vitamin A deficiency), intracellular and extracellular changes, and the symptomatic implications post exposure (please see section 6 for suggested age related pathologies post (Ro)accutane exposure)

- Suggested significant hippocampal cell loss during (Ro)accutane exposure in subjects :

In a mouse model, endogenous RA generated by synthetic enzymes in the meninges acts on hippocampal granule neurons, and chronic (3-week) exposure to a clinical dose of 13-cis RA is suggested to result in hippocampal cell loss and supress hippocampal cell division and proliferation [3 and 4].

- Vitamin A deficiency and effects on the hippocampus :

Vitamin A deficiency may in itself, without a prior significant cell loss contribute to a worsened function of the hippocampus. Vitamin A deficiency was found to decrease hippocampal acethylcholine release. Following 12 weeks of vitamin A-free diet, rats were trained to acquire a radial-arm maze task. Results show that a vitamin A deficient diet induced a severe deficit in the spatial learning and memory task. A significant decrease in hippocampal acetylcholine release induced by scopolamine, assessed using microdialysis technique, and a reduction in the size of hippocampal nuclei of CA1 region in vitamin-deficient rats, compared to rats fed with a vitamin A-sufficient diet [7]. A decreased learning capacity was also found in the (Ro)accutane exposed rats [4]. In rats, dietary folic acid deficiency dramatically increased blood homocysteine levels and significantly reduced the number of proliferating cells in the dentate gyrus of the hippocampus in adult mice [8].

- Effects of long-term thyroid deficiency on the hippocampus :

In the hippocampus, neurotrophins are involved in the modulation of synaptic transmission, including the induction of long-term potentiation (LTP) through the receptor TrkB. The expression levels of two (RS)-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits, GluR2 and GluR3, but not GluR1 or GluR4, were found to be significantly altered in the hippocampus of p75NTR-deficient mice. These results implicate p75NTR in activity-dependent synaptic plasticity and extend the concept of functional antagonism of the neurotrophin signaling system. [1].

In postnatal rats, thyroxin is found to insert an important function in the regulation of Trk and p75NTR- signaling. After thyroxine application, the mRNA expression of neurotrophins of the nerve-growth-factor (NGF) family is significantly upregulated both in septum and

hippocampus [2].

In a GH1 cell line, thyroid receptor expression is found to be suppressed by 50% in doses that could be expected in human subjects exposed to retinoic acid [5]. Inhibition of TR receptors, seems to be accompanied by inhibition of trkB receptors.

In an adult rat neuroblastoma cell line, induction of trkB mRNA is also caused by transient expression of either TR alpha 1 or beta 1 isoforms [6].

- The function of the hippocampus :

Neurogenesis in the adult hippocampus may play important roles in learning and memory, and in recovery from injury [9].

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(2005) Neuroreport. Jul 13;16(10):1055-1059.

J Neurol Sci. 2002 Aug 15;200(1-2):67-73. Related Articles, Links
Alteration of midkine expression in the ischemic brain of humans.

Wada M, Kamata M, Aizu Y, Morita T, Hu J, Oyanagi K.
Department of Neuropathology, Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai, Fuchu, Tokyo 183-8526, Japan.

Midkine (MK) is a heparin-binding growth factor that occurs as a product of the retinoic acid-inducible gene. Alteration of MK expression in ischemic brain lesions was examined

in humans immunohistochemically in nine patients and in two control subjects without neurological disorders. Some neurons were MK-immunopositive, but no evident MK-immunoreactivity was observed in astrocytes in brains of control subjects. In the ischemic lesions, significant elevation of MK-immunoreactivity in the astrocytes and depletion of the reactivity in neurons were seen, especially in the early period, where edema and eosinophilic neurons were prominent. On the other hand, MK-immunoreactivity was not observed in hypertrophic and fibrillary astrocytes in the later period. These findings suggest that the MK in astrocytes play some role in the repair process in the early period of the ischemic brain lesions in humans.

PMID: 12127679 [PubMed - indexed for MEDLINE]

Neurochem Int. 2005 Jul 5; [Epub ahead of print] Related Articles, Links
Hippocampal mitochondrial dysfunction with decreased mtNOS activity in prehepatic portal hypertensive rats.
Lores-Arnaiz S, Perazzo JC, Prestifilippo JP, Lago N, D'Amico G, Czerniczyniec A, Bustamante J, Boveris A, Lemberg A.
Laboratory of Free Radical Biology, School of Pharmacy and Biochemistry, University of Buenos Aires, Junin 956, 1113 Buenos Aires, Argentina.

Portal hypertension is a major complication of human cirrhosis that frequently leads to central nervous system dysfunction. In our study, rats with prehepatic portal hypertension developed hippocampal mitochondrial dysfunction as indicated by decreased respiratory rates, respiratory control and mitochondrial nitric oxide synthase (mtNOS) activity in mitochondria isolated from the whole hippocampus. Succinate-dependent respiratory rates decreased by 29% in controlled state 4 and by 42% in active state 3, and respiratory control diminished by 20%. Portal hypertensive rats showed a decreased mtNOS activity of 46%. Hippocampal mitochondrial dysfunction was associated with ultrastructural damage in the mitochondria of hippocampal astrocytes and endothelial cells. Swollen mitochondria, loss of cristae and rupture of outer and inner membrane was observed in astrocytes and endothelial cells of the blood-brain barrier in parallel with the ammonia gradient. It is concluded that the moderate increase in plasma ammonia that followed portal hypertension was the potential primary cause of the observed alterations.

PMID: 16005112 [PubMed - as supplied by publisher]

Neuroreport. 2005 Jul 13;16(10):1055-1059. Related Articles, Links
Folate deficiency inhibits proliferation of adult hippocampal progenitors.
Kruman II, Mouton PR, Emokpae R Jr, Cutler RG, Mattson MP.

1Sun Health Research Institute, 10515 West Santa Fe Drive, Sun City, AZ 85351 2Laboratory of Experimental Gerontology 3Laboratory of Neurosciences, National Institute on Aging Intramural Research Program, 5600 Nathan Shock Drive, Baltimore, MD 21224 4Department of Neuroscience, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA.

Neurogenesis in the adult hippocampus may play important roles in learning and memory, and in recovery from injury. As recent findings suggest, the perturbance of homocysteine/folate or one-carbon metabolism can adversely affect both the developing and the adult brain, and increase the risk of neural tube defects and Alzheimer's disease.

We report that dietary folic acid deficiency dramatically increased blood homocysteine levels and significantly reduced the number of proliferating cells in the dentate gyrus of the hippocampus in adult mice. In vitro, the perturbance of one-carbon metabolism repressed proliferation of cultured embryonic multipotent neuroepithelial progenitor cells and affected cell cycle distribution. Our results suggest that dietary folate deficiency inhibits proliferation of neuronal progenitor cells in the adult brain and thereby affects neurogenesis.

PMID: 15973147 [PubMed - as supplied by publisher]

2.1.3 Parts of the brain aff.: Blood-brain bar.

The blood-brain barrier (BBB) is a metabolic and physiological barrier important for maintaining brain homeostasis [5]. Integrity of the blood-brain barrier is essential for the normal functioning of CNS [6].

- Inhibition of insulin transport to the brain, inhibition of transcytosis and altered permeability of the blood brain barrier :

The Hoffman la Roche corporation itself, salesagent of (Ro)accutane, has admitted to the finding of an increased alkaline phosphatase in acne-subjects exposed to the toxin [0]. A positive correlation between ecto-ALP activity and (125)I-insulin incorporation ($r = 0.82$; $P < 0.0001$) was shown in cultured rat brain endothelial cells, suggesting that insulin entry into the blood-brain barrier may be modulated through ALP [1]. Cellular retinol binding protein (CRBP) was demonstrated in cells that form the blood brain barrier in humans and rats, specifically within endothelial cells of the brain microvasculature and in cuboidal epithelial cells of the choroid plexus. Translocation of retinol across the blood brain barrier is suggested to occur via RBP uptake from the plasma with subsequent transcellular movement of retinol as a complex with CRBP [6]. A disturbance in the continous translocation of retinol to the brain following a (Ro)accutane exposure is highly likely. Significant effects of hypervitaminosis A on the choroid plexus in humans was discovered allready in the 1960s [7]. Plasma transthyretin (TTR, formerly called prealbumin) is a 55-kd protein that participates in the plasma transport of both thyroxine and retinol (vitamin A). TTR concentrations are disproportionately high in human ventricular CSF, suggesting that TTR is either selectively transported across or synthesized de novo within the blood-CSF barrier [8].

The epithelial cells of the choroid plexus are also known to contain specific transport systems for thiamine, ascorbic acid, pyridoxine, folate and inositol [6].

Protein kinase C (PKC) isoforms have been found to modulate permeability of the blood brain barrier [5].

- Inhibition of glucose transport to the brain, worsening symptoms with age in (Ro)accutane exposed subjects :

Glucose transporter 1 (GLUT1) is the primary glucose transporter in the blood brain barrier [2]. In rats, it is concluded that alterations in cerebral GLUT-1 content in response to altered thyroid state are age-specific [3]. In human acne-subjects exposed to

(Ro)accutane, levels of thyroxine and triiodothyronine were significantly lower after exposure (p less than 0.05), indicating a (Ro)accutane induced clinical thyroid deficiency (hypothyroidism) [4].

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J Cell Sci. 2004 Oct 1;117(Pt 21):5071-8. Epub 2004 Sep 21. Related Articles, Links

Efficient transfer of receptor-associated protein (RAP) across the blood-brain barrier.

Pan W, Kastin AJ, Zankel TC, van Kerkhof P, Terasaki T, Bu G.
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We have sought to identify a high-capacity transport system that mediates transcytosis of proteins from the blood to the brain. The 39 kDa receptor-associated protein (RAP) functions as a specialized endoplasmic reticulum chaperone assisting in the folding and trafficking of members of the low-density lipoprotein (LDL) receptor family. RAP efficiently binds to these receptors and antagonizes binding of other ligands. Previous studies have shown that two large members of the LDL receptor family, LDL receptor-related protein 1 (LRP1) and LDL receptor-related protein 2 (LRP2 or megalin), possess the ability to mediate transcytosis of ligands across the brain capillary endothelium. Here, we tested

whether blood-borne RAP crosses the blood-brain barrier (BBB) by LRP1- or megalin-mediated transport by studying the pharmacokinetics of [125I]-RAP transport into the brain in intact mice and across cell monolayers in vitro. Our results show that [125I]-RAP is relatively stable in blood for 30 minutes and has a mean influx constant of 0.62+/-0.08 microl/g-minute from blood to brain. In situ brain perfusion in blood-free buffer shows that transport of [125I]-RAP across the BBB is a saturable process. Capillary depletion of brain homogenates indicates that 70% of [125I]-RAP is localized in the parenchyma rather than in the vasculature of the brain. Results of transport in stably transfected MDCK cells are consistent with the hypothesis that megalin mediates most of the apical-to-basolateral transport across polarized epithelial cells. The inhibition of [125I]-RAP influx by excess RAP and the involvement of megalin indicate the presence of a saturable transport system at the BBB. The higher permeability of RAP compared with that of melanotransferrin and transferrin show that the LRP receptor is a high capacity transport system. These studies suggest that RAP may provide a novel means of protein-based drug delivery to the brain.

PMID: 15383619 [PubMed - indexed for MEDLINE]

J Am Soc Nephrol. 2005 Jun 23; [Epub ahead of print] Related Articles, Links
Intraperitoneal Administration of Recombinant Receptor-Associated Protein Causes Phosphaturia via an Alteration in Subcellular Distribution of the Renal Sodium Phosphate Co-Transporter.
Yamagata M, Ozono K, Hashimoto Y, Miyauchi Y, Kondou H, Michigami T.
*Department of Environmental Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health; and Department of Developmental Medicine (Pediatrics), Osaka University Graduate School of Medicine, Osaka, Japan.

Megalin is a multifunctional endocytic receptor that is expressed in renal proximal tubules and plays critical roles in the renal uptake of various proteins. It was hypothesized that megalin-dependent endocytosis might play a role in renal phosphate reabsorption. For addressing the short-term effects of altered megalin function, a recombinant protein for the soluble form of 39-kD receptor-associated protein (RAP) was administered intraperitoneally to 7-wk-old mice. Histidine (His)-tagged soluble RAP (amino acids 39 to 356) lacking the amino-terminal signal peptide and the carboxy-terminal endoplasmic reticulum retention signal was prepared by bacterial expression (designated His-sRAP). After the direct interaction between His-sRAP and megalin was confirmed, mice were given a single intraperitoneal administration of His-sRAP (3.5 mg/dose). Immunostaining and Western blot analyses demonstrated the uptake of His-sRAP and the accelerated internalization of megalin in proximal tubular cells 1 h after administration. In addition, internalization of the type II sodium/phosphate co-transporter (NaPi-II) was observed. The effects of three sequential administrations of His-sRAP (3.5 mg/dose, three doses at 4-h intervals) then were examined, and increased urinary excretion of low molecular weight proteins, including vitamin D-binding protein, was found, which is consistent with findings reported for megalin-deficient mice. It is interesting that urinary excretion of phosphate was also increased, and the protein level of NaPi-II in the brush border membrane was decreased. Serum concentration of 25-hydroxyvitamin D was decreased, whereas the plasma level of intact parathyroid hormone was not altered by the administration of His-sRAP. The results suggest that the His-sRAP-induced acceleration of megalin-mediated endocytosis caused phosphaturia via altered subcellular distribution of NaPi-II.

PMID: 15976002 [PubMed - as supplied by publisher]

Am J Physiol Heart Circ Physiol. 2005 Jul 1; [Epub ahead of print] Related Articles, Links
Activation of PKC modulates blood-brain barrier endothelial cell permeability changes induced by hypoxia and post-hypoxic reoxygenation.
Fleegal MA, Hom S, Borg LK, Davis TP.
Medical Pharmacology, University of Arizona, Tucson, AZ, USA.

The blood-brain barrier (BBB) is a metabolic and physiological barrier important for maintaining brain homeostasis. The aim of these studies was to determine the role of protein kinase C (PKC) activation in BBB paracellular permeability changes induced by hypoxia and post-hypoxic reoxygenation using in vitro and in vivo BBB models. In rat brain microvessel endothelial cells (RMECs) exposed to hypoxia (1% O2; 24 h), a significant increase in total PKC activity was observed, and this was reduced by post-hypoxic reoxygenation (95% room air/ 5% CO2) for 2 h. The expression of PKCbetaII, PKCgamma, PKCeta, PKCmicro and PKClambda also increased following hypoxia (1% O2; 24 h) and these protein levels remained elevated following post-hypoxic reoxygenation (95% room air/ 5% CO2; 2 h). Increases in the expression of PKCepsilon and PKCzeta were also observed following post-hypoxic reoxygenation (95% room air/ 5% CO2; 2 h). Moreover, inhibition of PKC with chelerythrine chloride (10 microM) attenuated the hypoxia-induced increases in (14)C-sucrose permeability. Similar to what was observed in RMECs, total PKC activity was also stimulated in cerebral microvessels isolated from rats exposed to hypoxia (6% O2; 1 h) and post-hypoxic reoxygenation (room air; 10 min). In contrast, hypoxia (6% O2; 1 h) and post-hypoxic reoxygenation (room air; 10 min) significantly increased the expression levels of only PKCgamma and PKCtheta in the in vivo hypoxia model. These data demonstrate that hypoxia-induced BBB paracellular permeability changes occur via a PKC-dependent mechanism, possibly by differentially regulating the protein expression of the eleven PKC isozymes.

PMID: 15994856 [PubMed - as supplied by publisher]

J Neurochem. 2005 Jul;94(1):204-14. Related Articles, Links
Inhibition of phosphoinositide 3 kinase-Akt (protein kinase B)-nuclear factor-kappaB pathway by lovastatin limits endothelial-monocyte cell interaction.
Prasad R, Giri S, Nath N, Singh I, Singh AK.
Department of Pediatrics, Medical University of South Carolina, Charleston, SC 29425, USA.

Integrity of the blood-brain barrier is essential for the normal functioning of CNS. Its disruption contributes to the pathobiology of various inflammatory neurodegenerative disorders. We have shown that the HMG-CoA reductase inhibitor (lovastatin) attenuated experimental autoimmune encephalomyelitis (EAE, an inflammatory disease of CNS) in rodents by inhibiting the infiltration of mononuclear cells into the CNS. Here, using an in vitro system, we report that lovastatin inhibits endothelial-monocyte cell interaction by down-regulating the expression of vascular cell adhesion molecule-1 and E-selectin by inhibiting the phosphoinositide 3 kinase (PI3-kinase)/protein kinase B (Akt)/nuclear factor-kappa B (NF-kappaB) pathway in endothelial cells. It inhibits tumor necrosis factor alpha (TNFalpha)-induced PI3-kinase, Akt and NF-kappaB activation in these cells. Co-transfection of constitutively active forms of PI3-kinase and Akt reversed the lovastatin-mediated inhibition of TNFalpha-induced adhesion, as well as activation of NF-kappaB, indicating the involvement of the PI3-kinase/Akt pathway in the interaction of adhesion

molecules and the process of adhesion. This study reports that lovastatin down-regulates the pathway affecting the expression and interaction of adhesion molecules on endothelial cells, which in turn restricts the migration and infiltration of mononuclear cells thereby attenuating the pathogenesis of inflammatory diseases.

PMID: 15953363 [PubMed - in process]

Biochem Genet. 2005 Apr;43(3-4):175-87. Related Articles, Links

Glucose transporter 1, distribution in the brain and in neural disorders: its relationship with transport of neuroactive drugs through the blood-brain barrier.
Guo X, Geng M, Du G.

Department of Pharmacology, Marine Drug and Food Institute, Ocean University of China, Qingdao 266003, China.

Facilitative glucose transport is mediated by one or more of the members of the closely related glucose transporter (GLUT) family. Thirteen members of the GLUT family have been described thus far. GLUT1 is a widely expressed isoform that provides many cells with their basic glucose requirement. It is also the primary transporter across the blood-brain barrier. This review describes the distribution and expression of GLUT1 in brain in different pathophysiological conditions including Alzheimer's disease, epilepsy, ischemia, or traumatic brain injury. Recent investigations show that GLUT1 mediates the transport of some neuroactive drugs, such as glycosylated neuropeptides, low molecular weight heparin, and D-glucose derivatives, across the blood-brain barrier as a delivery system. By utilizing such highly specific transport mechanisms, it should be possible to establish strategies to regulate the entry of candidate drugs.

PMID: 15932065 [PubMed - in process]

2.1.4 Parts of the brain affected: Hypothalamus :

- Retinoid receptor expression and metabolism in the hypothalamus :

Components of the metabolic pathway for retinoids have been identified in adult brain tissues, suggesting that all-trans-retinoic acid (ATRA) can be synthesized in discrete regions of the brain. The distribution of retinoid receptor proteins in the adult nervous system is different from that seen during development; and suggests that retinoid signalling is likely to have a physiological role in adult cortex, amygdala, hypothalamus, hippocampus, striatum and associated brain regions [1].

- The retinoid X receptor and retinoid signaling in the neuroendocrine hypothalamus associated with adaptations to season and body-weight :

Siberian hamsters were maintained in long or short photoperiods that generate

physiological states of obesity or leanness. Microarray expression analysis first identified CRBP1 as a photoperiod-responsive gene, and then further studies using in situ hybridization and immunocytochemistry revealed that expression levels of several related retinoid-signaling genes were modulated in response to photoperiod changes. Genes of the retinoid-signaling pathway, encoding nuclear receptors (RXR/RAR) and retinoid binding proteins (CRBP1 and CRABP2) are photoperiodically regulated in the dorsal tuberomamillary nucleus (DTM): Their expression is significantly lower in short photoperiods and parallels body weight decreases. Studies in pinealectomized hamsters confirm that the pineal melatonin rhythm is necessary for these seasonal changes, and studies in testosterone-treated hamsters reveal that these changes in gene expression are not the secondary consequence of photoperiod-induced changes in steroid levels. Comparative studies using Syrian hamsters, which show divergent seasonal body weight responses to Siberian hamsters when exposed to short photoperiods, showed a distinct pattern of changes in retinoid gene expression in the DTM in response to a change in photoperiod. The DTM may be an important integrating center for photoperiodic control of seasonal physiology and suggest that the changes in retinoid X receptor gamma expression may be associated with seasonal changes in body weight and energy metabolism [2].

- Retinoic acid involved in rat GnRH release in the hypothalamus :

To complete

- Insulin signaling in the hypothalamus :

To complete

- References :

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Endocrinology. 2005 Jul 7; [Epub ahead of print] Related Articles, Links

Consumption of a fat-rich diet activates a pro-inflammatory response and induces insulin resistance in the hypothalamus.
De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boshero AC, Saad MJ, Velloso LA.
Departments of Internal Medicine and Physiology and Biophysics, State University of Campinas, 13084-970, Campinas - SP; Department of Physiology and Biophysics, University of Sao Paulo, 05508-900, Sao Paulo - SP, Brazil.

Obesity has reached epidemic proportions in several regions of the world. General changes in lifestyle, including consumption of fat-rich food, are among the most important factors leading to an unprecedented increase in the prevalence of this disease. Weight gain results from an imbalance between caloric intake and energy expenditure. Both of these parameters are under the tight control of specialized neurons of the hypothalamus that respond to peripheral anorexigenic and adipostatic signals carried by leptin and insulin. Here we show, by macroarray analysis, that high-fat feeding (hyperlipidic diet - HL) induces the expression of several pro-inflammatory cytokines and inflammatory responsive proteins in hypothalamus. This phenomenon is accompanied by increased activation of JNK and NFkappaB. In addition, HL feeding leads to impaired functional and molecular activation of the insulin-signaling pathway, which is paralleled by increased serine phosphorylation of the insulin receptor and IRS-2. Intracerebroventricular (icv) treatment of HL rats with a specific inhibitor of JNK (SP600125) restores insulin signaling and leads to a reduced caloric intake and weight loss. We conclude that HL feeding induces a local pro-inflammatory status in the hypothalamus, which results in impaired anorexigenic insulin signaling.

PMID: 16002529 [PubMed - as supplied by publisher]

Brain Res Mol Brain Res. 1998 Feb;54(1):74-84. Related Articles, Links
Retinoic acid regulates gonadotropin-releasing hormone (GnRH) release and gene expression in the rat hypothalamic fragments and GT1-1 neuronal cells in vitro.
Cho S, Cho H, Geum D, Kim K.
Department of Molecular Biology, Seoul National University, South Korea.

The present study attempts to examine the possible involvement of retinoic acid (RA) in the regulation of gonadotropin-releasing hormone (GnRH) release and gene expression in the rat hypothalamic fragments and GT1-1 neuronal cells in vitro. During a short-term period (2h), RA (0.01-1 microM) increased GnRH release in a dose-related manner. Time-course experiments showed that RA rapidly increased GnRH release by 30 min in both cells. RA-induced GnRH release was slowly attenuated in the next incubation period in hypothalamic fragments, but rapidly returned to control levels in GT-1 cells. In hypothalamic fragments, GnRH mRNA levels decreased, but in GT1-1 cells, no change in GnRH mRNA levels was observed. We then extended the incubation time to see any changes in GnRH mRNA levels by RA in GT1-1 cells. In a long term (up to 48 h), RA increased GnRH mRNA levels in a dose- and time-related manner. Significant increase in GnRH mRNA levels by RA (at higher than 10 nM) was observed within 12h. Transient transfection experiments with a luciferase reporter vector containing more than 3 kb of the rat GnRH 5'-flanking region (-3002 to +88) revealed that RA also increased the rat GnRH promoter activity in a similar dose-and time-dependent manner, suggesting that increases in GnRH mRNA levels are attributable, at least in part, to the enhanced gene transcription. The promoter analysis with the 5'-deletional constructs demonstrated that cis-elements responsible for the RA action may reside within -1640/-1438 of the rat GnRH promoter, where multiple direct or palindromic arrangements of the AGGTCA-related sequences exist. We also showed that GT1-1 cells as well as the hypothalamic tissues express mRNA for multiple subtypes of retinoid receptors, and that reporter plasmids with three copies of the strong retinoic acid response element (RARE) were activated by 80 folds upon treatment with RA in GT1-1 cells, suggesting that retinoid receptors in GT1-1 cells are functional. Taken together, the present study strongly suggests that RA is an important

regulator of the GnRH neurons.

PMID: 9526050 [PubMed - indexed for MEDLINE]

2.1.5 Parts of the brain affected: Other parts :

To complete

Stem Cells. 2005 Sep 1; [Epub ahead of print] Related Articles, Links
Role of Transcription Factors in the Motoneuron Differentiation of Adult Human Olfactory Neuroepithelial-Derived Progenitors.
Zhang X, Cai J, Klueber KM, Guo Z, Lu C, Winstead WI, Qiu M, Roisen FJ.
Department of Anatomical Sciences & Neurobiology, Louisville, Kentucky.

Neurosphere forming cell (NSFC) lines have been established from cultures of human adult olfactory neuroepithelium. Few of these cells ever express mature neuronal or glial markers in MEM supplemented with 10% FBS or defined medium. However these neural progenitors have the potential to differentiate along glial or neuronal lineages. To evaluate the potential of NSFCs to form motoneurons, transcription factors, Olig2, Ngn2 and HB9, were introduced into NSFCs to determine if their expression is sufficient for motoneuron specification and differentiation as has been shown in the early development of the avian and murine central nervous systems in vivo. NSFCs transfected with Olig2, Ngn2, and HB9 alone exhibited no phenotypic lineage-restriction. In contrast, simultaneous transfection of Ngn2 and HB9 cDNA increased the expression of Isl1/2 a motoneuron marker when the cells were maintained in medium supplemented with retinoic acid, forskolin, and sonic hedgehog (RFS). Furthermore, a population of Olig2-expressing NSFCs also expressed Ngn2. Co-transfection of NSFCs with Olig2 and HB9, but not Olig2 and Ngn2, increased Isl1/2 expression. Co-culture of NSFCs transfected with Ngn2-HB92, or Olig2 and HB9 with purified chicken skeletal muscle demonstrated frequent contacts that resembled neuromuscular junctions. These studies demonstrate that transcription factors governing the early development of chick and mouse motoneuron formation are able to drive human adult olfactory neuroepithelial progenitors to differentiate into motoneurons in vitro. Our long-term goal is to develop cell populations for future studies of the therapeutic utility of these olfactory-derived NSFCs for autologous cell replacement strategies for CNS trauma and neurodegenerative diseases.

PMID: 16141360 [PubMed - as supplied by publisher]

2.2 Affected neurotransmitters :

2.2.1 Neurotransmitter category: Serotonin

- (Ro)accutane induced signicicant inhibition of serotonergic biosynthesis in human subjects: Suggested significant inhibition of the tryptophan hydroxylase (TPH) through decreased promoter activity due to Sp1 and NF-Y dephosphorylation leading to

decreased binding affinity and formation of Sp proteins, partially through significant elevation of alkaline phosphatases :

Tryptophan hydroxylase (TPH) catalyses the rate-limiting reaction in the biosynthesis of serotonin. In humans, two different TPH genes exist, located on chromosomes 11 and 12, respectively, and encoding two enzymes (TPH1 and TPH2) with an overall sequence identity of 71% [1]. In rats nuclear transcription factor Y (NF-Y) is found to regulate TPH [2]and in rats, retinoic acid has found to be one factor that modulates NF-Y [3].

In human pinealocytes, NF-Y and Sp1 transactivators bind the inverted CCAAT box and GC-rich-region in the TPH gene, respectively [4]. Transcription of a 2.1-kb fragment of the human TPH promoter is induced by cAMP, although it lacks the canonical cAMP responsive element, CRE. An additional cis -acting sequence, the adjacent GC-rich region, cooperates with the inverted CCAAT box for the full activation of basal transcription, and both elements are essential for the full cAMP response. In small doses, retinoids induce the Retinoid receptors RARs/RXRs and physically interact with Sp1, potentiate Sp1 binding to the GC box motifs [5]. Unfortunately no publicly available study shows how NF-Y, Sp1 or its binding sites (GC box motifs) are affected by a dose of retinoic acid beyond the physiological limit. However an increased alkaline phosphatase as found in subjects exposed to (Ro)accutane, and is even admitted by Hoffman la Roche itself [0 and more] is found to dephosphorylate Sp proteins [6], which most likely leads to a significantly decreased promotion of the TPH-gene and lessened biosynthesis of serotonin. Further evidence for a dramatically reduced Sp promotion comes with the findings of dramatically increased homocysteine (Hcy) levels in subjects exposed to (Ro)accutane [0 and more]. Cystathionine beta-synthase (CBS) catalyzes the condensation of serine and homocysteine to form cystathionine, and is promoted by Sp1 and Sp3 [7] in a similiar manner to how the TPH gene is promoted.

In human NT2 cells, formation of the Sp1/Sp3 containing complex was inhibited by anti-RA receptor (RAR) antibodies [8]. This is probably due to a lessened promotion of Sp1, and a low degree of phosphorylation. Exposure of extremely high doses of retinoic acid, (Ro)accutane, in human acne-subjects, and thus inhibition of Sp1/Sp3 is highly likely due to inhibition of binding through high dose inhibition of phosphorylation. A bell shaped curve of Sp1 binding and activity is likely.

- Inhibition of serotonerg 5HT(1A) receptor expression and inhibtion of glycine responses through inhibition of several protein kinases and inhibition of phosphorylation of Sp1 :

The somatodendritic 5-HT(1A) autoreceptor has been considered a major determinant of the output of the serotonin (5-HT) neuronal system. 5-HT autoinhibition is critically regulated by the tryptophan hydroxylase-activating kinases calcium/calmodulin protein kinase II (CaMKII) and protein kinase A (PKA) [9]. The expression level of the 5-HT(1A) receptor gene (htr1a) in the central nervous system (CNS) is implicated in the aetiology and treatment of anxiety disorders and depression. In mice, htr1a have revealed that its proximal promoter is GC rich and TATA-less. Several functional transcription factor binding sites, including MAZ and SP1 recognition sequences, have been identified [10]. It is shown that inhibition of Sp1 phosphorylation causes a decreased binding affitnity for the transcription on promoter activity. Since the Sp1 is found to be a 5HT(1A) promoter, the

receptor expression is here suggested to be significantly decreased.

Mammalian alkaline phosphatases (AP) are glycosylphosphatidylinositol (GPI) anchored proteins that are localized on the outer layer of the plasma membrane. The GPI anchors are covalently attached to the C-termini of proteins and consist of a glycan chain bonded to phosphatidylinositol with two acyl chains anchored into the membrane bilayer [11]. In the the rat hippocampus, exposure to alkaline phosphatase and inhibition of PKC is found to inhibit glycine responses, which is affecting the serotonerg 5-HT receptors. PKC was found to be involved in the modulation of hippocampal glycine receptors, since the observed effect was more prominent when the phorbol ester PMA, an activator of PKC, was added [12]. In 10T1/2 cells, a decrease of total PKC activity was observed following exposure to 10(-5) M 13-cis-retinoic acid [13]. Inhibition of PKC is likely affecting many cell lines, including cell lines in the brain, and is suggested to logically result in inhibition of glycine responses, as well as lack of phosphorylation of Sp1 and NF-Y and thus promoter activity.

- Serotonerg transport :

1) Human serotonin transporter (hSERT) and AP-2

Retinoids activate AP2 transcription factors [14 and more]. Transcription factor AP-2 is essential for neuronal development and many genes involved in the brainstem monoaminergic systems have binding sites for AP-2 in their regulatory regions. The genotype of the AP-2beta isoform has been associated with e.g. anxiety-related personality traits and with platelet MAO activity [15].
Exon 1B of the human serotin transporter (hSERT) is surrounded by several elements potentially suitable for regulating serotonin transporter gene expression in vivo, including consensus sites for transcription factors AP-1, AP-2, CREB/ATF, and NF-kappaB [17]. All of these having in different studies been found to be significantly modultated by retinoic acid.

2) 5HTT

In humans, integrin beta 3 (ITGB3) is found to affect the serotonin transporter gene (5HTT) [18]. 1,25-dihydroxyvitamin D3 (D3) is found to accelerate beta 3 transcription, and avian beta 3 promoter is suggested to contain a vitamin D response element (VDRE) [19]. A significant fall in metabolites of 1,25-dihydroxyvitamin D3 were found to in acne-subjects during and post (Ro)accutane exposure in repeated studies [20 and more]. In raphe serotonergic neurons, a TATA-like motif and several potential binding sites for transcription factors, including two AP1, several AP2 and AP4 binding sites, CCAAT and GC boxes was found in the 5-HTT promoter[21].

An impaired function of serotonin transporter gene(5HTT) through alterations in the promoter region and decreased 1,25 dihyrdoxyvitamin D3, as a direct consequence of (Ro)accutane exposure is here suggested.

- (Ro)accutane induced inhibition of androgen receptors is suggested to be modulatory of 5-HT activity :

Inhibition of androgen receptors is suggested to result in an alteration in tryptophan residues, a precursor amino acid to serotonin. Binding to both types of DNA response element resulted in changes in the intrinsic fluorescence emission spectrum for four tryptophan residues within the AR-NTD and resulted in a more protease-resistant conformation [22].

- Inhibition of insulin transport to the brain, suggested to futher decrease 5-HT receptor activity :

The Hoffman la Roche corporation itself, salesagent of (Ro)accutane, has admitted to the finding of an increased alkaline phosphatase in acne-subjects exposed to the toxin [0]. A positive correlation between ecto-ALP activity and (125)I-insulin incorporation ($r = 0.82$; $P < 0.0001$) was shown in cultured rat brain endothelial cells, suggesting that insulin entry into the blood-brain barrier may be modulated through ALP [23]. A close connection between 5-HT activity in the brain and peripheral sensitivity to insulin has been suggested[24].

Six weeks of isotretinoin exposure caused a statistically significant 19% increase in suction blister fluid TGF-beta1 [4].

TPH-1 location: 11p15.1 (genatlas)

- Conclusions :

The tryptophan hydroxylase (TPH), the main enzyme that converts serotonin to its bioactive form, and serotonerg receptor subtype 5HT-(1A) is with highest certainity inhibited by (Ro)accutane exposure in human subjects. It is highly likely that more serotonerg receptor isoforms are inhibited. A significantly reduced Sp promoter activity, due to inhibition of Sp1 and NF-Y phosphorylation, is highly likely one of the major causes for the found significantly increased Hcy levels in human subjects exposed to (Ro)accutane, as well as the here suggestd significant inhibition of TPH and 5-HT-(1A) receptor isoform. Thus, an inhibited Sp1 promoter activity is an important mechanism contributing to a lessened synthesis of serotonin. Further inhibition of other isoforms of the sertotonerg receptor is highly likely. (Ro)accutane induces AP-2 which affects the human serotonin transporter and may exert effects on mao activity. Possible effects on decreased insulin transport to the brain and vitamin D activity may also modulate sertotonerg activity.

(Ro)accutane contributes to a multiple inhibition of serotonerg activity by inhibition of the tryptophan hydroxylase (TPH), inhibition of the serotonerg receptor isotype 5-HT(1A) and translocation of AP-2. Exposure may result in inhibition of cell proliferation and survival. These findings may exert one pathway in (Ro)accutane induced chemical depression.

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2.2.2 Neurotransmitter category: Neurosteroids :

- Vitamin A and the brain :

To complete...

- Vitamin D and the brain :

To complete...

- (Ro)accutane induced insulin resistance/ decreased entry through the blood brain barrier, and the brain :

Brain insulin resistance is suggested to contribute to cognitive impairment [6]. Reduced levels of insulin produced in brain can lead to death of key brain cells, and may contribute to pathologies like Alzheimers disease [3]. Insulin deficiency within the brain in insulin knockout mice (I(-/-)). The I(-/-) exhibited hyperphosphorylation of tau, at threonine 231, and neurofilament. In addition, we showed hyperphosphorylation of c-Jun N-terminal kinase (JNK) and glycogen synthase kinase 3 beta (GSK-3 beta) at serine 9. Extracellular signal-regulated kinase 1 (ERK 1) showed decrease in phosphorylation, whereas ERK 2 showed no changes. Ultrastructural examination demonstrated swollen mitochondria, endoplasmic reticulum, and Golgi apparatus, and dispersion of the nuclear chromatin. Microtubules showed decrease in the number of intermicrotubule bridges and neurofilament presented as bunches. Thus, lack of insulin brain stimulation induces JNK hyperphosphorylation followed by hyperphosphorylation of tau and neurofilament, and ultrastructural cellular damage, that over time may induce decrease in cognition and learning disabilities [4].

- Growth hormone and the brain :

A GH/IGF-1 deficiency is suggested due to vitamin A induced significant interaction with the GH axis. The IGF-1 receptors are expressed in cells in the brain.

- Thyroid hormone and the brain :

To complete...

- Progesterone and the brain :

To complete...

- Dihydrotestosterone and the brain: expression and binding in pituitary, hypothalamus, amygdala and cortex :

The enzyme 5alpha-reductase (5alpha-R) is present in many mammalian tissues, including the brain. The physiological importance of 5alpha-R in the brain derives from its capability to convert testosterone (T) to a more potent androgen, dihydrotestosterone (DHT), and to convert progesterone to its 5alpha-reduced derivative, precursors of allopregnanolone, potent allosteric modulator of the gamma-aminobutyric acid receptor (GABA(A)-R). 5alpha-R occurs as two isoforms, 5alpha-R type 1 (5alpha-R1) and 5alpha-R type 2 (5alpha-R2) [1].

Dihydrotestosterone (DHT) binding was measured in cytosols from brain regions and pituitary of adult female rats and, with the addition of ventral prostate, in adult male rats. Two types of binding were distinguished: one, saturable at concentration of DHT greater than or equal to 5 X 10(-9) M and an unsaturable component. In intact males saturable (limited capacity) binding was detected only in ventral prostate cytosol; 3 days after orchidectomy the saturable binding sites increase 3-fold in prostate and in pituitary, hypothalamus, amygdala and cortex to detectable levels in approximately the same abundance as in females. There were significant differences in the affinities of the limited capacity binding reactions in cytosols of different tissues though all were in the order of magnitude, 10(-9) M DHT. The affinity in pituitary cytosol was lower than in brain regions with the single exception of female amygdala in which the affinity was significantly lower than in cytosol of the same region from 3-day castrate males. The specificity of the limited capacity binding was investigated by competition between [3h]DHT and unlabelled steroids; the most effective competitors were potent androgen agonists and antagonists [2].

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2.2.3 Neurotransmitter category: GABA

In the central nervous system, glutamate serves as an excitatory neurotransmittor. Glutamate is also the precursor of the inhibitory neurotransmittor GABA, as well as glutamine, a potential mediator of hyperammonemic neurotoxicity [1].

- Altered synthesis of GABA :

Modulation of Sp1 activity by nuclear receptors is a novel mechanism by which fat-soluble hormones regulate gene expression. Northern blot analyses showed that in addition to urokinase, after induction of RARs, RA up-regulates GC-rich region-dependent mRNA expression of transglutaminase, TGF beta 1, and types I and II TGF beta receptors [2]. Glutamic acid decarboxylase (GAD) 65 is one of two homologous proteins responsible for the synthesis of gamma-aminobutyric acid, the most ubiquitous inhibitory neurotransmitter. Gel-shift assays and reporter gene assays suggest that Sp1 can bind to a region devoid of consensus Sp1 binding sites that are found on the GAD promoter [3].

Hoffman la Roche itself has admitted to the findings of a significantly increased alkaline phosphatase [0]. The ubiquitous enzyme TNAP (tissue non-specific alkaline phosphatase) is found in numerous tissues such as liver, kidney and bone, but little attention has been paid to its expression and role in the brain. Observations in TNAP-KO mice, which analyzed the role of this enzyme in osteogenesis, had suggested that TNAP might be involved in GABA neurotransmission. Apart from its presence in endothelial cells, there is a specific and strong alkaline phosphatase (AP) activity in the neuropile, matching the pattern of thalamo-cortical innervation in layer 4 of the primate sensory cortices (visual, auditory and somatosensory). Such a pattern is also evident in rodents and carnivores, making AP a powerful marker of primary sensory areas. Remarkably, AP activity is regulated by sensory experience as demonstrated by monocular deprivation paradigms in monkeys. The areal and laminar distribution of AP activity matches that of the GAD(65), the GABA synthesizing enzyme found in presynatic terminals. As our electron microscopic investigations indicate that AP is found at the neuronal membranes and in synaptic contacts, it is proposed that the neuronal AP isoform (NAP), may be a key enzyme in regulating neurotransmission and could therefore play an important role in developmental plasticity and activity-dependent cortical functions [5].

The role of uPAR in the adult brain is unknown. Mice with a targeted mutation of the gene encoding urokinase plasminogen activator receptor (uPAR), a key component in HGF/SF activation and function, have decreased levels of HGF/SF and a 50% reduction in neocortical GABAergic interneurons at embryonic and perinatal ages [6].

- GABA catabolism :

Possible interactions between massive doses of retinoic acid and GABA catabolic enzyme succinic semialdehyde dehydrogenase (SSADH) are unkown.

glutamate decarboxylase (GAD): GAD2 (GAD65) 10p11.23
aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase) ALDH5A1
glutamate dehydrogenase 1 (GLUD1) 10q23.3
-binding sites for AP1, AP2 and SP1

- Conclusions :

A significant interaction with GABA metabolism in human subjects exposed to (Ro)accutane is here suggested. Glutamic acid decarboxylase (GAD) 65 is one of two homologous proteins responsible for the synthesis of gamma-aminobutyric acid and has been shown to be regulated by transcription factor Sp1, which is heavily affected by retinoic acid. Alkaline phosphatase has also shown to modulate GAD. Glutamate is a precursor for GABA. Glutamate formation and transport is heavily affected by (Ro)accutane. Retinoic acid has been found to regulate the urokinase. The role of the urokinase plasminogen activator receptor (uPAR) in the human brain is not fully known.

Interaction may exist with the catabolic pathways of GABA. The succinic semialdehyde dehydrogenase may be affected.

Alteration of GABA metabolism may constitute one pathway to (Ro)accutane induced chemical depression.

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Phospho-dependent binding of the clathrin AP2 adaptor complex to GABAA receptors regulates the efficacy of inhibitory synaptic transmission
(endocytosis | phosphorylation)
Josef T. Kittler , Guojun Chen, Stephan Honing, Yury Bogdanov, Kristina McAinsh, I. Lorena Arancibia-Carcamo, Jasmina N. Jovanovic, Menelas N. Pangalos, Volker Haucke, Zhen Yan, and Stephen J. Moss

The efficacy of synaptic inhibition depends on the number of -aminobutyric acid type A receptors (GABAARs) expressed on the cell surface of neurons. The clathrin adaptor protein 2 (AP2) complex is a critical regulator of GABAAR endocytosis and, hence, surface receptor number. Here, we identify a previously uncharacterized atypical AP2 binding motif conserved within the intracellular domains of all GABAAR subunit isoforms. This AP2 binding motif (KTHLRRRSSQLK in the 3 subunit) incorporates the major sites of serine phosphorylation within receptor subunits, and phosphorylation within this site inhibits AP2 binding. Furthermore, by using surface plasmon resonance, we establish that a peptide (pep3) corresponding to the AP2 binding motif in the GABAAR 3 subunit binds to AP2 with high affinity only when dephosphorylated. Moreover, the pep3 peptide, but not its phosphorylated equivalent (pep3-phos), enhanced the amplitude of miniature inhibitory synaptic current and whole cell GABAAR current. These effects of pep3 on GABAAR current were occluded by inhibitors of dynamin-dependent endocytosis supporting an action of pep3 on GABAAR endocytosis. Therefore phospho-dependent regulation of AP2 binding to GABAARs provides a mechanism to specify receptor cell surface number and the efficacy of inhibitory synaptic transmission.

2.2.4 Neurotransmitter category: Dopa./norephin. :

- (Ro)accutane induced significant interaction with the dopamine beta hydroxylase :

Shared location between the dopamine beta hydroxylase and the retinoid x receptor alpha

Targets of retinoid regulation include dopamine D2 receptor and tyrosine hydroxylase and dopamine â-hydroxylase [6]. The dopamine beta-hydroxylase (DBH), is an enzyme that produces noradrenalin from dopamine [2]. The retinoid X receptor alpha is one of a number of retinoic acid receptors which are members of the steroid/thyroid hormone superfamily. Localization of RXRA was achieved using the polymerase chain reaction on a panel of somatic cell hybrids. A cosmid clone was isolated using the RXRA PCR product, and this was used to further localize the gene by fluorescence in situ hybridization to chromosome 9q34 distal to the dopamine beta hydroxylase gene (DBH) [1]. Since retinoid signalling is required for normal expression of D2 receptors, it is possible that 13-cis-RA has an effect on D2 receptors but this has not been tested [6].

- Transcription through AP-2 :

In adult mammals, AP-2 is expressed in both neural and non-neural tissues. However, the function of AP-2 in different neuronal phenotypes is poorly understood. In this study, transcriptional regulation of tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH) genes by AP-2 was investigated. AP-2 binding sites were identified in the upstream regions of both genes. Exogenous expression of AP-2 robustly transactivated TH and DBH promoter activities in non-catecholaminergic cell lines. While AP-2 regulates the DBH promoter activity via a single site, transactivation of the TH promoter by AP-2 appears to require multiple sites. In support of this, mutation of multiple AP-2 binding sites but not that of single site diminished the basal promoter activity of the TH gene in cell lines that express TH and abolished transactivation by exogenous AP-2 expression in cell lines that do not express TH. In contrast, mutation of a single AP-2 binding site of the DBH gene completely abolished transactivation by AP-2. [3].

- Transcription through angiotensin receptors :

Long-term AT(1) receptor blockade decreased adrenomedullary AT(1) receptor binding, AT(2) receptor binding and AT(2) receptor protein, rat tyrosine hydroxylase (TH) mRNA, norepinephrine (NE) content, Fos-related antigen 2 (Fra-2) protein, phosphorylated cAMP response element binding protein (pCREB), and ERK2 [4].

The dopamine D(2) receptor (D(2)R) is involved in the regulation of acethylcholin (ACh) release in the hippocampus [5].

Location Dopamine receptor D4: 11p15.5 (genatlas)

- Conclusions :

(Ro)accutane is suggested to affect both dopamine production and norephineprine production. There are several pathways where a clear interaction is found. The dopamine beta hydroxylase, the enzyme that converts norephinephrine from dopamine shares location with the retinoid x alpha receptor (RXRalpha). The angiotensin receptor 1 (AT-1) is suggested to be downregulated, which is PPARgamma mediated. AT-1 is suggested to be of importance for norephinephrine syntesis. Finally AP-2 transcription is suggested to be of importance for both the tyrosine hydroxylase and the dopamine beta hydroxylase.

These wide interactions may contribute to one of the pathways of (Ro)accutane induced chemical depression.

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2.2.5 Neurotransmitter category: Melatonin :

- Synthesis of melatonin :

Melatonin, an indoleamine neurohormone that is synthesized mainly in the pineal gland and derived from 5-HT, has many effects on a wide range of physio-pathological functions. Melatonin is synthesized in a pathway in which the first steps involve tryptophan hydroxylation and subsequent decarboxylation. These processes lead to the formation of serotonin (5-HT), which in turn is acetylated on the free amine and then O-methylated on the 5-hydroxyl position. The limiting step of this process is the alkylation of 5-HT, which is catalysed by AANAT. This process takes place mainly in the pineal gland, where AANAT is expressed, despite a recent report suggesting that it is present in other cells, such as mucosal enterochromaffin (EC) cells. Furthermore, melatonin can be synthesized by O-methylation of N-acetyl-5-hydroxytryptamine. This reaction appears to occur in the gut, where high levels of melatonin have been identified, which is not consistent with a pineal production of melatonin. AANAT has been cloned from various species, including humans [1].

Stimulation of melatonin biosynthesis by norepinephrine (NE) depends on the activation of the gene that encodes arylalkylamine N-acetyltransferase (AANAT). Dephosphorylation of pCREB by protein serine/threonine phosphatase (PSPs) is an essential mechanism for downregulation of Aanat transcription in the rat pineal gland [5].

- Receptor expression :

Some of the effects of melatonin are mediated by the interactions of melatonin with the two melatonin MT(1) and MT(2) receptors [1]. Melatonin has been shown to bind to the MT1 G protein-coupled receptor (GPCR) [2].

The two subtypes of retinoid Z receptor (RZR alpha and beta) and the three splicing variants of retinoid orphan receptor (ROR alpha 1, alpha 2, and alpha 3) form a subfamily within the superfamily of nuclear hormone receptors. It was found that the pineal gland hormone melatonin is a natural ligand of RZR alpha and RZR beta. Ligand-induced transcriptional control is therefore proposed to mediate physiological functions of melatonin in the brain where RZR beta is expressed, but also in peripheral tissues, where RZR alpha was found [4].

- Conclusions :

In other species, it has been shown that vitamin A is required to maintain the rhythm of melatonin synthesis [3]. The tryptophan hydroxylase is dependent on normal vitamin A status, since vitamin A interacts with the TPH promoter. 5-HT is necessary for the formation of melatonin. AANAT is the enzyme responsible for the formation of bioactive melatonin, and may also be dependent on vitamin A. It is therefore likely that a clinical vitamin A deficiency also results in a clinical melatonin deficiency.

Melatonin deficiency may constitute one pathway to (Ro)accutane induced chemical depression.

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2.2.6 Neurotransmitter cat.: Acetylch. and other :

- Vitamin A deficiency was found to decrease hippocampal acetylcholine release :

In rats fed with a vitamin A-deficient diet, a significant decrease in hippocampal acetylcholine release induced by scopolamine was found, assessed using microdialysis technique. A reduction in the size of hippocampal nuclei of CA1 region in vitamin A-deficient rats, compared to rats fed with a vitamin A-sufficient diet was also found [1].

- Suggested inhibition of choline acetyltransferase and Butyrylcholinesterase resulting in reduced formation of acetylcholine :

It is shown that ChAT (choline acetyltransferase), an ezyme active in the formation of acetylcholine, activity can be regulated in culture by retinoic acid, an active metabolite of vitamin A, and by sodium butyrate, an organic fatty acid [4]. Butyrylcholinesterase is a serine hydrolase biochemically related to the cholinergic enzyme acetylcholinesterase. It is capable of hydrolyzing esters of choline. Butyrylcholinesterase has unique enzymatic properties and is widely distributed in the nervous system, raising the possibility of its involvement in neural function [3].

Vitamin A and sodium butyrate have two major things in common. 1) They are found to upregulate the ChAT in small doses, which likely, as the butyrylcholinesterase is dependent on a serine hydrolase. 2) Since vitamin A is suggested to bind to serine residues, which possibly also is the case with sodium butyrate, a significant inhibition of the enzymes in larger doses can not be excluded. A possible chemical explanation to this suggested significant inhibition is that the residues get too heavy by the binding of retinoic acid for the hydrolysis to take place at an optimal degree.

Serine 27 of RXRalpha is an unique target for phosphorylation by protein kinase A (PKA) [5]. In the rat pheochromocytoma cell line PC12 The expression of the ChAT and VAChT genes is regulated coordinately at the transcriptional level, and a signaling pathway specifically involving PKA II plays an important role in this process [6].

- Suggested decreased promoter activity of the neural acetylcholine receptor :

The 4 subunit is a component of the neuronal nicotinic acetylcholine receptors which control catecholamine secretion in bovine adrenomedullary chromaffin cells. The promoter of the gene coding for this subunit was characterized. A proximal region (from 99 to 64) was responsible for the transcriptional activity observed in chromaffin, C2C12, and COS cells. Within this region two cis-acting elements that bind transcription factors Sp1 and NF-Y were identified. Mutagenesis of the two elements indicated that they cooperate for the basal transcription activity of the promoter. The human 4 promoter, that was also characterized, shared structural and functional homologies with the bovine promoter. Thus, two adjacent binding elements for Sp1 and NF-Y were detected. Whereas the Sp1 site was an important determinant of the promoter activity, the NF-Y site may have cell-specific effects [7].

- Opioid receptor activity :

Previously, several important cis-elements and trans-factors have been shown to play a functional role in the proximal promoter of mouse micro-opioid receptor (MOR) gene. In this study, we defined another functional element located the in -450 to -400 bp (translational start site designated as +1) region of the proximal promoter, which is also essential for the full promoter activity. It is designated as the morAP-2-like element for its sequence homologous to the consensus AP-2 element. Surprisingly, electrophoretic mobility shift analysis (EMSA) revealed that Sp1 and Sp3, but not AP-2 proteins, were specifically bound to the morAP-2-like element. Mutation of the morAP-2-like element, resulting in a loss of Sp binding, led to an approximately 35% decrease in activity, further confirming the positive role of the morAP-2-like element in MOR gene expression. Dephosphorylation of Sp proteins with alkaline phosphatase also decreased Sp binding to the morAP-2-like element in EMSA, suggesting phosphorylation of Sp is essential for its binding to this element. However, direct or indirect activation of PKA, a classical G-protein coupled signaling pathway, resulted in no significant change of Sp binding to the morAP-2-like element, nor of the promoter activity the SH-SY5Y cells, MOR expressing cells, suggesting that phosphorylation of Sp does not involve PKA. These results suggest that the binding of different phosphorylated forms of Sp proteins to the morAP-2-like element may contribute to the fine tuning of MOR expression in different cells [2].

- Conclusions :

(Ro)accutane is suggested to interact with the release of acethylcholine. A (Ro)accutane induced vitamin A deficiency is suggested to inhibit normal release of hippocampal acethylcholine, as is seen in rats fed with a diet with no vitamin A.

(Ro)accutane is suggested to inhibit the biosynthesis of acetylcholine through a PKA

dependent pathway, involving the excessive binding of serine residues, which slows down the required hydrolysis of choline esters. (Ro)accutane is also suggested to decrease promoter activity of neural acetylcholine receptors through decreased function of transcription factors Sp1 and NF-Y.

This pathway may also constitute one pathway by which a diet rich in butyrate like/forming fats (or unfavorable circulating fatty acid composition) inhibits function of memory, and other coginitive abilities.

(Ro)accutane is suggested to interact with the opioid receptor promoter through transcription factor Sp1 and AP-2 significant interation, and may decrease opioid receptor expression.

These mechanisms may be involved in (Ro)accutane induced chemical depression.

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Phosphorylation of AMPA receptor subunits is differentially regulated by phospholipase A(2) inhibitors.
Menard C, Patenaude C, Massicotte G.
Departement de chimie-biologie, Universite du Quebec a Trois-Rivieres, C.P. 500, Trois-Rivieres, Que., Canada G9A 5H7.

Our laboratory recently discovered that the phosphorylation of subunits forming the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) subtype of glutamate receptors is regulated by constitutive phospholipase A(2) (PLA(2)) activity in rat brain sections. In the present investigation, arachidonyl trifluoromethyl ketone (AACOCF3) and bromoenol

lactone (BEL) were used to compare the influence of calcium-dependent (cPLA(2)) and calcium-independent (iPLA(2)) enzymes on phosphorylation of AMPA and N-methyl-d-aspartate (NMDA) subtypes of glutamate receptors. Incubation of rat brain sections with 3muM BEL enhanced phosphorylation on the serine (Ser) 831 residue of the AMPA receptor GluR1 subunit in synaptosomal P2 fractions, whereas AACOCF3 at the same concentration resulted in increased phosphorylation on residues Ser880/891 of GluR2/3 subunits. These effects were restricted to the AMPA receptor subtype as no changes in phosphorylation were elicited on the NMDA receptor NR1 subunit. The effects of BEL and AACOCF3 were not occluded during blockade of protein phosphatases since AMPA receptor phosphorylation was still apparent in the presence of okadaic acid, indicating that the PLA(2) inhibitor-induced increase in AMPA receptor phosphorylation does not rely on a decrease in dephosphorylation reactions. However, pretreatment of rat brain sections with a cell-permeable protein kinase C (PKC) inhibitor prevented BEL- and AACOCF3-induced phosphorylation on the Ser831 and Ser880/891 sites of GluR1 and GluR2/3 subunits, respectively. These results suggest that constitutive cPLA(2) and iPLA(2) systems may differentially influence AMPA receptor properties and function in the rat brain through mechanisms involving PKC activity.

PMID: 16099093 [PubMed - as supplied by publisher]

Neuroscience. 2002;115(2):475-82. Related Articles, Links
Vitamin A deficiency produces spatial learning and memory impairment in rats.
Cocco S, Diaz G, Stancampiano R, Diana A, Carta M, Curreli R, Sarais L, Fadda F.
Department of Applied Science for Biosystems, Section of Physiology and Human Nutrition, University of Cagliari, Via Porcell 4, Italy.

Vitamin A and its derivatives (retinoids) play important roles in many physiological processes. The recent finding of high levels of cellular retinol-binding protein type 1 immunoreactivity, cellular retinoic acid-binding protein type 1 immunoreactivity and the presence of nuclear retinoid receptors in the central nervous system of adult rodents suggests that retinoids may carry out important roles in the adult brain. In consideration of the role of the hippocampus in spatial learning and memory we evaluated the effect of vitamin A deprivation in adult rats on these functions. Following 12 weeks of vitamin A-free diet, rats were trained to acquire a radial-arm maze task. Results show that this diet induced a severe deficit in the spatial learning and memory task. The cognitive impairment was fully restored when vitamin A was replaced in the diet. We also found a significant decrease in hippocampal acetylcholine release induced by scopolamine, assessed using microdialysis technique, and a reduction in the size of hippocampal nuclei of CA1 region in vitamin-deficient rats, compared to rats fed with a vitamin A-sufficient diet. These results demonstrate that vitamin A has a critical role in the learning and memory processes linked to a proper hippocampal functioning.

PMID: 12421614 [PubMed - indexed for MEDLINE]

Neuron. 2005 May 19;46(4):521-3. Related Articles, Links
An unconventional role of neurotransmission in synapse formation.
Xiong WC, Mei L.
Program of Developmental Neurobiology, Institute of Molecular Medicine and Genetics, Department of Neurology, Medical College of Georgia, Augusta 30912, USA.

How do presynaptic inputs regulate synapse formation? In this issue of Neuron, Lin et al. show that the neurotransmitter acetylcholine decreases the stability of AChR clusters. This dispersing activity, which requires the serine/threonine kinase Cdk5, cooperates with positive signals from motoneurons to ensure high concentration of AChRs at the neuromuscular junction.

Publication Types:
Comment

PMID: 15944118 [PubMed - in process]

Biochem Biophys Res Commun. 2000 Dec 29;279(3):853-7. Related Articles, Links
Serine 27, a human retinoid X receptor alpha residue, phosphorylated by protein kinase A is essential for cyclicAMP-mediated downregulation of RXRalpha function.
Harish S, Ashok MS, Khanam T, Rangarajan PN.
Department of Biochemistry, Indian Institute of Science, Bangalore, 560 012, India.

Retinoid X Receptor alpha (RXRalpha), a member of the steroid-thyroid hormone receptor super family, is phosphorylated in vitro by protein kinase A (PKA) and this phosphorylation is inhibited in presence of PKA inhibitory peptide. Analysis of various deletion mutants of RXRalpha indicate that the amino-terminal A/B domain is the target for PKA phosphorylation. An RXRalpha mutant in which serine residue 27 is mutated to alanine is no longer phosphorylated by PKA. In vivo transfection experiments in COS cells indicate that cyclic AMP represses retinoic acid-mediated transcriptional activation of RXRalpha and this repression is mediated by serine 27. These results indicate that serine 27 of RXRalpha is an unique target for phosphorylation by PKA in vitro and it has an important role in the crosstalk between RXRalpha and cyclic AMP signalling pathways.

PMID: 11162439 [PubMed - indexed for MEDLINE]

Mol Cell Neurosci. 2005 Jun 28; [Epub ahead of print] Related Articles, Links
Muscarinic acetylcholine receptor activation enhances hippocampal neuron excitability and potentiates synaptically evoked Ca(2+) signals via phosphatidylinositol 4,5-bisphosphate depletion.
Young KW, Billups D, Nelson CP, Johnston N, Willets JM, Schell MJ, Challiss RA, Nahorski SR.
Department of Cell Physiology and Pharmacology, Medical Sciences Building, University of Leicester, University Road, Leicester, LE1 9HN, UK.

Using single cell Ca(2+) imaging and whole cell current clamp recordings, this study aimed to identify the signal transduction mechanisms involved in mACh receptor-mediated, enhanced synaptic signaling in primary cultures of hippocampal neurons. Activation of M(1) mACh receptors produced a 2.48 +/- 0.26-fold enhancement of Ca(2+) transients arising from spontaneous synaptic activity in hippocampal neurons. Combined imaging of spontaneous Ca(2+) signals with inositol 1,4,5-trisphosphate (IP(3)) production in single neurons demonstrated that the methacholine (MCh)-mediated enhancement required activated G(q/11)alpha subunits and phospholipase C activity but did not require measurable increases in IP(3). Electrophysiological studies demonstrated that MCh treatment depolarized neurons from -64 +/- 3 to -45 +/- 3 mV and increased action potential generation. Depletion of plasma membrane phosphatidylinositol 4,5-

bisphosphate (PIP(2)) enhanced neuronal excitability and prolonged the action of MCh. These studies suggest that, in addition to producing the second messengers IP(3) and diacylglycerol, mACh receptor activation may directly utilize PIP(2) hydrolysis to regulate neuronal excitability.

PMID: 15996483 [PubMed - as supplied by publisher]

Nat Cell Biol. 2002 May;4(5):329-36. Related Articles, Links
The TRPM7 channel is inactivated by PIP(2) hydrolysis.
Runnels LW, Yue L, Clapham DE.
Howard Hughes Medical Institute, Children's Hospital, Harvard Medical School, Enders 1309, 320 Longwood Avenue, Boston, MA 02115, USA.

TRPM7 (ChaK1, TRP-PLIK, LTRPC7) is a ubiquitous, calcium-permeant ion channel that is unique in being both an ion channel and a serine/threonine kinase. The kinase domain of TRPM7 directly associates with the C2 domain of phospholipase C (PLC). Here, we show that in native cardiac cells and heterologous expression systems, G alpha q-linked receptors or tyrosine kinase receptors that activate PLC potently inhibit channel activity. Numerous experimental approaches demonstrated that phosphatidylinositol 4,5-bisphosphate (PIP(2)), the substrate of PLC, is a key regulator of TRPM7. We conclude that receptor-mediated activation of PLC results in the hydrolysis of localized PIP(2), leading to inactivation of the TRPM7 channel.

PMID: 11941371 [PubMed - indexed for MEDLINE]

Section 3 : Programmed cell death (apoptosis)

3. Introduction :

To complete...

3.1. Programmed cell death (apoptosis) :

To complete...

- References :

Biochim Biophys Acta. 2002 Feb 15;1570(1):9-18. Related Articles, Links
Cytochrome c forms complexes and is partly reduced at interaction with GPI-anchored alkaline phosphatase.
Dadak V, Janiczek O, Vrana O.
Department of Biochemistry, Faculty of Science, Masaryk University, 61137, Brno, Czech Republic.

Cytochrome (cyt) c forms complexes, undergoes a conformational change and becomes

partly reduced at interaction with membrane anchored alkaline phosphatase (AP), a glycoprotein which is released into the body fluid in forms differing in hydrophobicity. The proportion of products formed in the mixtures depends on pH, ionic strength, temperature and the buffer composition. The reaction terminates in an equilibrium between cyt c(FeII) and other cyt c conformers. Optimal conditions for the rate of the reaction are 100 mM glycine/NaOH, pH 9.7-9.9, at which 68-74% of cyt c is found in the reduced state. The interaction affects compactness of the haem cleft as shown by changes induced in CD spectra of the Soret region and changes in optical characteristics of phenylalanine, tyrosine and tryptophan residues. Differential scanning calorimetry of AP+cyt c mixtures revealed a creation of at least two types of complexes. A complex formed by non-coulombic binding prevails at substoichiometric AP/cyt c ratios, at higher ratios more electrostatic attraction is involved and at 1:1 molar ratio an apparent complexity of binding forces occurs. The rapid phase of the cyt c(FeII) formation depends on the presence of the hydrophobic alkylacylphosphoinositol (glycosylphosphatidylinositol) moiety, the protein part of the enzyme participates in an electrostatic and much slower phase of cyt c(FeII) creation. The results show that non-coulombic interaction may participate at interaction of cyt c with cellular proteins.

PMID: 11960683 [PubMed - indexed for MEDLINE]

Cell Death Differ. 2005 Jul 8; [Epub ahead of print] Related Articles, Links
Early mitochondrial alterations in ATRA-induced cell death.
Schmidt-Mende J, Gogvadze V, Hellstrom-Lindberg E, Zhivotovsky B.

[1] 1Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, Box 210, Stockholm SE-171 77, Sweden [2] 2Department of Medicine, Division of Hematology, Karolinska University Hospital Huddinge, Stockholm SE-141 86, Sweden.

All-trans retinoic acid (ATRA) induces differentiation and subsequent apoptosis in a variety of cell lines. Using the myeloid cell line P39, we show that ATRA disturbs mitochondrial functional activity long before any detectable signs of apoptosis occur. These early changes include diminished mitochondrial oxygen consumption, decreased calcium uptake by mitochondria and as a result, a lower mitochondrial matrix calcium concentration. Granulocyte colony-stimulating factor (G-CSF) increases mitochondrial respiration and calcium accumulation capacity and subsequently blocks ATRA-induced apoptosis. Nifedipine, a plasma membrane calcium channel blocker, inhibits apoptosis-related changes, such as the loss of the mitochondrial membrane potential and activation of caspases. Thus, the properties of ATRA and G-CSF to modulate mitochondrial respiration and intracellular calcium control are novel findings, which give insight into their precise molecular mode of action.Cell Death and Differentiation advance online publication, 8 July 2005; doi:10.1038/sj.cdd.4401715.

PMID: 16003389 [PubMed - as supplied by publisher]

3.2. The RAR/RXR receptor pathway :

To complete...

3.3. The "GPI complex pathway" :

To complete...

Section 4 : (Ro)accutane and the immune-defense

4. Introduction :

- Vitamin A extensively linked to various immune-functions :

The (Ro)accutane induced effects on immune function may be classified in two major categories:

1) The direct irreversible effects induced during heavy exposure which is likely to result in among other things deletion of N-glycosylation sites and thus a decreased funtion of the innate immune system

2) The secondary effects on immune function resulting from dyslipidemia, decreased NHR receptor expression and hormonal deficiencies

Vitamin A deficiency is associated with exacerbation of immunodeficiency, reduced or unbalanced levels of lymphocytes, and dysregulated production of antibodies. Animal experiments have shown that an adequate level of vitamin A is necessary to mount an efficient antibody response [1 and more]. Vitamin A status also plays an important role in reducing infectious disease morbidity and mortality by enhancing immunity, an effect that is partly mediated by macrophages [5].

The immune system can roughly be divided into two parts: the innate and adaptive. The innate immune system recognizes and initiates a response, and the adaptive part of the immune system executes the response. The principal immune effector cells of the innate immune system are monocytes/macrophages, dendritic cells (DC), natural killer cells (NK) and NK-T cells. These effector cells recognize pathogen associated molecular patterns, e.g., viral proteins, CpG DNA, or double-stranded viral RNA via a variety of pattern recognition receptors which include toll-like receptors (TLRs), NK cell receptors and mannose binding receptors. These cells then release a variety of proinflammatory cytokines and chemokines, which recruit cells to the site of infection and initiate infection and antiviral immune response. These soluble mediators also activate macrophages, NK cells and DC. Activation of the innate immune defense plays an instructive role (adjuvant effect) for the induction of virus specific, adaptive immune responses [6].

- Recent research on the immune-defense :

Recent research on the immune defense has brought us interestingly close to an

understanding of how (Ro)accutane disrupts immune-function. Altered IgE responses have been observed post exposure in (Ro)accutane exposed animal subjects, with a lack of primary immune response, and interestingly an elevated secondary immune response [2]. The significantly altered IgE responses are suggested to be only a fraction of the alterations in immune-function induced by (Ro)accutane exposure. Many variables are yet to be measured, where significant variations are likely to be found. The result does not point out a lack of possibilty of synthesis of IgE, but rather a failure in the signaling system (a failure of recognition by the receptors which are part of mediating the innate immune response) that recognizes when antibodies are to be synthesized and released.

"Homocysteine is an amino acid that can be generated in response to nutritionally deficient or nutritionally deficient diets. "

"When homocysteine levels increase in the blood, it is linked to massive inflammation and neurodegeneration!
In Alzheimer's disease and many other chronic diseases, we find a significant increase in homocysteine in blood tests. Since homocysteine is a potent excitotoxin and neurotoxin, high levels of homocysteine have been found to exacerbate the symptoms of Alzheimer's disease and other chronic diseases. Components of the metabolic degradation of homocysteine alter the NMDA (N-methyl-D-aspartate) receptor sites, resulting in multiple negative effects, including free radicals and a massive inflammatory cascade!
These free radicals and inflammation can trigger an autoimmune response in which the patient's immune system attacks the thyroid gland and / or other body systems. "

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5120102/

https://www.ncbi.nlm.nih.gov/pubmed/17852421

- IgE receptor subtypes - suggested reduced affinity to LPS after heavy exposure of retinoic acid due to deletion of N-glycosylation sites :

Toll-like receptor 4 (TLR4) belongs to the lipolysaccaride receptor (LPS). Mutants lacking two or more of the other N-glycosylation sites were deficient in interaction with LPS [Genecards database]. In Vero cells exposed to retinoic acid, when control and RA exposed samples were incubated with peptide N-glycosidase F (PNGase F), which removes N-glycosylated sugars, the molecular weights of the respective gB, gC and gD proteins produced were comparable in both the groups, indicating that RA did not alter the primary sequence of viral proteins during protein synthesis or increase viral protein proteolysis. RA treatment increased [3H]mannose incorporation into glycoproteins in HSV infected cells but did not change [3H]glucosamine incorporation. It is concluded that *RA exposure does not reduce the synthesis of three major viral envelope glycoproteins but alters their N-glycosylation and postulate that the inhibitory effect of RA is related to its action on N-glycosylation* [8].

- Interaction between nuclear receptors and Toll-like receptors (TLRs) :

Toll-like receptors (TLRs), play a role in recognizing when an immune response is to be

initiated (innate system) [2, 3]. Possible interactions between TLRs and IgE hyperresponses have been suggested not to exist [4]. However, there is data indicating the opposite. The nuclear receptors are found to interact significantly with the TLRs [5]. The role of TLRs in human immune function is not yet fully understood.

- Recent research leading to an understanding of (Ro)accutane induced alterations in immune-defense :

The function of other receptor types and transcriptional activity important for the immune responses are likely to be significantly altered, due to effects from the exposure and the hormonal deficiencies discovered in exposed subjects. Also, the binding of lipids to these immune-response mediating receptors may possibly alterate their binding affinity, and the significantly observed alterations in lipid profiles observed in (Ro)accutane exposed subjects may in a near future provide an extensive explanation to the induced malfunctions regarding the immune-defense.

- Conclusions :

Vitamin A is in numerous studies extensively linked to immune function. During heavy exposure, retinoic acid is found to have an antiviral effect on at least the herpes simplex virus, but likely also several other viruses. As with some other antiviral agents, this does not depend on interaction with the viral sequence, but altered N-glycosylation thus resulting in inhibition of viral replication.

However, after exposure lack of primary IgE responses with elevated secondary IgE responses are found, indicating a reduced function of the innate immune defense, or the initiation of viral responses. The results found in mice after exposure suggest that (Ro)accutane exposure results in deletion of N-glycosylation sites, and thus a lowered LPS affinity. Subjects exposed to (Ro)accutane are highly likely to be more sensitive to viral action of the herpes simplex virus, but also other viruses sensitive to related mechanisms.

- References :

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(2005) Proc Natl Acad Sci U S A. Aug
[2] **Barnett JB.** *Immunomodulating effects of 13-cis-Retinoic acid on the IgE response of BALB/c mice.*
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[6] **Iannello A, Debbeche O, Martin E, Attalah LH, Samarani S, Ahmad A.** *Viral strategies for evading antiviral cellular immune responses of the host.*

(2005) J Leukoc Biol. Oct 4
[Abstract PubMed]
[8] **Isaacs CE, Xu W, Pullarkat RK, Kascsak R.**
Retinoic acid reduces the yield of herpes simplex virus in Vero cells and alters the N-glycosylation of viral envelope proteins. (2000) Antiviral Res. Jul;47(1):29-40. **[Abstract PubMed]**

4.1. Significant inhibition of IgE :

- Lack of primary IgE response and elevated and sustained secondary IgE responses in subjects exposed to (Ro)accutane :

Repeated studies, in both mice and human subjects show the lack of a primary IgE response with elevated and sustained secondary IgE responses during and after 13-cRA exposure [1 and more]. Unstimulated human peripheral B cells express mRNA of the RA receptor alpha, beta, and gamma. Using retinoids with different receptor binding specificity (CD336, CD437, CD2019, CD367), dose-dependent inhibition of IgE synthesis was shown by all four derivates, but was most marked by an RA binding the alpha receptor with high specificity. Taken together, this study shows that RA inhibits IgE production of anti-CD40 + IL-4-stimulated B cells in vitro [2]. Retinoic acid is also found to modulate CD38 and BCR mediated B-cell proliferation [5].

- IgE receptors :

In horses, the gene Cepsilon is encoding the IgE heavy chain molecule and the gene FcepsilonR1 alpha is coding for the alpha subunit of the IgE receptor molecule [9]. However more isoforms of receptors that bind IgE are known in mammals. The low-affinity immunoglobulin E (IgE) receptor, CD23 (FcepsilonRII), binds both IgE and CD21 and, through these interactions, regulates the synthesis of IgE, the antibody isotype that mediates the allergic response [10].

Induction of an efficient immune-response requires the response of immunocompetent B-cells to the signals from the binding of antigens to the antigen receptor (BCR) as well as other events. Surface molecules such as CD38, CD19 and CD40 are known to function as receptors and costimulators of in the activation of B-cells [5].

CD40 gene expression is regulated by a TATA-less promoter, with Sp1 as a key transcription factor. Two Sp1 binding regions were identified in the mouse CD40 promoter at positions 59 to 50 and 74 to 66. Surprisingly, Sp1-mediated CD40 transcription was reduced following lipopolysaccharide stimulation and was associated with a time-dependent reduction in Sp1 DNA binding activity. This reduction seemed to be mediated by phosphorylation of the Sp1 molecule. CD40 expression in lipopolysaccharide-stimulated cells is up-regulated by NF-B through two distinct sites. One of these sites (128 to 119) was shown to bind p50 and p65 members of the NF-B family, while the other site (562 to 553) bound only p65. Transfectants of p65 were generated using RAW 264 cells, and it was shown that the up-regulation of CD40 mRNA expression was dependent on the presence of the p65 molecule [3].

In mesanglial cells, 10microM ATRA reduced both subunits p50 and p65 of [4]. It is suggested that specific blockade of NF-kappaB activation may be responsible for the

growth arrest and apoptosis of BCR-activated immature B-cells [6]. In mice, pharmacological inhibition of PKC isozymes and Ras revealed that the BCR-induced activation of NF-kappaB requires conventional PKCbeta, whereas that of NFAT may involve non-conventional PKCdelta and Ras pathways [7]. PKC delta, is activated during RA exposure in the NB-4 and HL-60 acute myeloid leukemia cell lines as well as the MCF-7 breast cancer cell line [8].

Similiar effects on B-cells are highly likely, as well as dose dependent possible inhibition of PKC delta.

- Conclusions :

To complete...

- References :

[1] **Barnett JB.** *Immunomodulating effects of 13-cis-Retinoic acid on the IgE response of BALB/c mice.* ***PubMed Abstract***
(1982) Int Arch Allergy Appl Immunol. 69(4):368-73.
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Retinoic acid inhibits CD40 + interleukin-4-mediated IgE production in vitro.
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[10] **The structure of human CD23 and its interactions with IgE and CD21.** *Hibbert RG, Teriete P, Grundy GJ, Beavil RL, Reljic R, Holers VM, Hannan JP, Sutton BJ, Gould HJ, McDonnell JM.* (2005) J Exp Med. Sep 19;202(6):751-60.

4.2. GlcNAc-transferase :

- Glycosyltransferases :

Carbohydrate structures are mainly determined by glycosyltransferases and glycosidases. Since the synthesis of oligosaccharides requires one enzyme for one glycosidic linkage, more than 100 kinds of glycosyltransferases seem to exist [1]. O-GlcNAc is an ubiquitous post-translational protein modification consisting of a single N-acetlyglucosamine moiety linked to serine or threonine residues on nuclear and cytoplasmic proteins. O-GlcNAc regulates cellular responses to hormones such as insulin, initiates a protective response to stress, modulates a cell's capacity to grow and divide, and regulates gene transcription. O-GlcNAc rapidly cycles on and off proteins on a time scale similar to that for phosphorylation/ dephosphorylation. O-GlcNAc is similar to a protein phosphorylation in that both modifications are found on serine and threonine residues, both are dynamically added and removed from proteins in response to cellular signals, and both alter the functions and associations of the modified protein. Many phosphorylation sites are also known *glycosylation sites*. However, the view that O-GlcNAc is simply reciprocal to phosphorylation is an overly simplistic model, since several proteins can be concomitantly phosphorylated and O-GlcNAcylated [2].

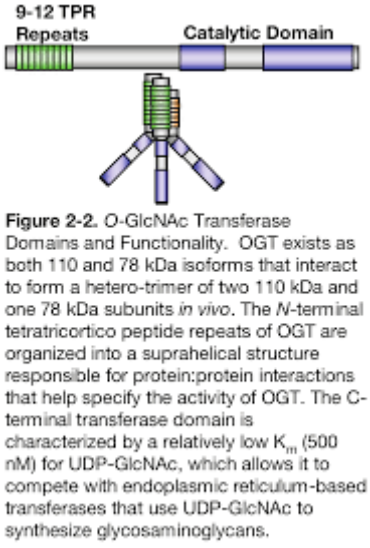

Figure 2-2. *O*-GlcNAc Transferase Domains and Functionality. OGT exists as both 110 and 78 kDa isoforms that interact to form a hetero-trimer of two 110 kDa and one 78 kDa subunits *in vivo*. The *N*-terminal tetratricortico peptide repeats of OGT are organized into a suprahelical structure responsible for protein:protein interactions that help specify the activity of OGT. The C-terminal transferase domain is characterized by a relatively low K_m (500 nM) for UDP-GlcNAc, which allows it to compete with endoplasmic reticulum-based transferases that use UDP-GlcNAc to synthesize glycosaminoglycans.

Figure 3. Slawson et al. (2005) *O-GlcNAc cycling: How a single sugar post-translational modification is changing the way we think about*

signaling networks

O-GlcNAc is dynamically added to and removed from serine and threonine residues by O-GlcNAc transferase (OGT) and O-GlcNAcase, respectively. The human OGT trimer consists of tetratricopeptide repeats (TPR) as well as the transferase domain. O-GlcNAcase consists of a -N-acetylglucosaminidase domain and a HAT domain with a caspase-3 cleavage site between them.

- Significant effects on GlcNAc in cells exposed to retinoic acid :

Normal hepatocytes are characterized by very low level of GlcNAc-transferase-V activity whereas hepatoma cells contained high activities. Hepatoma cells exposed to retinoic acid and 1alpha,2,5-dihydroxyvitamin D(3) (Vit-D(3)) resulted in an increase in GlcNAc-transferase-V activity [1].

- Relation between glycosyltransferases and transcription factor Sp1 :

Transcription factor Sp1 is O-glycosylated and contains N-acetylglucosamine side chains [Genecards database]. Sp1 is partially inhibited by O-linkage to glucosamine [GenAtlas]. O-glycosylation (O-GlcNAcylation), is the addition of single O-linked N-acetylglucosamine (O-GlcNAc) monosaccharides to serine or threonine residues [3]. O-GlcNAcylation of a chimeric transcriptional activator containing the second activation domain of Sp1 decreases its transcriptional activity both in an in vitro transcription system and in living cells, which is in concert with the observation that *O-GlcNAcylation of Sp1 activation domain blocks its in vitro and in vivo interactions with other Sp1 molecules and TATA-binding protein-associated factor II 110* [4].

In glomerular mesangial cells, decreasing O-GlcNAcylation by these means inhibited the ability of high glucose (HG) to increase endogenous PAI-1 mRNA and protein levels, the activity of a PAI-1 promoter-luciferase reporter gene, and Sp1 transcriptional activation [3].

- References :

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[Full Text PNAS]

- CXCL16

Int J Mol Med. 2005 Oct;16(4):661-665. Related Articles, Links
All-trans retinoic acid regulates CXCL16/SR-PSOX expression.
Wagsater D, Shelikine Y, Sirsjo A.
Division of Biomedicine, Department of Caring Sciences, University of Orebro, Orebro SE-701 82, Sweden.

Several studies have shown the ability of retinoids to modulate inflammatory response. CXCL16/SR-PSOX is a novel protein functioning as a chemokine and a scavenger receptor. We investigated effects of all-trans retinoic acid (atRA) on CXCL16/SR-PSOX expression in several cell types. Real-time PCR showed that atRA increased CXCL16/SR-PSOX mRNA expression in THP-1 and endothelial cells, which corresponded to increased release of CXCL16 protein from the cells, measured by ELISA. In THP-1 cells this effect was reduced by retinoic acid receptor (RAR) antagonist, which indicates receptor-mediated inhibition. RAR-alpha and RAR-gamma agonists increased CXCL16 release, which suggests RAR-mediated effect of atRA, which is not selective for a particular RAR subtype. In smooth muscle cells, up-regulation of CXCL16 mRNA was observed only after 96 h of treatment, while protein expression did not change. These findings suggest that retinoid signaling might be a pathway modulating inflammatory response by regulating CXCL16 expression in a cell-specific manner.

PMID: 16142401 [PubMed - as supplied by publisher]

-Toll-like-receptors (TLR) and macrophage activation :

Thirteen TLRs have been described to date, each which recognize specific pathogen-associated molecular patterns [2]. Macrophages recognize the presence of infection by using the Toll-like receptor (TLR) family of proteins that detect ligands on bacterial, viral, and fungal pathogens. TLR signaling, through the MyD88 adaptor, up-regulates transcription of the retinoic acid early inducible-1 (RAE-1) family of NKG2D ligands, but not H-60 or murine UL16-binding protein-like transcript-1 [1]. Mast cells mediate both IgE-dependent allergic reactions and protective responses against acute infections possibly through the activation of Toll-like receptors (TLRs). Antigen interacts synergistically with TLR2 and TLR4 ligands to markedly enhance production of cytokines in murine mast cell lines. The suggested regulation of cytokine production could be attributed to synergistic activation of mitogen activated protein kinases in addition to the engagement of a more effective repertoire of transcription factors for cytokine gene transcription. The synergistic interactions of TLR ligands and antigen might have relevance to the exacerbation of IgE-mediated allergic diseases by infectious agents [2].

While the Toll-like receptor (TLR) system is innate immunity's sensor of infectious danger, macrophages receive activating as well as inhibitory signals via the Jak-Stat pathway. IFNgamma is key to the control of infection particularly with intracellular pathogens and depends on functional Stat1 signal transduction. Stat3 signalling is activated by a range of cytokines, including IL-10, IL-6 and IL-27 [3].

-Significant interaction between the nuclear receptor family and the toll like receptors :

Cell. 2005 Sep 9;122(5):707-21. Related Articles, Links
Molecular Determinants of Crosstalk between Nuclear Receptors and Toll-like Receptors.
Ogawa S, Lozach J, Benner C, Pascual G, Tangirala RK, Westin S, Hoffmann A, Subramaniam S, David M, Rosenfeld MG, Glass CK.
Department of Cellular and Molecular Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093.

Nuclear receptors (NRs) repress transcriptional responses to diverse signaling pathways as an essential aspect of their biological activities, but mechanisms determining the specificity and functional consequences of transrepression remain poorly understood. Here, we report signal- and gene-specific repression of transcriptional responses initiated by engagement of toll-like receptors (TLR) 3, 4, and 9 in macrophages. The glucocorticoid receptor (GR) represses a large set of functionally related inflammatory response genes by disrupting p65/interferon regulatory factor (IRF) complexes required for TLR4- or TLR9-dependent, but not TLR3-dependent, transcriptional activation. This mechanism requires signaling through MyD88 and enables the GR to differentially regulate pathogen-specific programs of gene expression. PPARgamma and LXRs repress overlapping transcriptional targets by p65/IRF3-independent mechanisms and cooperate with the GR to synergistically transrepress distinct subsets of TLR-responsive genes. These findings reveal combinatorial control of homeostasis and immune responses by nuclear receptors and suggest new approaches for treatment of inflammatory diseases.

PMID: 16143103 [PubMed - in process]

Keio J Med. 2004 Jun;53(2):78-84. Related Articles, Links
Osteoclasts, mononuclear phagocytes, and c-Fos: new insight into osteoimmunology.
Matsuo K, Ray N.
Department of Microbiology and Immunology, School of Medicine, Keio University, Tokyo, Japan. matsuo@sc.itc.keio.ac.jp

Osteoimmunology is the emerging concept that certain molecules link the skeletal and immune systems. The transcription factor c-Fos, a component of activator protein-1 (AP-1), is essential for osteoclast differentiation. Mice lacking c-Fos are osteopetrotic owing to impaired osteoclast development. Recent studies suggest that in contrast to this positive role in osteoclastogenesis, c-Fos expression inhibits differentiation and activation of mononuclear phagocytes. Here, we focus on the contrasting roles of c-Fos in the bone and immune lineages. Both osteoclasts and mononuclear phagocytes are derived from common myeloid precursors. Osteoclasts resorb bone, whereas macrophages and myeloid dendritic cells phagocytose microbial pathogens, initiating innate and adaptive immunity. Differentiation of the common precursors into either bone or immune lineage is determined by ligand binding to cell-surface receptors, particularly receptor activator of NF-kappa B (RANK) for osteoclasts, or Toll-like receptors (TLRs) for mononuclear phagocytes. Both RANK and TLRs activate the dimeric transcription factors NF-kappa B and AP-1. Yet, c-Fos/AP-1 plays a positive role in osteoclasts but a negative role in macrophages and dendritic cells. Further study is necessary to clarify this dual role of c-Fos.

Publication Types:
Review

Review, Tutorial

PMID: 15247511 [PubMed - indexed for MEDLINE]

Mol Cell. 2003 Oct;12(4):805-16. Related Articles, Links
Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism.
Castrillo A, Joseph SB, Vaidya SA, Haberland M, Fogelman AM, Cheng G, Tontonoz P.
Howard Hughes Medical Institute, University of California, Los Angeles, Los Angeles, CA 90095, USA.

The liver X receptors (LXR) alpha and beta are regulators of cholesterol metabolism and determinants of atherosclerosis susceptibility. Viral and bacterial pathogens have long been suspected to be modulators of atherogenesis; however, mechanisms linking innate immunity to cholesterol metabolism are poorly defined. We demonstrate here that pathogens interfere with macrophage cholesterol metabolism through inhibition of the LXR signaling pathway. Activation of Toll-like receptors (TLR) 3 and 4 by microbial ligands blocks the induction of LXR target genes including ABCA1 in cultured macrophages as well as in aortic tissue in vivo. As a consequence of these transcriptional effects, TLR3/4 ligands strongly inhibit cholesterol efflux from macrophages. Crosstalk between LXR and TLR signaling is mediated by IRF3, a specific effector of TLR3/4 that inhibits the transcriptional activity of LXR on its target promoters. These findings highlight a common mechanism whereby bacterial and viral pathogens may modulate macrophage cholesterol metabolism and cardiovascular disease.

PMID: 14580333 [PubMed - indexed for MEDLINE]

Dev Cell. 2003 Nov;5(5):666-8. Related Articles, Links
Do macrophage innate immune receptors enhance atherogenesis?
Gordon S.
Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, England.

Macrophages play a central role in both innate immunity to infection and atherosclerosis. Castrillo and colleagues report that selected microbial agonists for Toll-like receptors strongly inhibit LXR-mediated cholesterol efflux from macrophages. TLR-LXR crosstalk could explain how nonspecific microbial infections promote atherogenesis.

PMID: 14602065 [PubMed - indexed for MEDLINE]

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Cutting edge: Toll-like receptor signaling in macrophages induces ligands for the NKG2D receptor.
(2004) J Immunol. Feb 15;172(4):2001-5.
[2] **Qiao H, Andrade MV, Lisboa FA, Morgan K, Beaven MA.** *Fc{epsilon}RI and Toll-Like Receptors Mediate Synergistic Signals to Markedly Augment Production of Inflammatory Cytokines in Murine Mast Cells.* (2005) Blood. Sep

[3] **Lang R.** *Tuning of macrophage responses by Stat3-inducing cytokines: molecular mechanisms and consequences in infection.* (2005) Immunobiology. 210(2-4):63-76.

Innate immune recognition of nucleic acids: Beyond toll-like receptors
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Keywords
Innate immunity • DNA • RNA • CpG motif • TLR • dsRNA

Abstract
During infection or tissue damage, the innate immune system detects and responds to nucleic acids released from pathogens or damaged host cells. Accumulating evidence has showed that specific sequences, modifications or structures of nucleic acids influence their immunomodulatory activities. Resulting innate immune modulations are regulated by Toll-like receptor (TLR)-dependent or -independent signaling pathways. The first step in host defense against foreign or unwelcome self nucleic acids may play important roles in immune responses against infectious organisms, as well as in clearance of unnecessary tissues, which may be linked to autoimmune diseases and possibly to other immunological disorders. Elucidating mechanisms of innate immune activation by nucleic acids will help future development of more efficient or safer nucleic acid-based immunotherapies and gene therapies. © 2005 Wiley-Liss, Inc.

- hCap18 :

Curr Issues Mol Biol. 2005 Jul;7(2):179-96. Related Articles, Links
The role of cathelicidins in the innate host defenses of mammals.
Zanetti M.
Department of Biomedical Sciences and Technology, University of Udine, 1-33100 Udine, Italy. zanetti@icgeb.trieste.it

The cathelicidin peptides comprise one of several families of antimicrobial peptides that are found in neutrophils and epithelia as components of the early host defenses of mammals against infection. All cathelicidin family members are synthesized and stored in cells as two-domain proteins. These are split on demand to produce a cathelin protein and an antimicrobial peptide. Accumulating evidence indicates that both the cathelin portion and the C-terminal peptide exert biological activities connected with host protection. This review presents an overview of the structure and biology of cathelicidins and discusses recent progress in cathelicidin research with emphasis on the functional properties and

role in host defense of the human cathelicidin hCAP18/LL-37. Although investigators initially concentrated their attention on antibiotic activity, it is becoming clear now that LL-37 is a multifunctional molecule that may mediate various host responses, and thus represents an essential component of the innate immune system in humans.

PMID: 16053249 [PubMed - in process]

- Production of cytokines and cytokine receptors :

To complete...

- References :

J Immunol. 2004 Nov 1;173(9):5343-8. Related Articles, Links
Mechanisms of soluble cytokine receptor generation.
Levine SJ.
Pulmonary-Critical Care Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA. levines@nhlbi.nih.gov

Soluble cytokine receptors regulate inflammatory and immune events by functioning as agonists or antagonists of cytokine signaling. As such, they act within complex receptor systems that include signaling receptors, nonsignaling decoy receptors, receptor-associated proteins, and soluble receptor antagonists. Soluble cytokine receptors can be generated by several mechanisms, which include proteolytic cleavage of receptor ectodomains, alternative splicing of mRNA transcripts, transcription of distinct genes that encode soluble cytokine-binding proteins, release of full-length receptors within the context of exosome-like vesicles, and cleavage of GPI-anchored receptors. Furthermore, the important role of soluble cytokine receptors in regulating host defense mechanisms is evidenced by viruses that encode soluble homologues of mammalian receptors and thereby evade innate host immune responses via the sequestration of essential cytokines.

Publication Types:
Review
Review, Tutorial

PMID: 15494479 [PubMed - indexed for MEDLINE]

Glia. 2005 Apr 1;50(1):21-31. Related Articles, Links
Retinoic acid inhibits expression of TNF-alpha and iNOS in activated rat microglia.
Dheen ST, Jun Y, Yan Z, Tay SS, Ling EA.
Department of Anatomy, Molecular Neurobiology Laboratory, Faculty of Medicine, National University of Singapore, Singapore. antstd@nus.edu.sg

The release of proinflammatory mediators such as tumor necrosis factor-alpha (TNF-alpha) and nitric oxide by microglia has been implicated in neurotoxicity in chronic neurodegenerative diseases such as Alzheimer's disease. As all-trans-retinoic acid (RA) has been reported to exert anti-inflammatory actions in various cell types, we have

examined its effects on the expression of TNF-alpha and inducible nitric oxide synthase (iNOS) in microglia activated by beta-amyloid peptide (Abeta) and lipopolysaccharide (LPS). Exposure of primary cultures of rat microglial cells to Abeta or LPS stimulated the mRNA expression level of TNF-alpha (6-116-fold) and iNOS (8-500-fold) significantly. RA acted in a dose-dependent manner (0.1-10 microM) by attenuating both TNF-alpha (29-97%) and iNOS (61-96%) mRNA expression in microglia exposed to Abeta or LPS. RA-induced inhibition of TNF-alpha and iNOS mRNA expression in activated microglia was accompanied by the concomitant reduction in release of iNOS and TNF-alpha proteins as revealed by nitrite assay and ELISA, respectively. The anti-inflammatory effects of RA were correlated with the enhanced expression of retinoic acid receptor-beta, and transforming growth factor-beta1 as well as the inhibition of NF-kappaB translocation. These results suggest that RA may inhibit the neurotoxic effect of activated microglia by suppressing the production of inflammatory cytokines and cytotoxic molecules. 2004 Wiley-Liss, Inc.

PMID: 15602748 [PubMed - indexed for MEDLINE]

Mutat Res. 2004 Jul 13;551(1-2):199-211. Related Articles, Links
Molecular imaging of the transcription factor NF-kappaB, a primary regulator of stress response.
Carlsen H, Alexander G, Austenaa LM, Ebihara K, Blomhoff R.
Department of Nutrition, Faculty of Medicine, University of Oslo, P.O. Box 1046 Blindern, N-0316 Oslo, Norway.

A wide range of environmental stress and human disorders involves inappropriate regulation of NF-kappaB, including cancers and numerous inflammatory conditions. We have developed transgenic mice that express luciferase under the control of NF-kappaB, enabling real-time non-invasive imaging of NF-kappaB activity in intact animals. We show that, in the absence of stimulation, strong, intrinsic luminescence is evident in lymph nodes in the neck region, thymus, and Peyer's patches. Treating mice with stressors, such as TNF-alpha, IL-1alpha, or lipopolysaccharide (LPS) increases the luminescence in a tissue-specific manner, with the strongest activity observable in the skin, lungs, spleen, Peyer's patches, and the wall of the small intestine. Liver, kidney, heart, muscle, and adipose tissue exhibit less intense activities. Exposure of the skin to a low dose of UV-B radiation increases luminescence in the exposed areas. In ocular experiments, LPS- and TNF-alpha injected NF-kappaB-luciferase transgenic mice exhibit a 20-40-fold increase in lens NF-kappaB activity, similar to other LPS- and TNF-alpha-responsive organs. Peak NF-kappaB activity occurs 6h after injection of TNF-alpha and 12h after injection of LPS. Peak activities occur, respectively, 3 and 6h later than that in other tissues. Mice exposed to 360J/m(2) of UV-B exhibit a 16-fold increase in NF-kappaB activity 6h after exposure, characteristically similar to TNF-alpha-exposed mice. Thus, in NF-kappaB-luciferase transgenic mice, NF-kappaB activity also occurs in lens epithelial tissue and is activated when the intact mouse is exposed to classical stressors. Furthermore, as revealed by real-time non-invasive imaging, induction of chronic inflammation resembling rheumatoid arthritis produces strong NF-kappaB activity in the affected joints. Finally, we have used the model to demonstrate NF-kappaB regulation by manipulating the Vitamin A status in mice. NF-kappaB activity is elevated in mice fed a Vitamin A deficient (VAD) diet, and suppressed by surplus doses of retinoic acid (RA). We thus demonstrate the development and use of a versatile model for monitoring NF-kappaB activation both in tissue homogenates and in intact animals after the use of classical activators, during disease

progression and after dietary intervention.

Publication Types:
Review

PMID: 15225593 [PubMed - indexed for MEDLINE]

Biochem Biophys Res Commun. 2000 Feb 16;268(2):255-61. Related Articles, Links
Evidence for translational repression of the SOCS-1 major open reading frame by an upstream open reading frame.
Schluter G, Boinska D, Nieman-Seyde SC.
Institute of Human Genetics, University of Gottingen, Heinrich-Duker-Weg 12, Gottingen, 37073, Germany. gschlue@gwdg.de

The suppressor of cytokine signalling 1 protein (SOCS-1) belongs to a novel family of cytokine inducible factors which function as inhibitors of the JAK/STAT pathway. While SOCS-1 previously has been described as a single-exon gene, here we present evidence for an additional 5' exon, separated by a 509 bp intron from exon 2. Exon 1 and part of exon 2 contain an open reading frame of 115 nt, ending one nucleotide upstream of the major reading frame. Using SOCS-1-promoter/luciferase constructs, we investigated which sequences are involved in the regulation of SOCS-1 expression. Serial promoter deletion clones indicate the localization and functionality of SP1, interferon-stimulated responsive elements (ISRE), and interferon-gamma-activated sites (GAS) promoter elements in the SOCS-1 5' flanking region. We present evidence that the upstream open reading frame (uORF) represses the translation of the downstream major open reading frame (mORF). Mutating the start codon of the uORF relieves this repression. Our data indicate that expression of the SOCS-1 protein is repressed on translational level by a mechanism, which bears similarities to that postulated for genes like retinoic acid receptor beta2 (RARbeta2), S-adenosylmethionine-decarboxylase (AdoMetDC), Bcl-2, and others.

PMID: 10679190 [PubMed - indexed for MEDLINE]

Proc Natl Acad Sci U S A. 2004 Jul 13;101(28):10422-7. Epub 2004 Jul 6. Related Articles, Links
Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse.
Ueki K, Kondo T, Tseng YH, Kahn CR.
Research Division, Joslin Diabetes Center and Department of Medicine, Harvard Medical School, Boston, MA 02215, USA.

Insulin resistance, obesity, diabetes, dyslipidemia, and nonalcoholic fatty liver are components of the metabolic syndrome, a disease complex that is increasing at epidemic rates in westernized countries. Although proinflammatory cytokines have been suggested to contribute to the development of these disorders, the molecular mechanism is poorly understood. Here we show that overexpression of suppressors of cytokine signaling (SOCS)-1 and SOCS-3 in liver causes insulin resistance and an increase in the key regulator of fatty acid synthesis in liver, sterol regulatory element-binding protein (SREBP)-1c. Conversely, inhibition of SOCS-1 and -3 in obese diabetic mice improves insulin

sensitivity, normalizes the increased expression of SREBP-1c, and dramatically ameliorates hepatic steatosis and hypertriglyceridemia. In obese animals, increased SOCS proteins enhance SREBP-1c expression by antagonizing STAT3-mediated inhibition of SREBP-1c promoter activity. Thus, SOCS proteins play an important role in pathogenesis of the metabolic syndrome by concordantly modulating insulin signaling and cytokine signaling.

PMID: 15240880 [PubMed - indexed for MEDLINE]

- ICAM-3 :

J Leukoc Biol. 2001 Mar;69(3):361-72. Related Articles, Links
Retinoic acid up-regulates myeloid ICAM-3 expression and function in a cell-specific fashion--evidence for retinoid signaling pathways in the mast cell lineage.
Babina M, Mammeri K, Henz BM.

Department of Dermatology, Charite, Humboldt-Universitat zu Berlin, Germany. magda.babina@charite.de

Investigation of mast cell responsiveness toward retinoic acid (RA) revealed selective promotion of ICAM-3 expression in the human mast cell line HMC-1. This process was dose- and time-dependent and detectable by flow cytometry, Western blot analysis, ELISA, and Northern blot analysis. ICAM-3 modulation was found to be cell-type dependent, detectable also for HL-60 cells and monocytes but not U-937 and only weakly for KU812 cells. Terminally differentiated skin mast cells also failed to up-modulate their ICAM-3, suggesting the requirement for some degree of immaturity for the process. RA-mediated effects on ICAM-1 expression, studied in parallel, were clearly distinct from those on ICAM-3. Investigation of retinoid receptor expression, known to mediate intracellular RA signaling, revealed presence of RAR alpha, RAR gamma, RXR beta, and RXR gamma transcripts in all cell lines studied, and HMC-1 cells were the only line lacking RXR alpha. RAR beta, not expressed at baseline, was induced by RA in a fashion obviously correlating with ICAM-3 up-regulation. Increased ICAM-3 expression was of functional significance, such that processes stimulated or co-stimulated via ICAM-3 (homotypic aggregation, IL-8 secretion) were clearly enhanced upon RA pretreatment, suggesting that RA may contribute via hitherto unrecognized pathways to immune function and host defense.

PMID: 11261782 [PubMed - indexed for MEDLINE]

- Integrins :

Eur J Immunol. 2003 Mar;33(3):616-25. Related Articles, Links
All-trans retinoic acid down-regulates expression and function of beta2 integrins by human monocytes: opposite effects on monocytic cell lines.
Babina M, Henz BM.
Department of Dermatology, Charite, Campus Mitte, Humboldt-Universitat zu Berlin, Schumannstrasse 20-21, D-10117 Berlin, Germany. magda.babina@charite.de

All-trans retinoic acid (ATRA) plays an important role in the differentiation of malignant myeloid cells but its effects on primary leukocytes have been poorly investigated. We report here that ATRA negatively affects expression and function of leukocyte integrins that play a key role in monocyte adhesive interactions. As evidenced by flow cytometry, ATRA (at 1 microM) clearly and donor-independently suppressed the expression of all integrin chains investigated (CD11a, CD11b, CD11c, and CD18), most strikingly of CD11a. Down-regulation was detectable after 24 and maximal after 72-96 h. Reverse transcription-PCR analysis revealed diminished steady-state concentrations of alpha specific transcripts but not of the common beta chain, suggesting that heterodimer expression was predominantly regulated through alpha chains. Results obtained with blood-derived monocytes were in sharp contrast to those for the leukemic cell lines THP-1 and U937, both of which showed marked increase in all integrin subunits in response to ATRA. ATRA-pretreated monocytes displayed significantly diminished beta(2) integrin-dependent homotypic aggregation, and adhesion to stimulated endothelial cells (EC), while ATRA-pretreated monocytic cell lines showed the opposite behavior displaying markedly enhanced aggregation and CD18-mediated adhesion to EC. Therefore, the level of leukocyte integrins was obviously a decisive factor for these adhesive interactions irrespective of the cellular source. Collectively, our data indicate a striking difference between leukemic cell lines and normal hematopoietic cells with regard to ATRA responsiveness. By acting on key adhesive structures of normal leukocytes, ATRA mediates processes that may be of substantially broad range applying to inflammation and immunity in addition to differentiation and proliferation.

PMID: 12616482 [PubMed - indexed for MEDLINE]

Endocr J. 1997 Jun;44(3):375-81. Related Articles, Links
Role of collagen in retinoic acid-induced differentiation and down-regulation of TGF-beta receptors in rat preosteoblastic RCT-1 cells.
Kodama Y, Takeuchi Y, Kikuchi T, Kurokawa T, Fujita T, Matsumoto T.
Department of Orthopedic Surgery, University of Tokyo School of Medicine, Japan.

Retinoic acid induces differentiation of preosteoblastic cells. We have demonstrated that osteoblastic differentiation and down-regulation of transforming growth factor (TGF)-beta receptors requires the interaction of type I collagen with alpha 2 beta 1 integrin (J Biol Chem 271: 3938-3944, 1996). The purpose of this study was to clarify the role of collagen in retinoic acid-induced differentiation and down-regulation of TGF-beta receptors using preosteoblastic RCT-1 cells. Retinoic acid enhanced the expression of alkaline phosphatase and type I collagen, and reduced TGF-beta receptors in these cells. Inhibiting collagen synthesis abolished these changes. Because TGF-beta inhibits osteoblastic differentiation, the changes described here may contribute to the osteoblastic differentiation by retinoic acid.

PMID: 9279512 [PubMed - indexed for MEDLINE]

- References :

To complete...

- CREB AP-1 IL-6 :

Mol Cell Endocrinol. 2003 Mar 28;201(1-2):47-56. Related Articles, Links
Transcriptional regulation of interleukin-6 in pituitary folliculo-stellate TtT/GF cells.
Nagashima AC, Giacomini D, Castro CP, Pereda MP, Renner U, Stalla GK, Arzt E.
Laboratorio de Fisiologia y Biologia Molecular, Departmento de Fisiologia y Biologia Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, PabellonII, Argentina.

Interleukin-6 (IL-6) secreted by pituitary folliculo stellate (FS) cells plays an important role in the control of pituitary function and proliferation. We demonstrate that in FS TtT/GF cells, estradiol (E(2)) inhibits dose dependently pituitary adenylate cyclase activating polypeptide (PACAP)-stimulated IL-6 secretion and transcription. We studied transcription factors involved in IL-6 stimulation by PACAP. Point mutations in kappaB, TRE, NF-IL-6 and CRE sites in the IL-6 promoter show that PACAP stimulates IL-6 through TRE and CRE sites. Accordingly, PACAP stimulated AP-1 and CREB transcriptional activity and E(2) inhibited TRE-LUC but not CRE-LUC activation. Thus, we demonstrate that transcription factors of the CREB and AP-1 family are critical for the stimulation of IL-6 by PACAP in TtT/GF cells and that estrogens block this stimulation by inhibiting AP-1 activity. The regulatory elements involved in IL-6 transcription in TtT/GF FS cells contribute to understand the specificity of the anterior pituitary gland paracrine pathways.

PMID: 12706293 [PubMed - indexed for MEDLINE]

- MAVS :

Cell. 2005 Sep 9;122(5):669-82. Related Articles, Links
Identification and Characterization of MAVS, a Mitochondrial Antiviral Signaling Protein that Activates NF-kappaB and IRF3.
Seth RB, Sun L, Ea CK, Chen ZJ.

Viral infection triggers host innate immune responses through activation of the transcription factors NF-kappaB and IRF3, which coordinately regulate the expression of type-I interferons such as interferon-beta (IFN-beta). Herein, we report the identification of a novel protein termed MAVS (mitochondrial antiviral signaling), which mediates the activation of NF-kappaB and IRF3 in response to viral infection. Silencing of MAVS expression through RNA interference abolishes the activation of NF-kappaB and IRF3 by viruses, thereby permitting viral replication. Conversely, overexpression of MAVS induces the expression of IFN-beta through activation of NF-kappaB and IRF3, thus boosting antiviral immunity. Epistasis experiments show that MAVS is required for the phosphorylation of IRF3 and IkappaB and functions downstream of RIG-I, an intracellular receptor for viral RNA. MAVS contains an N-terminal CARD-like domain and a C-terminal transmembrane domain, both of which are essential for MAVS signaling. The transmembrane domain targets MAVS to the mitochondria, implicating a new role of mitochondria in innate immunity.

PMID: 16125763 [PubMed - in process]

Section 5a : (Side)-effects in subjects exposed to retinoic acid and their suggested causes (A-Z)

Introduction :

- General effects decreased metabolism in all studied acne subjects after four monts of (Ro)accutane exposure :

A strong degeneration of several parts of the body and brain has been found in all examined subjects exposed to (Ro)accutane, whereas it is highly likely that these effects are general. Significantly decreased metabolism in the orbitofrontal cortex was found in *all human subjects examined* after exposure to (Ro)accutane (-21%) [1].

Figure 1. Bremner JD et al. *Functional brain imaging alterations in acne patients treated with isotretinoin.* (2005) Am J Psychiatry. May;162(5):983-91.

- Doubled oxidative stress measured by 8-OHdG allready at half the maximum dose in acne-subjects :

Human subjects with CA (n=18) were evaluated before and 45 days after Iso (0.5mg/kg per day) exposure and non-diseased controls (n=22) were tested only once. Plasma TAS levels and 8-OHdG were measured spectrophotometrically and with an immunoassay, respectively. Liver biochemical parameters and muscle enzymes were measured on a blood chemistry analyzer. Results: TAS levels were significantly ($p<0.0001$) lower in patients before treatment (921+/-124 mumol/L) compared with those after treatment (1335+/-93 mumol/L) and in controls (1536+/-126 mumol/L). In contrast, 8-OHdG serum levels were two-fold higher in patients after exposure (0.21+/-0.03 ng/mL) than before exposure (0.11+/-0.02 ng/mL) and three-fold higher than in controls (0.07+/-0.01 ng/mL; $p<0.0001$). Negative correlations were found between TAS and 8-OHdG ($r=-0.754$, $p<0.0001$) in patients before therapy and positive correlations were found between creatine kinase (CK) and 8-OHdG ($r=0.488$, $p<0.001$) and liver enzymes after isotretinoine exposure [4].

- Degeneration of bone mass in all exposed human acne subjects :

A degeneration of bone mass was seen in all human subjects examined that were exposed to the toxin. Bone density at the Ward triangle decreased a mean of 4.4% (P = . 03) after 6 months of isotretinoin exposure (1 mg/kg of body weight) [2].

- Slowed rhodospsin regeneration :

Even a single dose of isotretinoin slowed the recovery of rod signaling after exposure to an intense bleaching light, and that rhodopsin regeneration was markedly slowed [3].

To complete...

***Figure 4** : Lissamine green corneal staining of a dry eye patient taking isotretinoin. Photo courtesy of Eric Donnenfeld, M.D.*

To complete...

- References :

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf
[1] **Bremner JD, Fani N, Ashraf A, Votaw JR, Brummer ME, Cummins T, Vaccarino V, Goodman MM, Reed L, Siddiq S, Nemeroff CB.** *Functional brain imaging alterations in acne patients treated with isotretinoin.* (2005) Am J Psychiatry. May;162(5):983-91.
[2] **Leachman SA, Insogna KL, Katz L, Ellison A, Milstone LM.** *Bone densities in patients receiving isotretinoin for cystic acne.* (1999) Arch Dermatol. Aug;135(8):961-5.
[3] **Sieving PA, Chaudhry P, Kondo M, Provenzano M, Wu D, Carlson TJ, Bush RA, Thompson DA.** *Inhibition of the visual cycle in vivo by 13-cis retinoic acid protects from light damage and provides a mechanism for night blindness in isotretinoin therapy.* (2001) Proc Natl Acad Sci U S A. Feb 13;98(4):1835-40.
[4] **Georgala S, Papassotiriou I, Georgala C, Demetriou E, Schulpis KH.** *Isotretinoin therapy induces DNA oxidative damage.* (2005) Clin Chem Lab Med. 43(11):1178-82. **[Abstract PubMed]**

5.1 (Ro)accutane and allergic reactions :

- Hypoimmunoglobulinemia - Lack of IgE response :

Repeated studies, in both mice and human subjects show the lack of a primary IgE response with elevated and sustained secondary IgE responses during and after 13-cRA exposure [1 and more]. The recognition of allergy in subjects with hypoimmunoglobulinemia is very difficult because they have very low level or even lack of specific IgE antibodies. Sensitivity to cow's milk in 24 children and additionally in 4 of them sensitivity to gluten [2].

IgE binds to human insulin [3 and more], whereas it could be suspected that the natural anti-insulin responses are disrupted in subjects displaying partial or severe hypoimmunoglobulinemia. The SH2 domain proteins transmit intracellular signals initiated by activated tyrosine kinase-linked receptors, and are sensitive to IgE [4]. It is therefore a possibility that lack of IgE responses causes a disruption in Trk signaling.

- Discussion :

Significant research is done on the opposite condition, hyperimmunoglobulinemia, but little is known about long term effects in subjects with partial or severe deficits in IgE responses. It remains clear that the functions of IgE are wide and not fully discovered.

It is here suggested that lack of IgE response can alter insulin sensitivity by altering IgE mediated phosphorylation processes, and contibute to altering (decreasing) steroidal receptor binding affinity and lipid composition in cellular membranes.

To complete...

- References :

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf
[1] **Barnett JB.** *Immunomodulating effects of 13-cis-Retinoic acid on the IgE response of BALB/c mice.* (1982) Int Arch Allergy Appl Immunol. 69(4):368-73.
[2] **Siwinska-Golebiowska H, Czerwinska-Kartowicz I, Markiewicz K, Derentowicz P, Golebiowska-Wawrzyniak M.** *[Immunological confirmation of allergy in children with hypoimmunoglobulinemia]* (2003) Med Wieku Rozwoj. Apr-Jun;7(2):279-87.
[3] **Fireman P, Fineberg SE, Galloway JA.** *Development of IgE antibodies to human (recombinant DNA), porcine, and bovine insulins in diabetic subjects.* (1982) Diabetes Care. Nov-Dec;5 Suppl 2:119-25.
[4] **Ottinger EA, Botfield MC, Shoelson SE.** *Tandem SH2 domains confer high specificity in tyrosine kinase signaling.* (1998) J Biol Chem. Jan 9;273(2):729-35.

5.2 (Ro)accutane and bronchoconstriction, asthma :

- Several case reports of lung related effects :

Several case reports describe an aggravation of asthma, induced bronchoconstriction or induced bronchoconstriction during physical exercise in human acne-subjects exposed to (Ro)accutane [1, 2, 3 and more]. Hoffman la Roche itself has reported the associtation "bronchospasms, with or without history of asthma, respiratory infection and voice alteration" in human subjects exposed to (Ro)accutane [0]. The long term effects on lung function are not known. Suggested effects on the lungs include inhibition of cell proliferation, apoptosis, alteration of epithelial cell function and inhibition of angiogenesis, the formation of blood vessels, as seen in cancer therapy.

- Causal links :

HB-EGF :

The epidermal growth factor receptor (EGFR), an important signaling pathway in airway biology, is stimulated by compressive stress applied to human airway epithelial cells. Although the EGFR ligand, heparin-binding epidermal growth factor-like growth factor (HB-EGF), is known to be released as a result of this stimulation, whether compressive stress enhances expression of other EGFR ligands, and the duration of mechanical compression required to initiate this response, is not known [4]. Heparin affin regulatory peptide (HARP), also known as pleiotrophin or heparin-binding growth-associated molecule, is an 18-kDa growth factor that has a high affinity for heparin. It constitutes with midkine and retinoic acid heparin-binding protein, a family of structurally related heparin-binding growth factors. A growing body of evidence indicates that HARP is involved in the control of cellular proliferation, migration and differentiation and plays a significant role in tumor growth and angiogenesis [7].

Inhibition of angiogenesis, the formation of blood vessels, is a current cancer strategy. However, the effects on the healthy lung capacity is not fully evaluated. No measurments of capacity of oxygen uptake in (Ro)accutane exposed patients has been performed.

Histamine :

Marked basophilia and severe symptoms due to hyperhistaminemia have been reported after the exposure in APL subjects with all-trans retinoic acid [5].

TGF-beta1 :

While the addition of TGF-beta1 or retinoic acid to monkey normal lung bronchial 12MBr6 cells and human lung cancer NCI-H727 cells increased DENTT protein production, TGF-beta1 together with retinoic acid resulted in a more sustained increase in DENTT production than with TGF-beta1 or retinoic acid alone. Transient transfection studies showed that ectopic DENTT expression significantly increased TGF-beta1-responsive 3TP-Lux and CAGA12-Lux reporter transcription in 12MBr6 and NCI-H727 cells with TGF-beta1 addition, while ectopic DENTT expression had no significant effect on the transcription of a retinoic acid-responsive element reporter in the presence of retinoic acid or TGF-beta1 [6].

IgE dependent respiratory infections :

IgE dependent allergic diseases are initiated by multivalent binding of allergens to IgE that is bound to receptors with high affinity for IgE on mast cells. Mast cells also express functional Toll Like Receptors (TLRs) which may account for the protection conferred by mast cells against bacterial infections in animal models. Activated mast cells release an array of potent inflammatory mediators by rapid discharge of preformed mediators in granules, the generation of inflammatory lipids from arachidonic acid, and the production of numerous Th2-type cytokines and chemokines. Stimulation of mast cells via TLR2 and TLR4 results primarily in generation of cytokines such as IL-4, IL-5, IL-6, IL-10, IL-13 and TNFalpha [8].

- Conclusions :

To complete...

- References :

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5.3 (Ro)accutane and atrial tachycardia :

- Several clinical associtations to cardiovascular effects in human subjects exposed to (Ro)accutane :

Hoffman la Roche itself has reported the following associations of cardiovascular effects in human subjects exposed to (Ro)accutane: palpitation, tachycardia, vascular thrombotic disease and even stroke [0]. There are several independent case reports of premature atrial beats and episodes of nonsustained atrial tachycardia and other associated cardiac effects [1 and more].

- Several pathways involved both for direct interaction with cardiofunction and significantly increased predisposing risks in exposed human subjects, pathways for causal links :

Significant serotonergic effects and significant effects on the angiotensin/renin system - significantly decreased AT-1 receptor expression and angiotensin II synthesis

5-HT receptor subtypes (5-HT1, 5-HT2, 5-HT3, 5-HT4 and 5-HT7) in brainstem regions are found to be involved in cardiac control [2]. 5-HT(1A) is suggested to be inhibited due to a significantly decreased promoter activity of Sp1 and NF-Y. TPH is inhibited due to a similiar effect, resulting in a lessened biosynthesis of serotonin. It is a possiblility that more 5-HT receptor isotypes than only 5-HT(1A) is heavily affected, thus affecting cardiac control.

Angiotensin II (ANG II) internalization in some tissues is suggested to be megalin dependent and it is suggested that megalin may play a role in regulating proximal tubule ANG II levels [3]. The megalin receptors are suggested to be heavily affected due to a decreased phosphorylation of Sp1.

Recent evidence indicates that EGF receptor transactivation by heparin-binding EGF (HB-EGF) contributes to hypertrophic signaling in cardiomyocytes. HB-EGF operates in a spatially restricted circuit in the extracellular space within the myocardium, revealing the critical nature of the local microenvironment in intercellular signaling [4]. In transgenic epidermis, dnRARalpha dose-dependently inhibited tRA induction of suprabasal HB-EGF and subsequent basal cell proliferation [7]. A similiar effect is suggested to affect cardiomyocytes, thus contributing to inhibit cell signaling.

The individual actions of Ang II on EGF-R transactivation in specific cell types are related to differential involvement of MMP-dependent HB-EGF release. Stimulation of the angiotensin II (Ang II) type 1 receptor (AT1-R) causes phosphorylation of extracellularly regulated kinases 1 and 2 (ERK1/2) via epidermal growth factor receptor (EGF-R) transactivation-dependent or -independent pathways in Ang II target cells [5]. In rats a massive dose all-trans-RA, comparable to what is seen in human acne-subjects, influences the renal RAS in anti-Thy1.1 nephritis by decreasing ANG II synthesis and receptor expression [6]. PPAR-gamma suppressed angiotensin II type 1 receptor (AT1R) gene transcription in vascular smooth muscle cells (VSMCs) by the inhibition of Sp1 binding to the --58/--34 GC-box related element in the AT1R gene promoter region via a protein-protein interaction [8].

- (Ro)accutane induced CVD and CHF risk factors with age :

The results from the ULSAM study, started in 1970, suggest that insulin resistance predicts congestive heart failure (CHF) both in middle-aged and elderly men, and thus is a clearly defined risk factor [9].

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[Full Text PDF]

5.4 (Ro)accutane and back pain, chest pain :

- Lower back pain suggested to be due to (Ro)accutane induced effects on retinoid signaling in the spinal cord :

Several case reports describe back pain in association with (Ro)accutane exposure. Lower back pain was reported early in about 30% of patients (124) [1].

Lower back pain has been detected as a symptom in human subjects that have a dysfunctional spinal cord [2 and more]. Cells in the spinal cord have been demonstrated to be highly dependent on retinoid signaling in adult human subjects [3 and more]. Also the retinoid receptors are found to be widely expressed in the adult mammalian spinal cord [4 and more]. All PPAR (alpha, beta/delta and gamma) and RXR (alpha, beta and gamma) isotypes were detected and found to exhibit specific patterns of localization in the different areas of the rat brain and spinal cord [4]. An increase in COX-2 expression in the lumbar spinal cord was observed in animals exposed to ATRA [5].

Lower back pain is suggested to be yet another symptom demonstrating (Ro)accutane induced effects on the CNS and peripheral nervous system.

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5.5 (Ro)accutane and birth defects :

Hoffman la Roche itself has unconditionally been forced to admit to (Ro)accutane induced birth defects in child-bearing women exposed to the toxin [0]. (Ro)accutane is also, currently up to this date, used in subjects with infantile acne [4 and more]. In infantile exposure the dose of isotretinoin used ranged from 0.2 mg/kg/day to 1.5 mg/kg/day [4].

Birth defects have been verified by extensive independent case reports [1, 2, 3 and more]. However, it is not the aim of this forum to provide scientifical explanations for (Ro)accutane induced birth defects. The focus of this forum is solely directed to effects in the adult and adolescent subjects exposed to the toxin.

- References :

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5.6 (Ro)accutane and altered blood coagulation :

- Significant upregulation of thrombomodulin during (Ro)accutane exposure, unknown effects post exposure :

Thrombomodulin, a major anticoagulant proteoglycan of the endothelial cell membrane, is a thrombin receptor that acts as a cofactor for protein C activation. Retinoic acid induced significant increases in the total antigen level and in surface and intracellular thrombomodulin activities only in keratinocytes grown in a low-calcium medium. In these undifferentiated keratinocytes, quantification of mRNA transcripts showed a threefold increase after retinoic acid stimulation [1].

- References :

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Thrombomodulin, a functional surface protein on human keratinocytes, is regulated by retinoic acid.
(1997) Arch Dermatol Res. Feb;289(3):151-7.

Blood. 2001 Feb 15;97(4):946-51. Related Articles, Links
Regulation of human coagulation factor X gene expression by GATA-4 and the Sp family of transcription factors.
Hung HL, Pollak ES, Kudaravalli RD, Arruda V, Chu K, High KA.
Department of Pediatrics, University of Pennsylvania, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA.

Serine protease factor Xa plays a critical role in the coagulation cascade. Zymogen factor X is synthesized and modified in the liver. To understand the mechanisms governing the liver-specific expression of factor X, the proximal promoter of human factor X was previously characterized. Two crucial cis elements at -73 and -128 and their cognate binding proteins, HNF-4 and NF-Y, respectively, were identified. In this report, studies are extended to 3 additional cis elements within the factor X promoter. Using gel mobility shift assays, the liver-enriched protein GATA-4 was identified as the protein binding to the GATA element at -96. GATA-4 transactivates the factor X promoter 28-fold in transient transfection experiments. It was also determined that the Sp family of transcription factors binds 2 DNase I-footprinted sites at -165 and -195. Disruption of Sp protein binding at either site reduces the promoter activity by half. Simultaneous disruption of both sites reduces the promoter activity 8-fold. This is the first report indicating the involvement of GATA-4 in the regulation of clotting factor expression. These observations provide novel insight into mechanisms by which the vitamin K-dependent coagulation factors are regulated.

PMID: 11159521 [PubMed - indexed for MEDLINE]

Biochemistry. 1991 Feb 12;30(6):1571-7. Related Articles, Links
Intron-exon organization of the human gene coding for the lipoprotein-associated coagulation inhibitor: the factor Xa dependent inhibitor of the extrinsic pathway of coagulation.
van der Logt CP, Reitsma PH, Bertina RM.
Haemostasis and Trombosis Research Unit, University Hospital, Leiden, The Netherlands.

Blood coagulation can be initiated when factor VII(a) binds to its cofactor tissue factor. This factor VIIa/tissue factor complex proteolytically activates factors IX and X, which eventually leads to the formation of a fibrin clot. Plasma contains a lipoprotein-associated coagulation inhibitor (LACI) which inhibits factor Xa directly and, in a Xa-dependent manner, also inhibits the factor VIIa/tissue factor complex. Here we report the cloning of the human LACI gene and the elucidation of its intron-exon organization. The LACI gene, which spans about 70 kb, consists of nine exons separated by eight introns. As has been found for other Kunitz-type protease inhibitors, the domain structure of human LACI is reflected in the intron-exon organization of the gene. The 5' terminus of the LACI mRNA has been determined by primer extension and S1 nuclease mapping. The putative promoter was examined and found to contain two consensus sequences for AP-1 binding and one for NF-1 binding, but no TATA consensus promoter element.

PMID: 1993173 [PubMed - indexed for MEDLINE]

5.7 (Ro)accutane and altered blood pressure :

To complete ...

- References :

Diabetes Res Clin Pract. 2005 Jul 16; [Epub ahead of print] Related Articles, Links
Basal production of nitric oxide (NO) and non-NO vasodilators in the forearm microcirculation in Type 2 diabetes: Associations with blood pressure and HDL cholesterol.
Woodman RJ, Playford DA, Watts GF.
School of Public Health, Curtin University of Technology, Perth, Australia.

We examined basal forearm microcirculatory blood flow (FBF) using venous occlusive strain-gauge plethysmography in 47 middle-aged men and women [55+/-1 years] with Type 2 diabetes and 15 age-matched healthy individuals [52+/-3 years], all receiving aspirin. Blood flow was also measured following infusion of N(G)-monomethyl-l-arginine into the brachial artery to inhibit basal NO release (FBF+l-NMMA). Acetylcholine (ACh) and sodium nitroprusside (SNP) were administered to assess endothelium-dependent and endothelium-independent functions. Compared with controls, diabetic subjects had significantly lower vasodilatory responses to ACh and SNP ($p<0.05$ for each). Basal FBF and FBF+l-NMMA were increased in diabetic subjects compared with controls (2.4+/-0.2ml/100ml/min versus 1.7+/-0.2ml/100ml/min, $p=0.02$ and 1.9+/-0.1ml/100ml/min versus 1.2+0.1ml/100ml/min, $p=0.01$, respectively) whereas the change in FBF following l-NMMA was greater in the controls (-27% versus -19%, $p=0.05$). Amongst the diabetic subjects, pulse pressure and HDL cholesterol were independent predictors of FBF ($b=0.04+/-0.01$, adjusted $r(2)=0.21$ and $p=0.001$, and $b=3.3+/-1.2$, adjusted $r(2)=0.12$ and $p=0.007$, respectively) and FBF+l-NMMA ($b=0.03+/-0.01$, adjusted $r(2)=0.20$, $p=0.002$ and $b=2.1+/-0.9$, adjusted $r(2)=0.09$ and $p=0.02$, respectively). Diastolic blood pressure predicted the change in FBF with l-NMMA ($b=-1.02+/-0.32$, adjusted $r(2)=0.20$ and $p=0.003$). Our findings suggest that well controlled T2DM patients have impaired agonist-mediated vasodilatation of the forearm resistance arteries that is associated with impaired basal release of nitric oxide but an increase in the release of non-NO vasodilators. The latter may be a compensatory response to increased arterial stiffness and may be facilitated by an effect of HDL.

PMID: 16029909 [PubMed - as supplied by publisher]

Eur J Pharmacol. 2005 Jan 10;507(1-3):311-6. Epub 2004 Nov 23. Related Articles, Links
Hypothyroidism changes adrenoceptor- and muscarinic receptor-mediated blood pressure responses.
Iwata T, Honda H, Matsuda H, Kondo M, Taniguchi J, Miwa T, Kumasaka K, Moroe H, Notoya Y.
Second Department of Physiology, School of Medicine, Showa University, Hatanodai 1-5-8, Shinagawa-ku, Tokyo 142-8555, Japan.

Hypothyroidism was induced by the administration of 0.03% methimazole to drinking water for 1, 2 or 6 weeks to study whether there is a change in adrenoceptor- and muscarinic receptor-mediated blood pressure responses in hypothyroid rats. After 1, 2 and 6 weeks of treatment, the pressor response to norepinephrine was progressively suppressed, and after 6 weeks a significant suppression was observed as compared to control. The depressor response induced by isoprenaline, acetylcholine or sodium nitroprusside was not significantly different between control and hypothyroid rats at any time. The pressor response induced by N(G)-nitro-L-arginine (L-NOARG), an inhibitor of nitric oxide (NO) synthase, was significantly reduced in hypothyroid rats after 1, 2 or 6 weeks of treatment, and the magnitude of the reduction was almost the same for three groups. These results indicated that hypothyroidism causes a time-dependent decrease in pressor responses mediated by alpha-adrenoceptors, but a time-independent decrease in those induced by L-NOARG, and suggest that a progressive decrease in alpha-adrenoceptor-mediated pressor responses occurs in hypothyroidism; however, the decrease in basal NO production and/or release in the peripheral vasculature already occurs in hypothyroid rats at an early stage of the disease.

PMID: 15659322 [PubMed - indexed for MEDLINE]

5.8 (Ro)accutane and altered body fluid homeostasis :

Retinoids are known to produce water loss. Synthetic retinoids produce dose-dependent alterations in transepidermal water loss [4].

- TNF-alpha and the Na+-K+-2Cl- cotransporter :

Retinoic acid is known to in a dose dependent manner inhibit TNF-alpha in various cell types. In endothelial cells derived from microvessels, ATRA significantly inhibited TNFalpha-induced TF expression in HMEC-1 as well as HUVEC [2]. RA-induced inhibition of TNF-alpha and iNOS mRNA expression in activated microglia was accompanied by the concomitant reduction in release of iNOS and TNF-alpha proteins as revealed by nitrite assay and ELISA, respectively [3]. In cultured caco-2 cells, TNF-alpha inhibited net water and chloride absorption, down-regulated in both surface and crypt colonocytes the Na+-K+-2Cl- cotransporter, and reduced the protein expression and activity of the Na+-K+ ATPase. It was concluded that the effect of the cytokine on colonocytes is mediated via PGE2. By inhibiting the Na+-K+ ATPase, it reduces the Na+ gradient needed for NaCl absorption, and by down-regulating the expression of the Na+-K+-2Cl- symporter, it

reduces basolateral Cl- entry and luminal Cl- secretion. The inhibitory effect on absorption is more significant than the inhibitory effect on secretion resulting in a decrease in net electrolyte uptake and consequently in more water retention in the lumen [1].

to complete...

- References :

[1] **Markossian S, Kreydiyyeh SI.** *TNF-alpha down-regulates the Na+-K+ ATPase and the Na+-K+-2Cl-cotransporter in the rat colon via PGE2.* (2005)Cytokine. Jun 21;30(6):319-27.
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5.9. (Ro)accutane and chemical depression :

To complete

The administration of isotretinoin to humans has been shown to be associated with increased concentrations of homocysteine, which is a potential metabolic mechanism by which isotretinoin may promote depression ":

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3276716/

https://www.ncbi.nlm.nih.gov/pubmed/17707566

https://www.ncbi.nlm.nih.gov/pubmed/15863802

- Significant number of case reports indicating a causal relation between exposure to (Ro)accutane and various signs of significant depression in acne-subjects :

A significant number of case reports describe the association between (Ro)accutane exposure and depression in human subjects [1, 2, 3 and more]. Depression may continue in subjects after exposure. Potential targets for retinoid signalling in depression include

dopaminergic, serotonergic or noradrenergic pathways or a complex interaction between these neurotransmitter systems [5].

- Suggested chemical cause: Significant inhibition of serotonergic activity and sertonerg receptor (5-HT) expression :

The expression level of the 5-HT(1A) receptor gene (htr1a) in the central nervous system (CNS) is implicated in the aetiology and treatment of anxiety disorders and depression. In mice, htr1a have revealed that its proximal promoter is GC rich and TATA-less. Several functional transcription factor binding sites, including MAZ and SP1 recognition sequences, have been identified [22].

- Suggested chemical cause: Neurosteroids and depression :

To complete...

- Suggested chemical cause: Gaba and depression :

To complete...

- Suggested chemical cause: Norephinephrine, dopamine and depression :

To complete...

- References :

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5.10. (Ro)accutane and diabetes :

- Major alterations of insulin sensitivity, insulin release and secretion, insulin production and liver responses :

Various studies are pointing out clear interactions between (Ro)accutane and insulin function. The insulin patway is a *major* pathway in how (Ro)accutane mediates its effects, thus it should be considered an effect, and not a side-effect.

Significantly altered insulin release/secretion in response to glucose, and antiproliferative, possibly apoptotic effects on beta cells

Whereas it remains completely clear that insulin secretion is affected during exposure, it is uncertain how insulin release and secretion is affected after a (Ro)accutane exposure. Experiments in rats show that insulin secretion could be expected to be efficiently reduced , due to decreased lessened promoter activity of Sp1 and disruptions in the GC-box [11]. Another study of an INS-1 cell line points out the clear relation between the retinoid receptors and insulin release and suggests a significantly higher insulin release during exposure, but found cells to reaggregate to a single clump in massive doses [1]. The most probable is that insulin secretion, in massive doses of retinoic acid as seen in acne-subjects, is significantly impaired during exposure.

Retinoic acid in was found to induce the pyruvate decarboxylating activity in modest doses [19]. In rats the pyruvate decarboxylase is found to be dependent on Sp1 and NF-Y promoter activity [11]. It is therefore possible that a massive dose of retinoic acid, as seen in acne-subjects, inhibits the pyruvate decarboxylase through decreased Sp1 and NF-Y binding. The pyruvate carboxylase (PC) plays a crucial role in various metabolic pathways, including gluconeogenesis, lipogenesis, and glucose-induced insulin secretion. The pyruvate carboxylase gene is transcriptionally regulated by peroxisome proliferator-activated receptor-gamma (PPARgamma) [20].

In rats, pyruvate carboxylase plays diverse roles in different biosynthetic pathways, including glucose-induced insulin secretion in pancreatic beta-cells. Transient transfections of these constructs into INS-1 cells identified a CCAAT box and a GC box that are located at -65/-61 and -48/-41, respectively, as the important determinants. Disruption of the GC box resulted in a 4-fold reduction of the reporter activity. Electrophoretic mobility shift assays (EMSAs) and supershift EMSAs using nuclear extract from INS-1 cells demonstrated that Sp1 and Sp3 bind a GC box while the nuclear factor Y was shown to bind the proximal but not the distal CCAAT box [11].

In INS-1 cells retinoid receptors RAR and RXR were found to mediate insulin release [1]. In rat islets and in response to glucose (Ro)accutane (10(-4) M) stimulated insulin secretion at 9.7, 12.5, 16.7, and 27.7 mM glucose. Maximal effects of 13 cis-RA (174% of control) were evident during second phase release at 9.7 mM glucose. Thirteen cis-RA

(10(-7) and 10(-6) M) caused cells to adhere to each other, and at higher concentrations, 13 cis-RA caused dispersed cells to reaggregate into a single clump. These retinoid-induced clumps were perifused in a Bio-Gel P-2 gel column. Secretion from the clump was twofold greater than from an equal number of perifused dispersed cells [7]. Both 9cRA and ATRA inhibit INS-1 cell proliferation [1].

- Significant inhibition of response to insulin by SREBP-1c in liver :

Transcription of the gene encoding sterol regulatory element-binding protein 1c (SREBP-1c) is known to be activated by insulin in the liver [2]. Synthesis of fatty acids in the liver and adipose tissue in response to insulin is critically dependent on the transcription factor SREBP-1c [3]. The rat SREBP-1c promoter contains binding sites for: 1) LXR (liver X receptor), which binds to liver X response elements (LXREs) 2) transcription factor Sp1, that has a binding site on SREs 3) transcription factor NF-Y (nuclear factor-Y) that also has a binding site on SREs and 4) SREBP itself, which also binds to sterol response elements (SREs) [3].

Each of these sites is required for the full stimulatory response of the SREBP-1c promoter to insulin [3]. Complete insulin response (increase of 6- to 11-fold) requires two binding sites for liver X receptors (LXRs), which are nuclear receptors that are activated by oxygenated sterols. Disruption of these binding sites did not lower basal transcription but severely reduced the response to insulin. In contrast, disruption of the closely linked binding sites for SREBPs and nuclear factor Y lowered basal transcription drastically but still permitted a 4- to 7-fold increase in response to insulin. Arachidonic acid, an inhibitor of LXR activation, blocked the response to insulin [2].

- Significant inhibition of the insulin receptor, suggested inhibition of response to insulin due to inhibition of insulin receptor promoter :

The human insulin receptor promoter region (HINSR) contains six GGGCGG sequences [4]. Sp1 binds to canonical GGGCGG or its atypical hexanucleotide sequence, called "GC box" motif, of several cellular and viral genes and activates transcription of these genes by RNA polymerase II. Retinoid receptors RARs/RXRs physically interact with Sp1, potentiate Sp1 binding to the GC box motifs [5]. However, in massive doses of retinoic acid as seen in acne-subjects, Sp1 binding affinity to the GC box may be decreased, thus resulting in downregulation of the insulin receptor.

- Altered renal clearance of insulin :

Renal clearance is a major pathway for regulating the levels of insulin and other low molecular weight polypeptide hormones in the systemic circulation.
Reabsorption of insulin from the glomerular filtrate occurs by binding to megalin on the luminal surface of proximal tubule cells followed by endocytosis and degradation in lysosomes. An insulin binding site, megalin, was identified in renal microvillar membranes. Megalin is a large multiligand binding endocytic receptor that is abundantly expressed in clathrin-coated pits on the apical surface of proximal tubule cells. Megalin is able to

internalize insulin into endocytic vesicles. In ligand blotting assays, megalin also bound several other low molecular weight polypeptides, including beta2-microglobulin, epidermal growth factor, prolactin, lysozyme, and cytochrome c, suggesting that megalin may play a significant role as a renal reabsorption receptor for the uptake of insulin and other low molecular weight polypeptides from the glomerular filtrate [6].

- Suggested alteration of insulin production :

Well-orchestrated transcriptional regulation of pancreatic beta cells is essential for insulin production and glucose homeostasis. Pancreas duodenum homeobox-1 (PDX-1) is a key regulator of glucose-dependent insulin production and glucose metabolism. We find that PDX-1 interacts with the PDZ-domain coactivator Bridge-1 in yeast interaction trap assays. Rat Bridge-1 and PDX-1 interact directly in GST pull-down assays via Bridge-1 interactions with the amino-terminal transactivation domain of PDX-1. Bridge-1 also interacts with wild-type and mutant human PDX-1 (IPF-1) proteins and strongly interacts with the amino-terminal PDX-1 P63fsdelC (MODY4) mutant protein. Transcriptional activation by PDX-1 is increased by addition of Bridge-1 in multiple contexts, including synergistic activation of a Gal4 reporter by Gal4-Bridge-1 and Gal4-PDX-1 fusion proteins, activation of the somatostatin promoter TAAT1 enhancer, and synergistic activation of the rat insulin I promoter FarFlat enhancer by PDX-1, E12, and E47. We propose that the coactivator Bridge-1 modulates PDX-1 functions in the regulation of its target genes [8].

- Reduced resistin levels :

In small doses, Retinoic acid (RA), the acid form of vitamin A, exerts funtions a signal that inhibits the expression of resistin, an adipocyte-secreted protein previously proposed to act as an inhibitor of adipocyte differentiation and as a systemic insulin resistance factor. Both 9-cis and all-trans RA reduced resistin mRNA levels in white and brown adipocyte cell model systems; the effect was time- and dose-dependent, was followed by a reduced secretion of resistin, and was reproduced by selective agonists of both RA receptors and retinoid receptors. Association of CCAAT/enhancer-binding protein alpha (a positive regulator of the resistin gene) and its coactivators p300, cAMP response element-binding protein binding protein, and retinoblastoma protein with the resistin gene promoter was reduced in RA-treated adipocytes. RA administration to normal mice resulted in reduced resistin mRNA levels in brown and white adipose tissues, reduced circulating resistin levels, reduced body weight, and improved glucose tolerance. Resistin expression was also downregulated after dietary vitamin A supplementation in mice. The results raise the possibility that vitamin A status may contribute to modulate systemic functions through effects on the production of adipocyte-derived protein signals [9].

It is unknown how the levels of resistin are affected during a severe retinoic acid exposure and how resisitin levels are affected in a chronical vitamin A deficient condition.

- Significant interaction with the pancreatic secretin receptor :

The human secretin receptor (hSR) is an important glycoprotein receptor for regulating the secretion of pancreatic bicarbonate, water, and electrolytes. The secretin receptor is in humans suggested to be regulated by Sp1 and Sp3 binding to GC-box in the promoter [10]. A significant downregulation of the secretin receptor in association with (Ro)accutane

exposure is therefore here suggested.

- Alterations of glucose transport :

GLUT-1 :

In the development of diabetic nephropathy, angiotensin (Ang) II is thought to exert numerous actions on the glomerulus, and especially on the mesangium. However, the role(s) played by Ang II in the glucose metabolism per se in mesangial cells remains unclear. Ang II, at least via its type 1 receptor (AT1-R)-mediated effect, phosphorylates extracellular signal regulated kinase (ERK) by transactivation of epidermal growth factor receptors (EGF-Rs) via the Ca2+ or protein kinase C (PKC) pathways. Ang II upregulated GLUT1 mRNA accumulation in a time- and dose-dependent manner (peaking at 12 h; approximately 3.8-fold vs. control), and this upregulation was completely inhibited by the PKC inhibitor calphostin-C. The Ang II-induced GLUT1 expression was significantly inhibited by the EGF-R inhibitor AG1478 (approximately 80% inhibition), by inactivation of ERK by PD98059, and by pretreatment with heparin and the metalloproteinase (MMP) inhibitor batimastat [13]. In Xenopus oocytes, all six native cysteine residues of GLUT1 were changed to either glycine or serine residues by site-directed mutagenesis, resulting in a functional Glut1 construct with Cys mutated to Gly/Ser (C-less). The GLUT1 reporter molecule was engineered from C-less GLUT1 by creating a unique cleavage site for factor Xa protease within the central cytoplasmic loop and by eliminating the site of N-linked glycosylation [14]. The data suggests that the clusering of GLUT-1 is of importance for function.

GLUT-2 :

ATRA ralsed the GLUT 2 mRNA in a bell-shaped concentration response curve after 48 h [1]. This bell shaped curve is here suggested to possibly result in inhibition of GLUT-2 in higher doses of retinoic acid. In mice, the expression of type 2 glucose transporter isoform (GLUT2) could be regulated by PPAR-gamma in the liver through binding of PPARgamma to the GLUT-2 promoter [21].

Gene 33 is found to be regulated by both insulin and glucocorticoids. It is suggested that that the MEK-ERK, but not the phosphatidylinositol 3-kinase (PI3-K), pathway plays a direct role in insulin regulation of Gene33 transcription and protein expression. Gene33 is found to be involved in proliferation and differentiation of cells [16]. Inhibition of insulin signaling via the ERK pathway is thus suggested to contribute to apoptosis, and and pathway of (Ro)accutane induced hypometabolism.

- Significant downregulation of the farnesoid X receptor (FXR) :

Dyslipidemia and gallbladder diseases are two current anomalies observed in patients suffering from the metabolic syndrome and type 2 diabetes. The bile acid-activated nuclear receptor farnesoid X receptor (FXR) controls bile acid as well as lipid metabolism. Recent observations indicate a role for FXR also in carbohydrate metabolism. Hepatic FXR expression is altered in diabetic animal models in vivo and regulated by hormones and nutrients in vitro. At the molecular level, FXR activation modifies the transcriptional activity of different transcription factors controlling gluconeogenesis and lipogenesis, thus affecting

in concert bile acid, lipid and carbohydrate metabolism [12].

- Calpain :

Calpains have shown to be altered during the caspases, and modulate NF-kappB, both mechanisms that are involved in (Ro)accutane exposure.

The mRNA expression of type 2 diabetes-related genes in white blood cells (WBC) was examined before and after onset in Otsuka Long-Evans Tokushima Fatty (OLETF) rat. The level of the calpain 10 (CAPN10) transcript was significantly decreased compared to control animals in WBC before and after onset. Significant decreases in this gene expression were also found in the major insulin-target tissues as well as WBC before onset. These results suggest that gene expression in WBC could be a useful screening system for predicting the incidence of type 2 diabetes before onset in OLETF rats, and that CAPN10 represents a potential candidate gene for predicting type 2 diabetes in human [15].

- Autoimmune contribution to diabetic complications :

An overproduction of cytokines are normally connected with a cell destruction in autoimmune diabetes. Indeed (Ro)accutane may trigger such an event, by for example a significant elevation of TGFbeta1. However, (Ro)accutane may exert an opposite effect, inhibit cytokine production, which also may affect several pathways that may severely affect the beta cells, production, release and response to insulin.

Cytokines are important humoral mediators of beta cell destruction in autoimmune diabetes. Exposure of RINm5F cells to IL-1beta or to a cytokine mixture (IL-1beta, TNF-alpha, IFN-gamma) for 6 h resulted in the differential expression of a functional gene cluster. Apart from the well-known up-regulation of the cytokine-responsive genes iNOS, NF-kappaB, MnSOD and Hsp70, several genes that belong to the functional cluster of the endocytotic pathway were identified. These endocytotic genes comprised: clathrin, megalin, synaptotagmin and calcineurin, which were up-regulated by IL-1beta or the cytokine mixture [18].

- (Ro)accutane induced diabetes mellitus :

The insulin patway is a *major* pathway in how (Ro)accutane mediates its effects. (Ro)accutane exposure in human subjects may result in diabetes mellitus. The (Ro)accutane induced general effects share quite well the picture with a diabetic diagnosis, and depending on severity, diabetes mellitus may be adequate in some subjects.

The clinical picture of type 2 diabetes mellitus (T2DM) is formed by impairment in insulin secretion and resistance to insulin action. Sequence differences in a few genes have been associated, so far, with complex, polygenic forms of T2DM, for example, calpain 10, PPARgamma, KCJN11, and insulin. In addition, some evidence exists that genes, such as adiponectin, IRS-1, and some others may also influence the susceptibility to T2DM. It is expected that in the nearest future more T2DM susceptibility genes will be identified [17].

- Conclusions :

Insulin secretion and release are most definitely significantly affected during a (Ro)accutane exposure in human acne-subjects. The insulin receptors are found to be heavily affected by retinoid receptors, and their GC-boxes in the insulin receptor promoter area, show exact binding sites for Sp1 that are inhibited. The insulin receptors are with highest certainity significantly suppressed during a (Ro)accutane exposure, resulting in reduced insulin sensitivity. This is suggested not to be considered a side-effect, but an actual effect, which among other things is significantly antiproliferative. In the liver, the insulin response through SREBP-1 is suggested to be significantly inhibited.

Both renal clearance of insulin and angiotensin II are suggested to heavily affected, thus contibuting to inhibition of glutamate transporters. Glut-1 and Glut-2 via PPARgamma are found to be affected, but it is likely that more glutamate transporters are involved. Insulin production may be affected due to interaction with PDX promoters. A possible significant loss of insulin producing beta-cells can not be excluded.

Both resistin and secretin are heavily affected. (Ro)accutane induces, in the normal response profile, a significantly downregulated cytokine production, which may contribute to an atypical immune-dependent diabetes. The effects on the insulin-system are major, and may result in both type 1 and 2 diabetes, severe insulin resistance, or a combination.

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5.11. (Ro)accutane and oxidative DNA damage :

- Significantly elevated 8-OHdG in human subjects exposed to (Ro)accutane - highly likely one of the relevant markers for (Ro)accutane induced oxidative DNA damage :

High serum levels of 8-OHdG in acne-subjects exposed to isotretinoine may be due to a direct effect on liver, muscle and skin epidermal cells. Regular evaluation of 8-OHdG in sera of patients, especially of exposed women of reproductive age, could be a sensitive follow-up biomarker of DNA oxidation [1]. In the rat-brain, elevated 8-OHdG is a suggested marker for oxidative stress and correlated with degenerative changes [2].

Human subjects with CA (n=18) were evaluated before and 45 days after Iso (0.5mg/kg per day) exposure and non-diseased controls (n=22) were tested only once. Plasma TAS levels and 8-OHdG were measured spectrophotometrically and with an immunoassay, respectively. Liver biochemical parameters and muscle enzymes were measured on a blood chemistry analyzer. Results: TAS levels were significantly ($p<0.0001$) lower in patients before treatment (921+/-124 mumol/L) compared with those after treatment (1335+/-93 mumol/L) and in controls (1536+/-126 mumol/L). In contrast, 8-OHdG serum levels were two-fold higher in patients after exposure (0.21+/-0.03 ng/mL) than before exposure (0.11+/-0.02 ng/mL) and three-fold higher than in controls (0.07+/-0.01 ng/mL; $p<0.0001$). Negative correlations were found between TAS and 8-OHdG ($r=-0.754$, $p<0.0001$) in patients before therapy and positive correlations were found between creatine kinase (CK) and 8-OHdG ($r=0.488$, $p<0.001$) and liver enzymes after isotretinoine exposure [1].

- Significantly elevated 8-hydroxy-2'-deoxyguanosine (8-OHdG) as an index of oxidative stress in the brain of rats exposed to arsenic :

To clarify the association between oxidative DNA damage and the neurotoxicity of arsenic, the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) as an index of oxidative DNA damage in the brain was examined in mice fed with drinking water containing 1 or 2 ppm arsenic, using an HPLC-electrochemical detector and immunohistochemical method. 8-OHdG levels were significantly increased in the brain of mice given arsenic and its immunoreactivity was distributed in the cerebral and cerebellar cortexes. Cerebral cortex neurons and Purkinje cells in the cerebellar cortex showed degenerative changes in accordance with the distribution of 8-OHdG immunoreactivity. The levels of arsenic in this study were lower than those reported in epidemiological studies. Thus, it is concluded that environmentally relevant levels of arsenic induce pathological changes through oxidative DNA damage in the brain tissues in vivo and that cerebral and cerebellar cortex neurons seem to be the major targets of arsenic neurotoxicity [2].

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5.12 (Ro)accutane, eating disord., addictive disorder :

- (Ro)accutane and eating disorders :

To complete...

- (Ro)accutane and addictive disorders :

It is here suggested that (Ro)accutane exposure may result in alcoholism, through a disruption of retinoid signaling and metabolism in the brain. Etanol is found to increase retinoid metabolism in the brain, which, in the human (Ro)accutane exposed subjects , there is a suggested lack of.

Astrocytes are the predominant source of postnatal RA synthesis in the cerebellum. They express both retinaldehyde dehydrogenase 1 and 2. In vitro cytosolic preparations of astrocytes, as well as live cell preparations, have an increased capacity to synthesize RA in the presence of ethanol [1].

- References :

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Endocrinology. 2004 Aug;145(8):3935-40. Epub 2004 May 6. Related Articles, Links
Evidence indicating that renal tubular metabolism of leptin is mediated by megalin but not by the leptin receptors.
Hama H, Saito A, Takeda T, Tanuma A, Xie Y, Sato K, Kazama JJ, Gejyo F.
Department of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951-8510, Japan.

Leptin is secreted by adipocytes and is a circulating factor that regulates food intake and energy expenditure. Its serum level is elevated in patients with renal failure and has been suggested to be associated with malnutritional factors in these patients. Leptin has been suggested to be primarily metabolized by the kidneys, although the precise molecular mechanisms are not known. The purpose of this study was to determine the nephron segments and potential receptors involved in renal leptin metabolism. To determine the segment involved in leptin uptake, we performed histoautoradiography of kidney sections obtained from rats that had been injected iv with (125)I-leptin. The ability of megalin, a multiligand endocytic receptor in the proximal tubules, to bind and endocytose leptin was examined by ligand blotting analysis, quartz-crystal microbalance, and degradation assays using megalin-expressing rat yolk sac L2 cells. Immunohistochemistry was performed to

localize leptin receptors (LEP-R) in the rat kidney using two antibodies that recognize different epitopes on the LEP-R proteins. Circulating (125)I-leptin was filtered by glomeruli and internalized by proximal convoluted tubules. Megalin bound leptin in the presence of Ca(2+) and mediated its cellular internalization and degradation. On immunohistochemistry, LEP-R were localized in the proximal straight tubules, loops of Henle, distal tubules, and collecting ducts. In conclusion, circulating leptin was filtered by glomeruli and taken up by proximal convoluted tubules, where megalin likely mediates its binding and uptake. The localization of LEP-R suggests that they are not primarily involved in leptin metabolism in the proximal tubules.

PMID: 15131016 [PubMed - indexed for MEDLINE]

5.13 (Ro)accutane and epileptic seizures :

Several case reports describe the initiation of epileptic seizures in association with (Ro)accutane exposure in human acne-subjects [1, 2 and more].

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5.14 (Ro)accutane and facial musculature :

To complete...

- References :

5.15 (Ro)accutane and reduced fertility :

- Vitamin A deficiency in male rodents results in infertility :

The molecular mechanisms leading to male infertility in vitamin A deficient (VAD) rodents have never been fully elucidated. An interaction between BMP4 and retinoid signaling pathways in germ cells may help clarify the biochemical basis of VAD. Adult germ cells, in particular spermatogonia, expressed BMP4 at both the mRNA and protein levels. BMP4 expression was significantly up-regulated in the testes of VAD mice and was down-regulated in freshly isolated germ cells and VAD testes by retinol, but not retinoic acid. The retinoid-responsive gene, RARbeta, was not induced in germ cells following retinoid treatment. Examination of BMP4 promoter usage in spermatogonia and the VAD testis revealed that germ cells utilize the recently characterized BMP4 intron 2 promoter, in addition to the classical 1A and 1B promoters. The observed decrease in BMP4 in response to retinol was mediated by the 1A and intron 2 promoters of the BMP4 gene. A direct requirement for retinoids by germ cells for the resumption of spermatogenesis in

VAD animals via mechanisms that involve the suppression of BMP4 expression is necessary [2].

- Cellular retinol binding protein (CRBP) distribution in the testis :

The distribution of cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP) in rat testis and epididymis was examined by the peroxidase-antiperoxidase immunolocalization technique. In the testis, cellular retinol-binding protein was localized exclusively in the Sertoli cells. Staining varied with the stages of the seminiferous epithelium cycle and was maximal prior to the maturation divisions. Cellular retinoic acid-binding protein was localized exclusively in the germinal cells in the adluminal compartment. The results suggest that retinoic acid may be the retinoid form used by the germinal cells, and that Sertoli cells may use the cellular retinol-binding protein to transfer retinol from the basal to the adluminal compartment. In the epididymis, cellular retinol-binding protein was localized in the cytoplasm and stereocilia of the principal cells in the proximal caput epididymidis, while cellular retinoic acid-binding protein was localized in the spermatozoa and the stereocilia of the principal cells throughout the epididymis and in the epithelial cells of the distal vas deferens. Sperm staining intensity decreased from the initial segment to the cauda. The presence of high levels of cellular retinol-binding protein in the epithelial cells and high levels of cellular retinoic acid-binding protein in the spermatozoa of the caput epididymidis, known to be involved in the synthesis and secretion of factors necessary for sperm maturation, suggests that vitamin A may have a role in this process [1]. A similiar distribution is highly likely to be present in humans.

ODF3
Outer dense fiber of sperm tails 3
Location: 11p15.5

Source: genatlas

To complete...

- References :

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Localization of cellular retinol-binding protein and cellular retinoic acid-binding protein in the rat testis and epididymis. (1985) J Androl. May-Jun;6(3):197-212.
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Vitamin A regulation of BMP4 expression in the male germ line. (2005) Dev Biol. Aug 22

Dev Biol. 2005 Aug 31;285(1):49-56 [Epub ahead of print] Related Articles, Links
Growth factors sustain primordial germ cell survival, proliferation and entering into meiosis in the absence of somatic cells.
Farini D, Scaldaferri ML, Iona S, La Sala G, De Felici M.
Department of Public Health and Cell Biology, Section of Histology and Embryology, University of Rome "Tor Vergata", Via Montpellier 1, Rome 00173, Italy.

It is known that mammalian primordial germ cells (PGCs), the precursors of oocytes and prospermatogonia, depend for survival and proliferation on specific growth factors and other undetermined compounds. Adhesion to neighboring somatic cells is also believed to

be crucial for preventing PGC apoptosis occurring when they loss appropriate cell to cell contacts. This explains the current impossibility to maintain isolated mouse PGCs in culture for periods longer than a few hours in the absence of suitable cell feeder layers producing soluble factors and expressing surface molecules necessary for preventing PGC apoptosis and stimulating their proliferation. In the present paper, we identified a thingytail of soluble growth factors, namely KL, LIF, BMP-4, SDF-1, bFGF and compounds (N-acetyl-l-cysteine, forskolin, retinoic acid) able to sustain the survival and self-renewal of mouse PGCs in the absence of somatic cell support. We show that under culture conditions allowing PGC adhesion to an acellular substrate, such growth factors and compounds were able to prevent the occurrence of significant levels of apoptosis in PGCs for 2 days, stimulate their proliferation and, when LIF was omitted from the thingytail, allow most of them to enter into and progress through meiotic prophase I. These results consent for the first time to establish culture conditions for purified mammalian PGCs in the absence of somatic cell support and should make easier the molecular dissection of the processes governing the development of such cells crucial for early gametogenesis.

PMID: 16139834 [PubMed - as supplied by publisher]

Biol Reprod. 2005 Apr;72(4):898-907. Epub 2004 Dec 15. Related Articles, Links
Identification, characterization, and functional analysis of sp1 transcript variants expressed in germ cells during mouse spermatogenesis.
Thomas K, Sung DY, Yang J, Johnson K, Thompson W, Millette C, McCarrey J, Breitberg A, Gibbs R, Walker W.
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The SP family of zinc-finger transcription factors are important mediators of selective gene activation during embryonic development and cellular differentiation. SP-binding GC-box domains are common cis-regulatory elements present in the promoters of several genes expressed in a developmentally specific manner in differentiating mouse germ cells. Four Sp1 cDNAs were isolated from a mouse pachytene spermatocyte cDNA library and characterized by DNA sequence analysis. Northern blot studies revealed that these cDNAs corresponded to 3 full-length Sp1 transcripts (4.1, 3.7, and 3.2 kilobases [kb]) and an additional 1.4-kb 5'-truncated Sp1 transcript that are temporally expressed during spermatogenesis. Quantitative real-time polymerase chain reaction studies verified that the highest levels of Sp1 transcript expression of 4.1, 3.7, and 3.2 kb occur in the primary spermatocytes. The spatial and temporal expression patterns of these Sp1 transcripts and their encoded 60-kDa and 90-kDa SP1 proteins were demonstrated using in situ hybridization and immunohistochemical analyses. To assess the transcriptional properties of these SP1 transcription factors, SP-deficient Drosophila SL2 cells were stably transfected with the respective Sp1 cDNA expression vectors and cotransfected with either Ldh2, Ldh3, or Creb promoter/luciferase reporter constructs. The levels of SP-mediated luciferase expression observed depended on the structure of the glutamine-rich transactivation domains and the number of GC-box elements present in the respective promoters. The alterations observed in germ cells in the patterns of expression of the Sp1 transcripts encoding the 60-kDa and 90-kDa SP1 isoforms suggest that these SP1 factors may be involved in mediating stage-specific and cell type-specific gene expression during mouse spermatogenesis.

PMID: 15601926 [PubMed - in process]

5.16 (Ro)accutane and fatigue, loss of energy :

- References :

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf

5.17 (Ro)accutane and gallstone formation :

There are several case reports of gall-stone formation in association with (Ro)accutane exposure in human subjects [4 and more]. The long term effects on gall-stone formation are unknown.

Activation of the retinoid x receptor (RXR), in a similiar manner as of (Ro)accutane induced activation, is found to antagonize (inhibit) the transcription of the farnoid x receptor (FXR) [2 and more] which could increase the susceptibility to pathologies like gallstone disease in human subjects exposed to the toxin.

Cholesterol gallstone disease is characterized by several events, including cholesterol precipitation in bile, increased bile salt hydrophobicity and gallbladder inflammation. FXR agonists (the opposite of (Ro)accutane) are suggested to be a possible remedy for gallstone disease due to FXR-dependent increases in biliary bile salt and phospholipid concentrations, which restored cholesterol solubility and thereby prevented gallstone formation [1, 3 and more].

- Retinoid X receptor (RXR) antagonism of the farnesoid X receptor (FXR) :

RXR agonist LG100268 antagonizes induction of BSEP expression mediated by endogenous and synthetic FXR ligands, CDCA and GW4064, respectively. Moreover, this antagonism is a general feature of RXR agonists and is attributed to a decrease in binding of FXR/RXR heterodimers to the BSEP-FXRE coupled with the inability of RXR agonists to recruit coactivators to FXR/RXR. FXR/RXR is suggested to be a conditionally permissive heterodimer and is the first example of RXR ligand-mediated antagonism of FXR activity. Because FXR agonists lower triglyceride levels, suggesting a novel role for RXR-mediated antagonism of FXR activity in the development of hypertriglyceridemia observed with RXR agonists in rodents and humans [2].

- References :

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Hepatology. Jul;42(1):218-21.
[2] **Kassam A, Miao B, Young PR, Mukherjee R.** *Retinoid X receptor (RXR) agonist-induced antagonism of farnesoid X receptor (FXR) activity due to absence of coactivator recruitment and decreased DNA binding.*
(2003) J Biol Chem. Mar 21;278(12):10028-32.
[3] **Doggrell SA.** *Farnesoid X receptor agonism -- a new approach to the treatment of cholesterol gallstone disease.* (2005) Expert Opin Investig Drugs. Apr;14(4):535-8.
[4] **Aurousseau MH, Levacher S, Beneton C, Blaise M, Pourriat JL.** *[Transient dysfibrinogenemia and thrombocytopenia associated with recurrent acute pancreatitis in the course of isotretinoin therapy]*
(1995) Rev Med Interne. 16(8):622-5.

Genes Dev. 2004 Jan 15;18(2):157-69. Epub 2004 Jan 16. Related Articles, Links
Peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1alpha) regulates triglyceride metabolism by activation of the nuclear receptor FXR.
Zhang Y, Castellani LW, Sinal CJ, Gonzalez FJ, Edwards PA.
Department of Biological Chemistry, University of California at Los Angeles, Los Angeles, CA 90095, USA.

Peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1alpha) has been shown to regulate adaptive thermogenesis and glucose metabolism. Here we show that PGC-1alpha regulates triglyceride metabolism through both farnesoid X receptor (FXR)-dependent and -independent pathways. PGC-1alpha increases FXR activity through two pathways: (1) it increases FXR mRNA levels by coactivation of PPARgamma and HNF4alpha to enhance FXR gene transcription; and (2) it interacts with the DNA-binding domain of FXR to enhance the transcription of FXR target genes. Ectopic expression of PGC-1alpha in murine primary hepatocytes reduces triglyceride secretion by a process that is dependent on the presence of FXR. Consistent with these in vitro studies, we demonstrate that fasting induces hepatic expression of PGC-1alpha and FXR and results in decreased plasma triglyceride levels in wild-type but not in FXR-null mice. Our data suggest that PGC-1alpha plays an important physiological role in maintaining energy homeostasis during fasting by decreasing triglyceride production/secretion while it increases fatty acid beta-oxidation to meet energy needs.

PMID: 14729567 [PubMed - indexed for MEDLINE]

Biochem Biophys Res Commun. 2005 Apr 1;329(1):391-6. Related Articles, Links
Regulation of pyruvate dehydrogenase kinase expression by the farnesoid X receptor.
Savkur RS, Bramlett KS, Michael LF, Burris TP.
Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, IN 46285, USA.

The pyruvate dehydrogenase complex (PDC) functions as an important junction in intermediary metabolism by influencing the utilization of fat versus carbohydrate as a source of fuel. Activation of PDC is achieved by phosphatases, whereas, inactivation is catalyzed by pyruvate dehydrogenase kinases (PDKs). The expression of PDK4 is highly regulated by the glucocorticoid and peroxisome proliferator-activated receptors. We demonstrate that the farnesoid X receptor (FXR; NR1H4), which regulates a variety of genes involved in lipoprotein metabolism, also regulates the expression of PDK4. Treatment of rat hepatoma cells as well as human primary hepatocytes with FXR agonists

stimulates the expression of PDK4 to levels comparable to those obtained with glucocorticoids. In addition, treatment of mice with an FXR agonist significantly increased hepatic PDK4 expression, while concomitantly decreasing plasma triglyceride levels. Thus, activation of FXR may suppress glycolysis and enhance oxidation of fatty acids via inactivation of the PDC by increasing PDK4 expression.

PMID: 15721319 [PubMed - indexed for MEDLINE]

Scand J Gastroenterol Suppl. 2004;(241):60-9. Related Articles, Links
Relevance of hereditary defects in lipid transport proteins for the pathogenesis of cholesterol gallstone disease.
vanBerge-Henegouwen GP, Venneman NG, Portincasa P, Kosters A, van Erpecum KJ, Groen AK.
Gastrointestinal Research Unit, Dept. of Gastroenterology, UMC Utrecht, The Netherlands. gbergehe@azu.nl

In the formation of cholesterol gallstones, cholesterol hypersecretion into bile causing cholesterol supersaturation and crystallization appears to be the primary factor, with disturbed gallbladder and intestinal motility as secondary factors. Although intestinal uptake mechanisms have not yet been fully elucidated, the HDL receptor scavenger receptor B1 (SRB1) may be involved. Since HDL-cholesterol, both from the intestine and peripheral sources, is the preferred type of cholesterol for biliary secretion, increased HDL transport to the liver can also cause cholesterol hypersecretion in bile. In the hepatocyte, bile formation is regulated by several transmembrane proteins, all belonging to the ABC family. A change in the activity in one of these proteins can have a profound impact on biliary lipid secretion. The bile salt export pump (BSEP or ABCB11) regulates the excretion of bile salts into bile and mutations cause severe cholestasis. The second ABC transporter, ABCB4 (MDR3) regulates the secretion in bile of phosphatidylcholine (PC), while ABCG5/G8 is active in the excretion of cholesterol and sterols into bile. These transporters also facilitate transport of sterols back into the intestinal lumen. Mutations in either of these genes cause sitosterolaemia with increased absorption of plant sterols and cholesterol. Until now, evidence for a genetic background of human gallstone disease is mostly indirect and based on ethnic differences. Only two single gene defects are associated with gallstones. One is an ABCB4 mutation which causes a deficiency in biliary PC secretion and the other is a CYP7A1 mutation, the rate-limiting enzyme in the synthesis of bile salts from cholesterol in the liver. Recently, several common DNA polymorphisms in the ABCG8 gene were discovered that are associated with variations in plasma sterols, which could also influence biliary cholesterol secretion, but there is still a paucity of human studies.

Publication Types:
Review
Review, Tutorial

PMID: 15696852 [PubMed - indexed for MEDLINE]

5.18 (Ro)accutane and gastrointestinal side-effects :

To complete...

- References :

[0] **No author** *Roche official complete US Accutane Product information*
www.rocheusa.com/products/accutane/pi.pdf

J Pharm Sci. 2005 Feb;94(2):363-72. Related Articles, Links
Expression of PPAR, RXR isoforms and fatty acid transporting proteins in the rat and human gastrointestinal tracts.
Wang Q, Herrera-Ruiz D, Mathis AS, Cook TJ, Bhardwaj RK, Knipp GT.
Department of Pharmaceutics, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 160 Frelinghuysen Rd, Piscataway, New Jersey 08854-8020, USA.

Dietary fatty acid (FA) absorption across the gastrointestinal (GI) tract is of critical importance for sustenance, however, excessive FA absorption has also been linked to metabolic syndrome and associated disorders. The expression of isoforms that regulate the dietary FA absorption are not as well characterized in the GI tract as they are elsewhere. Peroxisome proliferator-activated receptors (PPARalpha, beta, and gamma) and 9-cis-retinoic acid receptors (RXRalpha, beta, and gamma) are nuclear hormone transcription factors that control FA homeostasis, in part through the regulation of expression of membrane-bound FA transporting proteins. The present study was designed to elucidate the expression of PPAR and RXR isoforms and FA transporting proteins (FABPpm and FAT/CD36) in the rat and human GI tracts using reverse transcriptase-polymerase chain reaction (RT-PCR), immunoblotting, and immunohistochemical staining. The results revealed rat GI expression of all the PPAR and RXR isoforms, FABPpm and FAT/CD36. PPARalpha, PPARbeta, PPARgamma, RXRalpha, FABPpm, and FAT/CD36 isoforms exhibited ubiquitous expression in human GI tract, whereas RXRbeta was not detected. RXRgamma was observed in a majority of the human GI samples. These results provide a physiological foundation for rational drug design and drug delivery for the mitigation of metabolic syndrome and associated disorders to normalize intestinal FA absorption. Copyright 2004 Wiley-Liss, Inc.

PMID: 15614817 [PubMed - indexed for MEDLINE]

5.19 (Ro)accutane and glucose-6-phosphate deficiency :

Follicular and sebaceous gland cell differentiation was investigated during treatment of acne patients with isotretinoin. Sebaceous glands were significantly reduced in volume and showed decreased metabolic activity as measured by glucose-6-phosphate dehydrogenase and succinic dehydrogenase enzyme activities [1]. These decreased enzymatic activities are likely to be present in several other areas and types of cells. G6Pase is one of the enzymes involved in whole body glucose homeostasis [4].

The enzyme glucose-6-phosphatase catalyzes the dephosphorylation of glucose-6-phosphatase to glucose, the final step in the gluconeogenic and glycogenolytic pathways. Expression of the glucose-6-phosphatase gene is induced by glucocorticoids and elevated

levels of intracellular cAMP. The effect of cAMP in regulating glucose-6-phosphatase gene transcription was corroborated by the identification of two genetic motifs CRE1 and CRE2 in the human and murine glucose-6-phosphatase gene promoter that resemble cAMP response elements (CRE) [3]. Glucose production represents the net contribution of gluconeogenesis and glycogenolysis. However, a portion of glucose entering the liver by means of phosphorylation of glucose is also a substrate for dephosphorylation by means of glucose-6-phosphatase (G6Pase, encoded by G6pc), creating a futile cycle [4].

Measurement of free NADP in ultrafiltrates confirms that in normal erythrocytes almost all NADP is bound to cytosolic proteins. In glucose-6-phosphate dehydrogenase-deficient erythrocytes unbound NADP is significantly higher than in normal red cells and the NADP+/NADPH ratio is largely in favor of the oxidized form. In normal and glucose-6-phosphate dehydrogenase-deficient erythrocytes essentially all NAD (bound and unbound) is in the oxidized state. About 50% of the total amount of NAD (NAD+ + NADH) is free in the cytosol, with a NAD+/NADH ratio greater than 100 [2].

- References :

[1] **Dalziel K, Barton S, Marks R.** *The effects of isotretinoin on follicular and sebaceous gland differentiation.* (1987) Br J Dermatol. Sep;117(3):317-23.
[2] **Canepa L, Ferraris AM, Miglino M, Gaetani GF.**
Bound and unbound pyridine dinucleotides in normal and glucose-6-phosphate dehydrogenase-deficient erythrocytes. (1991) Biochim Biophys Acta. May 24;1074(1):101-4.
[3] **Thiel G, Al Sarraj J, Stefano L.** *cAMP response element binding protein (CREB) activates transcription via two distinct genetic elements of the human glucose-6-phosphatase gene.* (2005) BMC Mol Biol. Jan 19;6(1):2.
[4] **Lam TK, Gutierrez-Juarez R, Pocai A, Rossetti L.**
Regulation of blood glucose by hypothalamic pyruvate metabolism. (2005) Science. Aug 5;309(5736):943-7.

5.20 (Ro)accutane and growth failure :

- Premature epihyseal closure and decreased growth velocity :

Hoffman la Roche itself has found premature epiphyseal closure in association with the exposure of (Ro)accutane in human subjects [0]. Several independent case reports indicate (Ro)accutane induced premature epiphyseal closure and a slower growth velocity [1 and more].

- References:

[0] **No author** *Roche official complete US Accutane Product information*
www.rocheusa.com/products/accutane/pi.pdf
[1] **Inamo Y, Suzuki T, Mugishima H.** *A case of growth failure caused by 13-CIS-retinoic acid administration after bone marrow transplantation for neuroblasoma.* (1999) Endocr J. Mar;46 Suppl:S113-5.

5.21 (Ro)accutane and hairloss/hair-thinning :

Hair loss, reversible and irreversible, have been found in human acne-subjects exposed to (Ro)accutane [1 and more].

J Invest Dermatol. 2005 Jun;124(6):1119-26. Related Articles, Links
Towards dissecting the pathogenesis of retinoid-induced hair loss: all-trans retinoic acid induces premature hair follicle regression (catagen) by upregulation of transforming growth factor-beta2 in the dermal papilla.
Foitzik K, Spexard T, Nakamura M, Halsner U, Paus R.
Department of Dermatology, University Hospital Hamburg-Eppendorf, University of Hamburg, Hamburg, Germany.

Diffuse hair loss ranks among the most frequent and psychologically most distressing adverse effects of systemic therapy with retinoids, which severely limits their therapeutic use even where clinically desired. Since the underlying mechanisms of retinoid-induced effluvium are as yet unknown, we have investigated the influence of the prototypic retinoid all-trans retinoic acid (ATRA, tretinoin) on the growth of human scalp hair follicles (HF) in culture. HF in the anagen VI stage of the hair cycle were cultured in the presence of 10(-8) or 10(-10) M ATRA. Compared with controls, hair shaft elongation declined significantly already after 2 d in the ATRA-treated group, and approximately 80% of the ATRA-treated HF had prematurely entered catagen-like stage at day 6, compared with 30% in the control group. This corresponded to an upregulation of apoptotic and a downregulation of Ki67-positive cells in ATRA-treated HF. Since transforming growth factor (TGF)-beta has been implicated as a key inducer of catagen, we next studied whether ATRA treatment had any effect on follicular expression. TGF-beta2 immunoreactivity was detected in the outer root sheath of anagen VI scalp HF. In catagen follicles, TGF-beta2 was also expressed in the regressing epithelial strand. After 4 d of ATRA treatment, TGF-beta2 was significantly upregulated in anagen HF in the dermal papilla (DP) and the dermal sheath, 7, and TGF-beta neutralizing antibody partially abrogated at RA induced hair growth inhibition. Real-time PCR confirmed a significant upregulation of TGF-beta2 transcripts in ATRA-treated hair bulbs. This study is the first to provide direct evidence that ATRA can indeed induce a catagen-like stage in human HF and suggests that this occurs, at least in part, via upregulation of TGF-beta2 in the DP. Therefore, topical TGF-beta2/TGF-beta receptor II antagonists deserve to be explored for the prevention and management of retinoid-induced hair loss.

PMID: 15955085 [PubMed - indexed for MEDLINE]

The regression phase of the hair cycle (catagen) is an apoptosis-driven process accompanied by terminal differentiation, proteolysis, and matrix remodeling. As an inhibitor of keratinocyte proliferation and inductor of keratinocyte apoptosis, transforming growth factor beta1 (TGF-beta1) has been proposed to play an important role in catagen regulation. This is suggested, for example, by maximal expression of TGF-beta1 and its receptors during late anagen and the onset of catagen of the hair cycle. We examined the potential involvement of TGF-beta1 in catagen control. We compared the first spontaneous entry of hair follicles into catagen between TGF-beta1 null mice and age-matched wild-type littermates, and assessed the effects of TGF-beta1 injection on murine anagen hair

follicles in vivo. At day 18 p.p., hair follicles in TGF-beta1 -/- mice were still in early catagen, whereas hair follicles of +/+ littermates had already entered the subsequent resting phase (telogen). TGF-beta1-/- mice displayed more Ki-67-positive cells and fewer apoptotic cells than comparable catagen follicles from +/+ mice. In contrast, injection of TGF-beta1 into the back skin of mice induced premature catagen development. In addition, the number of proliferating follicle keratinocytes was reduced and the number of TUNEL + cells was increased in the TGF-beta1-treated mice compared to controls. Double visualization of TGF-beta type II receptor (TGFRII) and TUNEL reactivity revealed colocalization of apoptotic nuclei and TGFRII in catagen follicles. These data strongly support that TGF-beta1 ranks among the elusive endogenous regulators of catagen induction in vivo, possibly via the inhibition of keratinocyte proliferation and induction of apoptosis. Thus, TGF-betaRII agonists and antagonists may provide useful therapeutic tools for human hair growth disorders based on premature or retarded catagen development (effluvium, alopecia, hirsutism) [2].

- Nail dystrophy :

Nail dystrophy has been seen in association with (Ro)accutane exposure in human acne subjects. Median canaliform nail dystrophy typically appears as a central nail groove, beginning at or distal to the proximal nail fold, from which small lateral fissures may be found [3 and more].

- References :

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[2] **Foitzik K, Lindner G, Mueller-Roever S, Maurer M, Botchkareva N, Botchkarev V, Handjiski B, Metz M, Hibino T, Soma T, Dotto GP, Paus R.** *Control of murine hair follicle regression (catagen) by TGF-beta1 in vivo.* (2000) FASEB J. Apr;14(5):752-60.
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Familial median canaliform nail dystrophy. (2005) Cutis. Mar;75(3):161-5.

5.22 (Ro)accutane and hearing impairment /tinnitus :

Hoffman la Roche itself has found tinnitus and hearing impairment to be associated with (Ro)accutane exposure in human subjects [0].

One result from (Ro)accutane exposure in acne subjects may be lasting tinnitus, or hearing impairment. The thyroid receptor isoform beta2 (TRbeta2) was found to be inhibited by 50-70 % [1]. The TRbeta2 isoform also is highly expressed in the inner ear and is involved in the regulation of auditory function [2]. A decrease in amplitudes for both ears were found in 3 out of 33 acne-subjects (~10%) after three weeks of exposure. It is suggested that these subclinical changes may be due to an isotretinoin-induced synaptic malfunction or to a conduction defect in the auditory nerve fibers [3]. It remains to be answered what frequencies and degrees of severity that are reached when measured after 4 months of exposure in human acne-subjects.

- Divergent roles for thyroid hormone receptor beta isoforms in the endocrine axis and auditory system :

J Clin Invest. 1999 Aug;104(3):291-300.
Abel ED, Boers ME, Pazos-Moura C, Moura E, Kaulbach H, Zakaria M, Lowell B, Radovick S, Liberman MC, Wondisford F.
Thyroid Unit, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215, USA.

Thyroid hormone receptors (TRs) modulate various physiological functions in many organ systems. The TR alpha and TR beta isoforms are products of 2 distinct genes, and the beta 1 and beta 2 isoforms are splice variants of the same gene. Whereas TR alpha 1 and TR beta 1 are widely expressed, expression of the TR beta 2 isoform is mainly limited to the pituitary, triiodothyronine-responsive TRH neurons, the developing inner ear, and the retina. Mice with targeted disruption of the entire TR beta locus (TR beta-null) exhibit elevated thyroid hormone levels as a result of abnormal central regulation of thyrotropin, and also develop profound hearing loss. To clarify the contribution of the TR beta 2 isoform to the function of the endocrine and auditory systems in vivo, we have generated mice with targeted disruption of the TR beta 2 isoform. TR beta 2-null mice have preserved expression of the TR alpha and TR beta 1 isoforms. They develop a similar degree of central resistance to thyroid hormone as TR beta-null mice, indicating the important role of TR beta 2 in the regulation of the hypothalamic-pituitary-thyroid axis. Growth hormone gene expression is marginally reduced. In contrast, TR beta 2-null mice exhibit no evidence of hearing impairment, indicating that TR beta 1 and TR beta 2 subserve divergent roles in the regulation of auditory function.

PMID: 10430610 [PubMed - indexed for MEDLINE]

- References :

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf
[1] **Perez P, Sanchez-Pacheco A, Pascual A, Aranda A.** *Retinoic acid decreases thyroid hormone receptor expression in pituitary GH1 cells.* (1991) Biochem Biophys Res Commun. Nov 27;181(1):9-15.
[2] **Abel ED, Boers ME, Pazos-Moura C, Moura E, Kaulbach H, Zakaria M, Lowell B, Radovick S, Liberman MC, Wondisford F.** *Divergent roles for thyroid hormone receptor beta isoforms in the endocrine axis and auditory system.* (1999) J Clin Invest. Aug;104(3):291-300.
[3] **Nikiforidis G, Tsambaos D, Karamitsos D, Koutsojannis C, Georgiou S.** *Effects of oral isotretinoin on human auditory brainstem response.* Dermatology. 1994;189(1):62-4.

Dermatology. 1994;189(1):62-4. Related Articles, Links
Effects of oral isotretinoin on human auditory brainstem response.
Nikiforidis G, Tsambaos D, Karamitsos D, Koutsojannis C, Georgiou S.

Department of Medical Physics, University of Patras, Greece.

BACKGROUND: Accumulating evidence suggests that synthetic retinoids may be capable

of affecting the differentiation and growth of nervous tissue in vivo and in vitro. On the other hand, adverse reactions concomitant with brainstem involvement definitely or probably related to oral retinoid therapy have been reported in a small number of patients. OBJECTIVE: The purpose of the present study was to substantiate the possible effects of oral isotretinoin on the synaptic activity and propagation of action potentials along the nerve fibers. METHODS: The auditory brainstem response of 33 patients with severe nodulocystic acne before and 3 weeks after the onset of oral isotretinoin administration was investigated using auditory evoked potentials. RESULTS: The paired analysis of the response variables before and after treatment failed to reveal any statistically significant differences. However, a marked increase in latencies and interpeak latencies and a decrease in amplitudes for both ears were found in 3 patients after therapy. CONCLUSIONS: It seems reasonable to suggest that these subclinical changes may be due to an isotretinoin-induced synaptic malfunction or to a conduction defect in the auditory nerve fibers.

PMID: 8003790 [PubMed - indexed for MEDLINE]

5.23 (Ro)accutane and a weakened immune-defense :

Hoffman la Roche itself has found delayed wound healing, decreases in white and red blood cell parameters and a number of inflammations occuring a different organs [0]. It is here suggested that a (Ro)accutane exposure in human subjects may permanently affect the immune-defense. Vitamin A status plays an important role in reducing infectious disease morbidity and mortality by enhancing immunity, an effect that is partly mediated by macrophages [1].

- Effects on macrophage function :

J774 macrophages efficiently take up chylomicron remnant retinyl esters and retinol-binding protein (retinol-RBP) bound retinol by specific and saturable mechanisms. The binding of (125)I-RBP to plasma membrane vesicles demonstrated that the macrophage receptor had a similar binding affinity, as was discovered previously for other cells. The B(max) for the macrophages was smaller than the values reported for placenta, bone marrow, and kidney, but larger than that reported for liver. The J774 cells also bound and took up [(3)H]retinol-RBP. Approximately 50 to 60% of the uptake may compete with excess unlabeled retinol-RBP and approximately 30 to 40% with excess transtyrethin. Following the uptake of [(3)H]retinol-RBP, an extensive esterification occurred: After 5 hours of incubation, 77.8 +/- 3.9% (SD; n = 3) of the cellular radioactivity was recovered as retinyl esters. The J774 cells also demonstrated saturable binding of chylomicron remnant [(3)H]retinyl esters, and a continuous uptake at 37 degrees C followed by an extensive hydrolysis of the retinyl esters. Binding could be inhibited by approximately 50% by excess unlabeled low density lipoprotein (LDL). In addition, lipoprotein lipase increased the binding of chylomicron remnant [(3)H]retinyl esters by approximately 30% and the uptake of chylomicron remnant [(3)H]retinyl ester by more than 300%. Furthermore, because sodium chlorate reduced binding with 40% and uptake with 55%, the results suggest that proteoglycans are involved in the uptake. Thus, the results suggest that both LDL receptor and LDL-related protein are involved in the uptake of chylomicron remnant [(3)H]retinyl

ester in macrophages [1].

- References :

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www.rocheusa.com/products/accutane/pi.pdf
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(1999) J Nutr Biochem. Jun;10(6):345-52.

5.24 (Ro)accutane and intracranial hypertension :

To complete...

- References :

5.25 (Ro)accutane and joint inflammation :

To complete...

- References :

[0] **No author** *Roche official complete US Accutane Product information*
www.rocheusa.com/products/accutane/pi.pdf

Mutat Res. 2004 Jul 13;551(1-2):199-211. Related Articles, Links
Molecular imaging of the transcription factor NF-kappaB, a primary regulator of stress response.
Carlsen H, Alexander G, Austenaa LM, Ebihara K, Blomhoff R.
Department of Nutrition, Faculty of Medicine, University of Oslo, P.O. Box 1046 Blindern, N-0316 Oslo, Norway.

A wide range of environmental stress and human disorders involves inappropriate regulation of NF-kappaB, including cancers and numerous inflammatory conditions. We have developed transgenic mice that express luciferase under the control of NF-kappaB, enabling real-time non-invasive imaging of NF-kappaB activity in intact animals. We show that, in the absence of stimulation, strong, intrinsic luminescence is evident in lymph nodes in the neck region, thymus, and Peyer's patches. Treating mice with stressors, such as TNF-alpha, IL-1alpha, or lipopolysaccharide (LPS) increases the luminescence in a tissue-specific manner, with the strongest activity observable in the skin, lungs, spleen, Peyer's patches, and the wall of the small intestine. Liver, kidney, heart, muscle, and adipose tissue exhibit less intense activities. Exposure of the skin to a low dose of UV-B radiation increases luminescence in the exposed areas. In ocular experiments, LPS- and TNF-alpha

injected NF-kappaB-luciferase transgenic mice exhibit a 20-40-fold increase in lens NF-kappaB activity, similar to other LPS- and TNF-alpha-responsive organs. Peak NF-kappaB activity occurs 6h after injection of TNF-alpha and 12h after injection of LPS. Peak activities occur, respectively, 3 and 6h later than that in other tissues. Mice exposed to 360J/m(2) of UV-B exhibit a 16-fold increase in NF-kappaB activity 6h after exposure, characteristically similar to TNF-alpha-exposed mice. Thus, in NF-kappaB-luciferase transgenic mice, NF-kappaB activity also occurs in lens epithelial tissue and is activated when the intact mouse is exposed to classical stressors. Furthermore, as revealed by real-time non-invasive imaging, induction of chronic inflammation resembling rheumatoid arthritis produces strong NF-kappaB activity in the affected joints. Finally, we have used the model to demonstrate NF-kappaB regulation by manipulating the Vitamin A status in mice. NF-kappaB activity is elevated in mice fed a Vitamin A deficient (VAD) diet, and suppressed by surplus doses of retinoic acid (RA). We thus demonstrate the development and use of a versatile model for monitoring NF-kappaB activation both in tissue homogenates and in intact animals after the use of classical activators, during disease progression and after dietary intervention.

Publication Types:
Review

PMID: 15225593 [PubMed - indexed for MEDLINE]

Arthritis Rheum. 2005 May;52(5):1381-91. Related Articles, Links
Elevated CXCL16 expression by synovial macrophages recruits memory T cells into rheumatoid joints.
van der Voort R, van Lieshout AW, Toonen LW, Sloetjes AW, van den Berg WB, Figdor CG, Radstake TR, Adema GJ.
Nijmegen Center for Molecular Life Sciences, University Medical Center St. Radboud, Nijmegen, The Netherlands.

OBJECTIVE: Directional migration of leukocytes is orchestrated by the regulated expression of chemokine receptors and their ligands. The receptor CXCR6 is abundantly expressed by Th1-polarized effector/memory lymphocytes accumulating at inflammatory sites. This study was undertaken to examine the presence of CXCR6+ T cells and of CXCL16, the only ligand for CXCR6, in the joints of patients with rheumatoid arthritis (RA). METHODS: Flow cytometry analysis of the expression of CXCR6 by peripheral blood and synovial fluid (SF) T cells. In addition, by performing conventional and real-time reverse transcriptase-polymerase chain reaction, immunohistochemistry, and enzyme-linked immunosorbent assay, we determined the expression of CXCL16 and its protease ADAM-10 within synovium and by cultured macrophages. SF T cell migration was studied with the Transwell system. RESULTS: Accumulation of CXCR6+ T cells within RA SF coincided with highly elevated levels of CXCL16+ macrophages. In vitro studies revealed that monocytes started to express CXCL16 upon differentiation into macrophages, and that RA SF and tumor necrosis factor (TNF) enhanced CXCL16 expression. Moreover, RA patients responding to anti-TNF therapy showed a strongly decreased CXCL16 expression, whereas nonresponding patients did not. Interestingly, ADAM-10, a recently identified protease of CXCL16, was abundantly expressed by CXCL16+ macrophages in vitro and in RA in vivo, which resulted in increased levels of cleaved CXCL16 in RA SF relative to controls. Finally, CXCR6+ T cells from RA SF were attracted by CXCL16. CONCLUSION:

These data provide evidence that enhanced production of CXCL16 in RA synovia leads to recruitment of CXCR6+ memory T cells, thereby contributing to the inflammatory cascade associated with RA pathology.

PMID: 15880344 [PubMed - indexed for MEDLINE]

5.26 (Ro)accutane and decreased libido :

- Male libido :

Decreased erections, decreased potence, possible erectile dysfunction

Hoffman la Roche itself has not seen a clear association between human exposure to (Ro)accutane and decreased potence [0]. In rats, androgens influenced the penile reflex arc, corpus cavernosum, and the perineal striated muscles. In reflex erection, erectile response to ES and penile NOS activity in the rat, T seems to be first converted to DHT, the more active androgen modality [1]. It is shown in various studies that the 5-alpha-r is inhibited in human subjects exposed to (Ro)accutane, and the inhibition was measured up to 50% in doses comparable to what is used in acne "therapy" [3]. The 5-alpha-r converts testosterone to the more potent androgen dihydrotestosterone. Nitric Oxide Synthase (NOS) has also found to be significantly impaired by supraphysiological doses of retinoic acid [4].

Under all penile conditions, systemic 5-HT levels were higher than those registered in the cavernous serum. Although 5-HT does not appear to be involved in postsynaptic transmission in the HCC, our results may provide evidence for a physiological significance of 5-HT in the control of penile flaccidity and detumescence [2]. Both systemic 5-HT levels and 5-HT receptor expression is suggested to be significantly lower in human subjects exposed to (Ro)accutane, due to inhibition of Sp1 and NF-Y phosphorylation, two transcription factors that are found to bind to the promoter regions of the TPH gene, and the 5-HT-receptors.

Sperm production, sperm count and sperm quality

Studies in the 1980s have frequently denied effects on spermatogenesis mediated by (Ro)accutane. One study found that "the percentage of morphologically normal spermatozoa was slightly reduced at the end of therapy; 12 weeks after completion of therapy it was significantly reduced (P less than 0.05)."

The authors conclude that "This could, however, be due to the short elimination half-life of 13-cis retinoic acid and have nothing to do with the drug. On impulse cytophotometry the spermatozoa DNS showed no significant changes. According to the results of the cytophotometric examination, contraceptive measures for men during treatment with 13-cis-retinoic-acid need not be made obligatory" [5].

Later research has found more clear links between retinoic acid and the spermatogenesis. Targeted mutagenesis of the retinoic acid receptor alpha (RAR alpha) gene has revealed its essential role in spermatogenesis. Although cells in all stages of spermatogenesis were detected in RAR alpha(-/-) testes, there was an increase in degenerating pachytene spermatocytes and a temporary developmental arrest in step 8-9 spermatids in the first wave of spermatogenesis, a delay in the onset of the second wave, and a temporary arrest in preleptotene to leptotene spermatocytes in the first, second, and third waves [6]. Also in other species a clear relation between retinoids and spermatogenesis has been found. The effect of retinoids on

spermatogenesis in adult male gerbils (Gerbillus cheesemani) was studied using light and electron microscopy. Treatment with either 13-cis-retinoic acid or retinol acetate was given for 6 weeks and their effects were compared with controls. It was found that 13-cis-retinoic acid induced almost complete cessation of spermatogenesis and produced alterations in the cytoplasm of Leydig cells [7].

- Female libido :

Hoffman la Roche itself has found abnormal menses in association with exposure in human female subjects [0].

- Conclusions :

Male potency is with high certainity significantly decreased due to exposure of (Ro)accutane. Contibuting effects are suggested to be a significantly lessened formation of dihydrotestosterone, inhibition of NOS, inhibition of TPH and serotonerg receptor expression and possibly also downregulation of androgen receptors.

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5.27 (Ro)accutane and liver degeneration :

To complete...

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www.rocheusa.com/products/accutane/pi.pdf

Biochim Biophys Acta. 1984 Feb 9;792(2):110-7. Related Articles, Links

Activities of fatty acid desaturases and fatty acid composition of liver microsomes in rats fed beta-carotene and 13-cis-retinoic acid.

Alam SQ, Alam BS, Chen TW.

The fatty acid composition of microsomal lipids and the activities of delta 9- and delta 6-desaturases in liver microsomes of rats fed diets supplemented with beta-carotene and two levels of 13-cis-retinoic acid were studied. Four groups of male, weanling rats were fed semipurified diets containing 0 or 100 mg beta-carotene per kg diet, and 20 or 100 mg 13-cis-retinoic acid per kg diet. After 11 weeks of feeding, the rats were killed, liver microsomes were prepared and assayed for delta 9-desaturase and delta 6-desaturase activities. The activity of delta 9-desaturase was lower in liver microsomes of rats fed beta-carotene-supplemented diet or the diet supplemented with the higher level of 13-cis-retinoic acid. Microsomal delta 6-desaturase activity was, however, higher in liver of rats fed 13-cis retinoic acid; there was no effect of beta-carotene on delta 6-desaturase activity. The fatty acid compositional data on total lipids of liver microsomes were consistent with the diet-induced changes in fatty acid desaturases. Phospholipid composition of liver microsomes was also altered as a result of feeding beta-carotene or 13-cis-retinoic acid-containing diets. The proportions of phosphatidylethanolamine were generally higher, whereas those of phosphatidylcholine were lower in the experimental groups as compared with the control.

PMID: 6582937 [PubMed - indexed for MEDLINE]

J Biochem (Tokyo). 2005 Jun;137(6):703-9. Related Articles, Links
Analysis of human UDP-glucose dehydrogenase gene promoter: identification of an Sp1 binding site crucial for the expression of the large transcript.
Vatsyayan J, Peng HL, Chang HY.
Institute of Molecular Medicine, National Tsing Hua University, Hsin Chu 300, Taiwan..

UDP-glucose dehydrogenase (UGDH) catalyzes the conversion of UDP-glucose to UDP-glucuronic acid, which is required in liver for the excretion of toxic compounds, and for the biosynthesis of complex carbohydrates, such as hyaluronan, in many cell types. Analysis of a human EST database, as well as the results of a 5'-RACE experiment, have revealed the presence of two transcription start sites approximately 160 bp apart in the human UGDH gene confirming previous Northern hybridization results. To delineate the regions in the UGDH promoter required for regulating the expression of the gene, in particular the synthesis of the large transcript, serial deletions of the 2.1-kb UGDH promoter region were constructed and their activities determined by the firefly luciferase reporter gene assay. Our results indicate that the region from nucleotide position -486 to -632 relative to the start of the small transcript contains positive regulatory elements that contribute to gene expression. Mithramycin A, an inhibitor of transcription factor Sp1, abrogates the promoter activity, suggesting the involvement of this specific protein in UGDH expression. By using site-directed mutagenesis, we analyzed the functional contribution of three putative Sp1 binding elements within this region. A mutation at position -564 demonstrated that this site serves as an enhancing element in both HepG2 and HeLa cells. The complex formation pattern revealed by an electrophoretic mobility shift assay as well as an anti-Sp1 antibody-mediated supershift assay confirmed the identity of this GC box as an Sp1 binding motif. Our results thus identify an alternative transcription start site on the UGDH promoter, and locate the cis-element that greatly enhances the basal transcriptional activity of UGDH gene.

PMID: 16002992 [PubMed - in process]

Infect Immun. 2005 Jul;73(7):4007-16. Related Articles, Links
Involvement of up-regulated CXC chemokine ligand 16/scavenger receptor that binds phosphatidylserine and oxidized lipoprotein in endotoxin-induced lethal liver injury via regulation of T-cell recruitment and adhesion.
Xu H, Xu W, Chu Y, Gong Y, Jiang Z, Xiong S.

Department of Immunology and Key Laboratory of Molecular Medicine of Ministry of Education, Shanghai Medical College of Fudan University, 138 Yi Xue Yuan Road, Shanghai 200032, People's Republic of China.

A murine model of endotoxin-induced lethal liver injury induced by Mycobacterium bovis BCG plus lipopolysaccharide (LPS) has been widely accepted and used. It has been reported that T cells play an important role in the pathogenesis of liver damage in this model. However, the precise mechanisms involved in regulation of the trafficking of effector T cells need to be elucidated. In the present study, we first reported that CXCL16/SR-PSOX (CXC chemokine ligand 16/scavenger receptor that binds phosphatidylserine and oxidized lipoprotein), a chemokine containing both membrane-anchored and soluble forms, was strongly up-regulated and predominantly distributed in the vascular endothelium in the injured liver tissue in the model. The secretory and membrane-anchored CXCL16/SR-PSOX functioned as a chemokine and an adhesive molecule, respectively, to attract T cells to a tumor necrosis factor alpha-activated endothelial cell line (SVEC) in vitro. To further identify the pathophysiological roles of CXCL16/SR-PSOX in the liver injury, the anti-CXCL16 antibody was administered to the BCG-primed mice before LPS challenge in vivo. Significant protection effects were observed with 70% of mice regarding lethality, the massive necrosis in the liver was reduced, and the intrahepatic infiltrating T cells were significantly inhibited. Taken together, these findings strongly suggest that functional CXCL16/SR-PSOX, as both a chemokine and an adhesion molecule, may be involved in the pathogenesis of the endotoxin-induced lethal liver injury via recruitment and adhesion of activated T cells to the vascular endothelium.

PMID: 15972488 [PubMed - indexed for MEDLINE]

5.28 (Ro)accutane and memory impairment :

A decreased hippocampal cell-survival, and loss of hippocampal cells, have been found in rats exposed to (Ro)accutane [1 and 4]. The hippocampus is an area that in human is suggested to be involved in short term memory storage and retrieval [2, 3 and more].

To complete...

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5.29 (Ro)accutane and loss of muscle mass :

There are frequent case reports of muscle damage and loss of muscle mass in association with (Ro)accutane exposure in human subjects [3]. The underlying causes are likely several. There is no data of long term effects on ratios of muscle mass or muscular dystrophy compared to control in subjects exposed to the toxin. A long-term relative loss of muscle mass is here suggested.

- Loss of muscle mass through inhibition of laminin mediated structural changes in the muscle extracellular matrix :

Laminin-2, is found to be essential for the structural integrity of muscle extracellular matrix, and a modulation of laminin results in muscle dystrophy [1]. Retinoic acid exposure decreased the synthesis of fibronectin and laminin and inhibited the migration of all three mesotheliomas on substrates of fibronectin and laminin of three mesothelioma cell cultures of different histotype [2].

To complete...

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5.30 (Ro)accutane and numbness in extremities :

To complete...

- References :

5.31 (Ro)accutane and ocular (side)-effects :

- Observed wide ocular effects, short and also suggested long term degenerative effects :

Some of the Accutane induced ocular side affects are not reversible when the drug is stopped [10 and more]. Hoffman la Roche itself sees the following associations related to vision in human subjects exposed to (Ro)accutane: corneal opacities, decreased night vision which may persist, cataracts, color vision disorder, conjunctivitis, dry eyes, eyelid inflammation, keratitis, optic neuritis, photophobia and visual disturbances [0]. Non published data suggest (Ro)accutane induced severe tunnelvision in a number of patients. The mechanism for this event is unknown. Lasting increased UV-sensitivity has been observed [10 and more].

Figure 4 : *Lissamine green corneal staining of a dry eye patient taking isotretinoin. Photo courtesy of Eric Donnenfeld, M.D.*

Figure 5 : *Fluorescein staining of the central cornea in a patient taking isotretinoin. Photo courtesy of Eric Donnenfeld, M.D.*

Isotretinoin is currently used in human subjects for severe recalcitrant nodular acne and has a variety of by independent research well known associated ocular side effects [1 and more]. Observations demonstrate that some of the Accutane induced ocular side affects are not reversible when the drug is stopped [10 and more]. Accumulating evidence is also pointing out that eye-related side-effects may worsen with time after a (Ro)accutane exposure in human subjects.

Subjects exposed to these drugs often show side effects resembling the symptoms of hypovitaminosis A, namely, among other things night blindness and decreased plasma retinol levels [5].

- Possible oculomotor deficit developing with time after (Ro)accutane exposure in subjects :

Thyroid function is found to be altered in human subjects exposed to retinoic acid, and significant fall in plasma concentrations of thyroxine and triiodthyronine have been found in human subjects exposed to the toxin [6]. This fall is likely explained by a found significant inhibition of thyroid receptor expression in various cell lines in doses related to what could be expected in acne subjects [7]. The long term effects of a hypothyroid condition on vision are not fully evaluated. Thyroid function is suggested to be of importance for oculomotor function and is associtated with ophthalmopathy (ocular-motor deficit) [8, 9].

In adult cats, the Trk receptor isoforms are suggested to be of importance for oculomotor function and exert an influence on the normal operation of the oculomotor circuitry. A similiar function is likely to be present in human. TrkA, TrkB, and TrkC immunopositive cells were found in similar percentages in the oculomotor and in the trochlear nuclei. In the abducens nucleus, however, a significantly higher percentage of cells expressed TrkB than the other two receptors, among both motoneurons (81.8%) and internuclear neurons (88.4%) [11]. Trk receptor isoform gene-expression is suggested to be modulated by among other things thyroxin [12 and 13].

1,25 dihydroxyvitamin D metabolites were found to be significantly lower in human subjects exposed to (Ro)accutane after exposure, implicating a clinical vitamin D deficiency [14 and more]. In the human eye the vitamin D receptor (VDR) was found to be expressed in several areas. Human retinal photoreceptors express vitamin D receptor (VDR), plasma membrane calcium pump and calcium-binding protein epitopes were detected in the outer nuclear layer. VDR epitopes were also seen in lens epithelium. Some immunostaining for VDR, PMCA and calbindin-D28k also was present in the endothelium and in the basal epithelium of the cornea. All three proteins were detected in some cells of the ganglion cell layer, the inner nuclear layer, and the retinal pigment epithelium [16]. Except for inducing the VDR receptor, vitamin D is found to induce Trk receptor isotypes in various tissue [15]. This may also be valid for Trk receptors that are related to the eye. The long-term effects of a clinically significant vitamin D-deficiency on the retinal photoreceptors, the eye-lens, and eye function are unknown.

Little is known about possible long-term ocular side-effects, or possible additional ocular effects appearing with time after exposure. There are no studies showing how photreceptor cell

maintenance and signaling is affected long term, no studies on how oculo-motor capacity is affected long term, and no studies of how the crystal lens structure is affected long-term in subjects exposed to retinoic acid.

- Degeneration of crystal lens structure :

Vitamin A has been found to be inducing regeneration in the lens of adult mammals [17]. A state with vitamin A insufficiency, both circulating and lack of adequate metabolism may therefore affect the lens negative long term.

- Night blindness :

Isotretinoin can cause nyctalopia (night blindness) [2, 5 and more]. Even a single dose of isotretinoin slowed the recovery of rod signaling after exposure to an intense bleaching light, and that rhodopsin regeneration was markedly slowed. When only a single dose was given, rod function recovered to normal within several days. Rods and cones both showed slow recovery from bleach after isotretinoin in rats and in mice [3].

Retinitis pigmentosa (RP) is a heterogeneous group of retinal dystrophies characterized by photoreceptor cell degeneration. RP causes night blindness, a gradual loss of peripheral visual fields, and eventual loss of central vision [4].

- Dry eye syndrome :

Persistent dry eye syndrome in human subjects has been observed [10 and more].
Location alkaline phosphatase: 2q37.1
Ophthalmic Genet. 2001 Sep;22(3):133-54. Related Articles, Links
Update on the molecular genetics of retinitis pigmentosa.
Wang Q, Chen Q, Zhao K, Wang L, Wang L, Traboulsi EI.
Center for Molecular Genetics, Lerner Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195, USA. wangq2@ccf.org

Retinitis pigmentosa (RP) is a heterogeneous group of retinal dystrophies characterized by photoreceptor cell degeneration. RP causes night blindness, a gradual loss of peripheral visual fields, and eventual loss of central vision. Advances in molecular genetics have provided new insights into the genes responsible and the pathogenic mechanisms of RP. The genetics of RP is complex, and the disease can be inherited in autosomal dominant, recessive, X-linked, or digenic modes. Twenty-six causative genes have been identified or cloned for RP, and an additional fourteen genes have been mapped, but not yet identified. Eight autosomal dominant forms are due to mutations in RHO on chromosome 3q21-24, RDS on 6p21.1-cen, RP1 on 8p11-21, RGR on 10q23, ROM1 on 11q13, NRL on 14q11.1-11.2, CRX on 19q13.3, and PRKCG on 19q13.4. Autosomal recessive genes include RPE65 on chromosome 1p31, ABCA4 on 1p21-13, CRB1 on 1q31-32.1, USH2A on 1q41, MERTK on 2q14.1, SAG on 2q37.1, RHO on 3q21-24, PDE6B on 4p16.3, CNGA1 on 4p14-q13, PDE6A on 5q31.2-34, TULP1 on 6p21.3, RGR on 10q, NR2E3 on 15q23, and RLBP1 on 15q26. For X-linked RP, two genes, RP2 and RP3 (RPGR), have been cloned. Moreover, heterozygous mutations in ROM1 on 11q13, in combination with heterozygous mutations in RDS on 6p21.1-cen, cause digenic RP (the two-locus mechanism). These exciting molecular discoveries have defined the genetic pathways underlying the pathogenesis of retinitis pigmentosa, and have raised the hope of genetic testing for RP and the development of new avenues for therapy.

Publication Types:

Review

PMID: 11559856 [PubMed - indexed for MEDLINE]

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Dev Growth Differ. 2002 Oct;44(5):391-4. Related Articles, Links

Expression and role of retinoic acid receptor alpha in lens regeneration.

Tsonis PA, Tsavaris M, Call MK, Chandraratna RA, Del Rio-Tsonis K.
Laboratory of Molecular Biology, Department of Biology, University of Dayton, Dayton, OH 45469-2320, USA. Panagiotis.Tsonis@notes.udayton.edu

The role of retinoids in eye development has been well studied. Retinoids and their receptors regulate gene expression and morphogenesis of the eye. In this study, a highly specific antagonist of retinoic acid receptor (RAR)-alpha was used in an attempt to study

its function in lens regeneration. It was found that this antagonist inhibited lens regeneration and lens fiber differentiation. It was also shown that RAR-alpha is expressed in the lens during the process of regeneration. These results indicate that different RAR might have unique as well as redundant effects and patterns of expression in the regenerating lens.

PMID: 12392572 [PubMed - indexed for MEDLINE]

J Cataract Refract Surg. 2005 Mar;31(3):595-606. Related Articles, Links
Upregulation of alphavbeta6 integrin, a potent TGF-beta1 activator, and posterior capsule opacification.
Sponer U, Pieh S, Soleiman A, Skorpik C.
Department of Ophthalmology and Optometrics, General Hospital, University of Vienna, Austria.
PURPOSE: To identify the predominant activation pathway of transforming growth factor (TGF)-beta1 in the lens capsule, studying the spatial and temporal expression pattern of alphavbeta6 and thrombospondin-1. Other PCO-related proteins were also studied. SETTING: Departments of Ophthalmology and Optometrics and Clinical Pathology, Medical School, University of Vienna, Vienna, Austria. METHODS: The lens capsules of 12 human donor eyes were cultivated in a protein-free medium for up to 28 days (cultivated lens capsules [CLCs]) after lens extraction. Ten intact lenses (ILs) served as the control group and were also cultured. During the culture period, cell dynamics were observed by phase-contrast microscopy. Proteins were detected by double immunofluorescence on frozen sections. RESULTS: In ILs, alphavbeta6 was absent but 91.6% of the CLCs showed extensive staining. Remnant lens epithelial cells (LECs) expressed alphavbeta6 immediately after lens extraction. The alphavbeta6 was detected throughout the culture period in all regions of the capsule. Thrombospondin-1 was absent in ILs and CLCs, suggesting that this protein is not significant in TGF-beta1 activation in the lens. Transforming growth factor-beta1 was abundantly expressed in all ILs and CLCs, slightly decreasing during intensive LEC proliferation and migration. The TGF-beta receptor II (RII) was expressed equally in all specimens, decreasing with culture time. Nonresident extracellular matrix proteins and alpha-smooth muscle actin were partially detected in CLCs but not in ILs. Latent TGF-beta binding protein 1 and collagen III were absent in all specimens. All cells found in the cultures expressed vimentin and alphaB-crystallin (LEC markers). CONCLUSION: Alphavbeta6 is the main activator of TGF-beta1 in the lens capsule and represents a new target for PCO prevention.

PMID: 15811751 [PubMed - indexed for MEDLINE]

Prog Retin Eye Res. 2005 Jan;24(1):87-138. Related Articles, Links
The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina.
SanGiovanni JP, Chew EY.
Division of Epidemiology and Clinical Research, National Eye Insitute, National Institutes of Health, 31 Center Drive, Building 31, Room 6A52, MSC 2510, Bethesda, MD 20892-2510, USA. jpsangio@nei.nih.gov

In this work we advance the hypothesis that omega-3 (omega-3) long-chain polyunsaturated fatty acids (LCPUFAs) exhibit cytoprotective and cytotherapeutic actions

contributing to a number of anti-angiogenic and neuroprotective mechanisms within the retina. omega-3 LCPUFAs may modulate metabolic processes and attenuate effects of environmental exposures that activate molecules implicated in pathogenesis of vasoproliferative and neurodegenerative retinal diseases. These processes and exposures include ischemia, chronic light exposure, oxidative stress, inflammation, cellular signaling mechanisms, and aging. A number of bioactive molecules within the retina affect, and are effected by such conditions. These molecules operate within complex systems and include compounds classified as eicosanoids, angiogenic factors, matrix metalloproteinases, reactive oxygen species, cyclic nucleotides, neurotransmitters and neuromodulators, pro-inflammatory and immunoregulatory cytokines, and inflammatory phospholipids. We discuss the relationship of LCPUFAs with these bioactivators and bioactive compounds in the context of three blinding retinal diseases of public health significance that exhibit both vascular and neural pathology. How is omega-3 LCPUFA status related to retinal structure and function? Docosahexaenoic acid (DHA), a major dietary omega-3 LCPUFA, is also a major structural lipid of retinal photoreceptor outer segment membranes. Biophysical and biochemical properties of DHA may affect photoreceptor membrane function by altering permeability, fluidity, thickness, and lipid phase properties. Tissue DHA status affects retinal cell signaling mechanisms involved in phototransduction. DHA may operate in signaling cascades to enhance activation of membrane-bound retinal proteins and may also be involved in rhodopsin regeneration. Tissue DHA insufficiency is associated with alterations in retinal function. Visual processing deficits have been ameliorated with DHA supplementation in some cases. What evidence exists to suggest that LCPUFAs modulate factors and processes implicated in diseases of the vascular and neural retina? Tissue status of LCPUFAs is modifiable by and dependent upon dietary intake. Certain LCPUFAs are selectively accreted and efficiently conserved within the neural retina. On the most basic level, omega-3 LCPUFAs influence retinal cell gene expression, cellular differentiation, and cellular survival. DHA activates a number of nuclear hormone receptors that operate as transcription factors for molecules that modulate reduction-oxidation-sensitive and proinflammatory genes; these include the peroxisome proliferator-activated receptor-alpha (PPAR-alpha) and the retinoid X receptor. In the case of PPAR-alpha, this action is thought to prevent endothelial cell dysfunction and vascular remodeling through inhibition of: vascular smooth muscle cell proliferation, inducible nitric oxide synthase production, interleukin-1 induced cyclooxygenase (COX)-2 production, and thrombin-induced endothelin 1 production. Research on model systems demonstrates that omega-3 LCPUFAs also have the capacity to affect production and activation of angiogenic growth factors, arachidonic acid (AA)-based vasoregulatory eicosanoids, and MMPs. Eicosapentaenoic acid (EPA), a substrate for DHA, is the parent fatty acid for a family of eicosanoids that have the potential to affect AA-derived eicosanoids implicated in abnormal retinal neovascularization, vascular permeability, and inflammation. EPA depresses vascular endothelial growth factor (VEGF)-specific tyrosine kinase receptor activation and expression. VEGF plays an essential role in induction of: endothelial cell migration and proliferation, microvascular permeability, endothelial cell release of metalloproteinases and interstitial collagenases, and endothelial cell tube formation. The mechanism of VEGF receptor down-regulation is believed to occur at the tyrosine kinase nuclear factor-kappa B (NFkappaB). NFkappaB is a nuclear transcription factor that up-regulates COX-2 expression, intracellular adhesion molecule, thrombin, and nitric oxide synthase. All four factors are associated with vascular instability. COX-2 drives conversion of AA to a number angiogenic and proinflammatory eicosanoids. Our general conclusion is that there is consistent evidence to suggest that omega-3 LCPUFAs may act in a protective role against ischemia-, light-, oxygen-, inflammatory-, and age-associated

pathology of the vascular and neural retina.

Publication Types:
Review

PMID: 15555528 [PubMed - indexed for MEDLINE]

Brain Res. 2005 Jun 7;1046(1-2):207-15. Related Articles, Links
Calpain and N-methyl-d-aspartate (NMDA)-induced excitotoxicity in rat retinas.
Chiu K, Lam TT, Ying Li WW, Caprioli J, Kwong Kwong JM.
Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong.

Calpain-mediated proteolysis has been implicated as a major process in neuronal cell death in both acute insults and the chronic neurodegenerative disorders in the central nerves system. However, activation of calpain also plays a protective function in the early phase of excitotoxic neuronal death. The exact role of calpains in neuronal death and recovery after exposure to N-methyl-D-aspartate (NMDA) is not clearly known. The purpose of present study was to examine the involvement of mu- and m-calpain in NMDA-induced excitotoxicity in the adult rat retina. Increased immunoreactivity of mu-calpain was noted in RGC layer cells and in the inner nuclear layer with maximal expression at 12 h after NMDA injection. This was further confirmed with Western blotting. TdT-mediated biotin-dUTP nick end labeling (TUNEL) positive cells in the inner retina co-localized with moderate or intense mu-calpain immunoreactivity. In contrast, there was no remarkable change in m-calpain immunoreactivity at any time point after NMDA injection. Simultaneous injection of 2 nmol of a calpain inhibitor (calpain inhibitor II) significantly reduced the number of TUNEL-positive cells in the inner retina at 18 h after NMDA injection and preserved RGC-like cells counted at 7 days after injection. The results of this study showed that mu-calpain may be involved in mediating NMDA-induced excitotoxicity in the rat retina and calpain inhibitors may play a therapeutic role in NMDA related disease.

PMID: 15878434 [PubMed - in process]

Cell Death Differ. 2005 Jul;12(7):796-804. Related Articles, Links
Multiple death pathways in retina-derived 661W cells following growth factor deprivation: crosstalk between caspases and calpains.
Gomez-Vicente V, Donovan M, Cotter TG.
1Cell Development and Disease Laboratory, Department of Biochemistry, Biosciences Research Institute, University College Cork, Cork, Ireland.

During development of the mammalian retina, neurons that do not succeed in establishing functional synaptic connections are eliminated by apoptosis, allowing the formation of a finely tuned network. Growth factors play a crucial role in controlling the balance between apoptosis and survival signals not only at developmental stages but also in long-term preservation of retinal functions. In the present work, we explore the apoptotic mechanisms triggered by growth factor deprivation of retina-derived 661W cells. Under serum starvation conditions, these cone photoreceptors underwent cell death with participation of caspase-9, -3 and -12. Interestingly, inhibition of caspases did not prevent apoptosis but only resulted in a temporary delay. We show m-calpain activation in parallel

with caspases, indicating that more than one execution pathway is available to cone photoreceptors. Moreover, crosstalk of the caspase and calpain pathways was detected, suggesting a loop that may act to amplify the apoptotic cascade.Cell Death and Differentiation (2005) 12, 796-804. doi:10.1038/sj.cdd.4401621 Published online 22 April 2005.

PMID: 15846377 [PubMed - in process]

Photochem Photobiol. 2004 Jul-Aug;80:61-71. Related Articles, Links
Identification of genes responsive to UV-A radiation in human lens epithelial cells using complementary DNA microarrays.
Andley UP, Patel HC, Xi JH, Bai F.
Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, 660 S. Euclid Avenue, Campus Box 8096, St. Louis, MO 63110, USA. andley@vision.wustl.edu

UV-A radiation produces cataract in animals, enhances photoaging of the lens and skin and increases the phototoxicity of drugs. However, the nature of genes that are activated or repressed after cellular exposure to UV-A radiation remains enigmatic. Because lens epithelial cells exposed to UV-A radiation undergo apoptosis 4 h after exposure to the stress, we sought to establish the change in gene expression in cells by UV-A radiation using gene expression profiling using complementary DNA microarrays containing about 12 000 genes. We identified 78 genes abnormally expressed in UV-A-irradiated cells (showing >2.5-fold change at $P < 0.05$). These genes are implicated in various biological processes, including signal transduction and nucleic acid binding, and genes encoding enzymes. A majority of the genes were downregulated. Our analysis revealed that the expression of genes for the transcription factors ATF-3 and Pilot increased four-fold, whereas the gene for the apoptosis regulator NAPOR-1 decreased five-fold. These changes were confirmed by real-time quantitative reverse transcriptase-polymerase chain reaction. The calpain large polypeptide 3 (CANP3) gene also increased nine-fold after UV-A radiation. In addition, peroxisomal biogenesis factor 7, glucocorticoid receptor-alpha and tumor-associated calcium signal transducer genes decreased three- to eight-fold. Western blot analysis further confirmed the increase in protein expression of ATF-3 and CANP3 and decreased expression of glucocorticoid receptor-alpha in the irradiated cells. Surprisingly, most of these genes had not been previously shown to be modulated by UV-A radiation. Our results show that human lens epithelial cells respond to a single dose of UV-A radiation by enhancing or suppressing functionally similar sets of genes, some of which have opposing functions, around the time at which apoptosis occurs. These studies support the intriguing concept that activation of competing pathways favoring either cell survival or death is a means to coordinate the response of cells to UV-A stress.

PMID: 15339208 [PubMed - indexed for MEDLINE]

J Zhejiang Univ Sci. 2004 Jun;5(6):743-8. Related Articles, Links
Expression and proteolytic activity of calpain in lens epithelial cells of oxidative cataract.
Xu W, Yao K, Sun ZH, Wang KJ, Shentu XC.
Eye Center, Second Affiliated Hospital, Medical College, Zhejiang University, Hangzhou 310009, China.

OBJECTIVE: To study the role of calpain in the mechanism of oxidative cataract through detecting the level of intracellular free Ca(2+), the expression and proteolytic activity of calpain in the lens epithelial cells (LECs) of H(2)O(2)-induced cataract. METHODS: Rat lenses were cultured in vitro and cataract was induced by H(2)O(2). The level of intracellular free Ca(2+) was measured by fluorescence determination with fura-2/AM. The expression of m-calpain protein in LECs was detected with immunohistochemical method. The proteolytic activity in LECs was measured using a fluorogenic synthetic substrate. RESULTS: There were significant differences of the level of intracellular free Ca(2+) (P=0.001, 0.000, 0.000), the expression of m-calpain (P=0.001, 0.000, 0.000) and the proteolytic activity of calpain (P=0.001, 0.000, 0.000) between H(2)O(2)-induced and control group at 6, 12 and 24 h, respectively. CONCLUSIONS: H(2)O(2) can increase intracellular free Ca(2+), then enhance the expression and proteolytic activity of calpain which may play a role in the mechanism of oxidative cataract of rat.

PMID: 15101113 [PubMed - indexed for MEDLINE]

5.32 (Ro)accutane and orbitofrontal cortex metabolism :

- Significant decrease in metabolism in orbitofrontal cortex in (Ro)accutane exposed human subjects :

Picture 1. Bremner JD et al. *Functional brain imaging alterations in acne patients treated with isotretinoin.* (2005) Am J Psychiatry. May;162(5):983-91.

- Function of the orbitofrontal cortex :

To complete...

References:

To complete...

5.33 (Ro)accutane and osteoporosis :

A loss of bone density in all acne-subjects exposed to (Ro)accutane can be expected. Compared with that in healthy control subjects, mean bone density was lower at all sites (spine, femoral neck, and Ward triangle) and was considerably more variable at the spine in young men with cystic acne even before treatment. Bone density at the Ward triangle decreased a mean of 4.4% (P = .03) after 6 months of isotretinoin use (1 mg/kg of body weight). Four patients showed decreased density of more than 9% at the Ward triangle. The difference between the mean change in bone density in the patient group and in the control group was significant at the Ward triangle (P = .04) but not at the other sites [1].

Hoffman la Roche itself has reported bone demineralization in association with exposure of (Ro)accutane in human subjects [0].

To complete...

- References :

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf
[1] **Leachman SA, Insogna KL, Katz L, Ellison A, Milstone LM.** *Bone densities in patients receiving isotretinoin for cystic acne.* (1999) Arch Dermatol. Aug;135(8):961-5.

FASEB J. 2003 Feb;17(2):247-9. Epub 2002 Dec 3. Related Articles, Links

Hypocalcemia and osteopathy in mice with kidney-specific megalin gene defect.
Leheste JR, Melsen F, Wellner M, Jansen P, Schlichting U, Renner-Muller I, Andreassen TT, Wolf E, Bachmann S, Nykjaer A, Willnow TE.
Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany.

Megalin is an endocytic receptor highly expressed in the proximal tubules of the kidney. Recently, we demonstrated that this receptor is essential for the renal uptake and conversion of 25-OH vitamin D3 to 1,25-(OH)2 vitamin D3, a central step in vitamin D and bone metabolism. Unfortunately, the perinatal lethality of the conventional megalin knockout mouse model precluded the detailed analysis of the significance of megalin for calcium homeostasis and bone turnover in vivo. Here, we have generated a new mouse model with conditional inactivation of the megalin gene in the kidney by using Cre recombinase. Animals with a renal-specific receptor gene defect were viable and fertile. However, lack of receptor expression in the kidney results in plasma vitamin D deficiency, in hypocalcemia and in severe bone disease, characterized by a decrease in bone mineral content, an increase in osteoid surfaces, and a lack of mineralizing activity. These features are consistent with osteomalacia (softening of the bones) as a consequence of hypovitaminosis D and demonstrate the crucial importance of the megalin pathway for systemic calcium homeostasis and bone metabolism.

PMID: 12475886 [PubMed - indexed for MEDLINE]

5.34 (Ro)accutane and altered oxygen uptake :

- The Epo gene :

Erythropoietin (Epo) is the humoral regulator of red blood-cell production. Low oxygen tension increases the Epo levels by enhancing transcription, through the hypoxia-inducible factor (HIF)-1, a transcriptional modulator in oxygen-regulated gene expression. In the present work, a cooperative interaction between hypoxia, mediated by the HIF-1 complex, and transforming growth factor-beta (TGF-beta), mediated by Smad3/4, was revealed in the Epo gene [1].

J Mol Biol. 2004 Feb 6;336(1):9-24. Related Articles, Links
Erratum in:
J Mol Biol. 2004 Mar 19;337(2):499. Rodriguez-Sanz, Francisco [corrected to Sanz-Rodriguez, Francisco].
A cross-talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SP1 and Smads.
Sanchez-Elsner T, Ramirez JR, Sanz-Rodriguez F, Varela E, Bernabeu C, Botella LM.
Centro de Investigaciones Biologicas, CSIC Ramiro de Maeztu, 9, Madrid 28040, Spain.

Erythropoietin (Epo) is the humoral regulator of red blood-cell production. Low oxygen tension increases the Epo levels by enhancing transcription, through the hypoxia-inducible factor (HIF)-1, a transcriptional modulator in oxygen-regulated gene expression. In the present work, a cooperative interaction between hypoxia, mediated by the HIF-1 complex, and transforming growth factor-beta (TGF-beta), mediated by Smad3/4, was revealed in the Epo gene. This cooperation is due to physical interaction between Smad3/4 and HIF-1alpha. The Smad3/4 binding site is located within the 3' Epo enhancer, downstream from the HRE consensus, and immediately adjacent to the orphan hepatic nuclear factor receptor (HNF-4). HNF-4 is interacting also with Smad3 and the HIF-1 complex, to potentiate further the cooperative effect between both factors. Moreover, Sp1 has been identified as the factor binding the promoter necessary for the full hypoxia inducibility of the EPO gene. However, this full induction is achieved only if the TGF-beta pathway is mediating a cross-talk between promoter (Sp1) and enhancer (HIF-1alpha) regions through Smad3. We show that Sp1 binding to the proximal promoter is relevant for Epo transcription, and contributes to the Epo induction by hypoxia. A functional cooperation among the transcription factors mediating hypoxia (HIF-1, Sp1), the TGF-beta pathway (Smad3/4), and tissue-specific HNF-4 is proposed for the regulation of the Epo gene. In this model, the physical contact between the upstream promoter and the 3' downstream enhancer is mediated by Sp1 and Smad3 factors, and would occur upon bending of the DNA intervening sequences. Thus, Sp1 would reinforce the promoter/enhancer contact, while Smad3 would stabilize the multifactorial complex by interacting with HIF-1/Sp1/HNF-4 and the coactivator CBP/p300. This model may be extended to other genes where collaboration between TGF-beta and hypoxia takes place.

PMID: 14741200 [PubMed - indexed for MEDLINE]

Cell Death Differ. 2005 Jul 8; [Epub ahead of print] Related Articles, Links
Early mitochondrial alterations in ATRA-induced cell death.
Schmidt-Mende J, Gogvadze V, Hellstrom-Lindberg E, Zhivotovsky B.

[1] 1Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, Box 210, Stockholm SE-171 77, Sweden [2] 2Department of Medicine, Division of Hematology, Karolinska University Hospital Huddinge, Stockholm SE-141 86, Sweden.

All-trans retinoic acid (ATRA) induces differentiation and subsequent apoptosis in a variety of cell lines. Using the myeloid cell line P39, we show that ATRA disturbs mitochondrial functional activity long before any detectable signs of apoptosis occur. These early changes include diminished mitochondrial oxygen consumption, decreased calcium uptake by mitochondria and as a result, a lower mitochondrial matrix calcium concentration. Granulocyte colony-stimulating factor (G-CSF) increases mitochondrial respiration and

calcium accumulation capacity and subsequently blocks ATRA-induced apoptosis. Nifedipine, a plasma membrane calcium channel blocker, inhibits apoptosis-related changes, such as the loss of the mitochondrial membrane potential and activation of caspases. Thus, the properties of ATRA and G-CSF to modulate mitochondrial respiration and intracellular calcium control are novel findings, which give insight into their precise molecular mode of action.Cell Death and Differentiation advance online publication, 8 July 2005; doi:10.1038/sj.cdd.4401715.

PMID: 16003389 [PubMed - as supplied by publisher]

- References :

To complete...

5.35 (Ro)accutane and altered pigmentation :

Hoffman la Roche itself has found both hypo- and hyperpigmentation in associtation with (Ro)accutane exposure in human subjects [0]. Pigmentation may be permanently altered.

To complete...

- References :

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf

5.36 (Ro)accutane, proteinuria, albuminuria, micturition :

To complete...

5.37 (Ro)accutane induced irreversible proteinuria :

Hoffman la Roche itself has seen an association between proteinuria and exposure of (Ro)accutane in human subjects [0]. It is here suggested to be an overall effect rather than a side-effect. An important part of all the (Ro)accutane induced major alterations of *endocrine* function are with high certainity due to significant interference with/inhibition of/aggregation of the cubulin/megalin receptors, predominantly located in renal proximal tubes (in certain kidney cells).

(Ro)accutane is found to cause a statistically significant induction of TGF-beta1 in several independent studies. Six weeks of isotretinoin treatment caused a statistically significant 19% increase in suction blister fluid TGF-beta1 [1]. There are no measured values of the cumulative effect after 3-4 months exposure, which is common in acne-subjects. Various studies have shown that significantly increased TGF-beta1 correlate with with the amount of urinary protein excretion (proteinuria and albumineria), in a *dose*, but also importantly *time* dependent manner. This due to failure in the kidney, of renal proximal tubular protein reabsorbtion. This is of major importance,

because a significant failure in reabsorbtion leads to the loss of important vitamins, hormones and amino-acids [2].

Findings in the urinary system that Hoffman la Roche itself reports include glomerulonephritis and nonspecific urogenital findings [0].

5.38 (Ro)accutane induced alterations in control of micturition and urine storage suggested to be partially due to effects on the spinal cord and GABA :

The micturition reflex is one of the autonomic reflexes, but the release of urine is regulated by voluntary neural mechanisms that involve centers in the brain and spinal cord. The micturition reflex is a bladder-to-bladder contraction reflex for which the reflex center is located in the rostral pontine tegmentum (pontine micturition center: PMC). There are two afferent pathways from the bladder to the brain. One is the dorsal system and the other is the spinothalamic tract. Afferents to the PMC ascend in the spinotegmental tract, which run through the lateral funiculus of the spinal cord. The efferent pathway from the PMC also runs through the lateral funiculus of the spinal cord to inhibit the thoracolumbar sympathetic nucleus and the sacral pudendal nerve nucleus, while promoting the activity of the sacral parasymapathetic nucleus. Inhibition of the sympathetic nucleus and pudendal nerve nucleus induces relaxation of the bladder neck and the external urethral sphincter, respectively. There are two centers that inhibit micturition in the pons, which are the pontine urine storage center and the rostral pontine reticular formation. In the lumbosacral cord, excitatory glutamatergic and inhibitory glycinergic/GABAergic neurons influence both the afferent and efferent limbs of the micturition reflex. The activity of these neurons is affected by the pontine activity. There are various excitatory and inhibitory areas co-existing in the brain, but the brain has an overall inhibitory effect on micturition, and thus maintains continence. For micturition to occur, the cerebrum must abate its inhibitory influence on the PMC [3].

To complete...

- References :

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Transforming growth factor-beta1 reduces megalin- and cubilin-mediated endocytosis of albumin in proximal-tubule-derived opossum kidney cells. (2003) J Physiol. Oct 15;552(Pt 2):471-81.

[3] **Sugaya K, Nishijima S, Miyazato M, Ogawa Y.**
Central nervous control of micturition and urine storage. (2005) J Smooth Muscle Res. Jun;41(3):117-32.

J Am Soc Nephrol. 2005 Jun;16(6):1673-83. Epub 2005 Apr 13. Related Articles, Links Suppressors of cytokine signaling regulate angiotensin II-activated Janus kinase-signal transducers and activators of transcription pathway in renal cells.
Hernandez-Vargas P, Lopez-Franco O, Sanjuan G, Ruperez M, Ortiz-Munoz G, Suzuki Y, Aguado-Roncero P, Perez-Tejerizo G, Blanco J, Egido J, Ruiz-Ortega M, Gomez-Guerrero C.

Renal and Vascular Research Laboratory, Fundacion Jimenez Diaz, Autonoma University, Avda Reyes Catolicos 2, Madrid, Spain 28040. cgomez@fjd.es

Suppressors of cytokine signaling (SOCS) family is constituted by cytokine-inducible proteins that modulate receptor signal transduction via tyrosine kinases, mainly the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway. Differential SOCS expression was noted in renal cells that were incubated with inflammatory stimuli, but the role of SOCS in the pathogenesis of renal diseases is not yet well defined. Because angiotensin II (Ang II) plays a key role in renal disease, SOCS proteins were studied as a novel mechanism involved in the negative regulation of Ang II-mediated processes. Systemic Ang II infusion for 3 d increased the renal mRNA expression of SOCS-3 and SOCS-1. SOCS protein synthesis was found in glomerular mesangial area and tubules. In cultured mesangial cells and tubular epithelial cells, Ang II induced a rapid and transient SOCS-3 and SOCS-1 expression in parallel with JAK2 and STAT1 activation. In both cell types, overexpression of SOCS proteins prevented the STAT activation in response to Ang II. SOCS expression observed in Ang II-infused rats and in Ang II-stimulated cells was significantly inhibited by treatment with AT(1) but not AT(2) receptor antagonist and was attenuated in mesangial cells from AT(1a)-deficient mice, demonstrating the implication of AT(1) in those responses. In SOCS-3 knockdown studies, antisense oligonucleotides inhibited the expression of SOCS-3 and increased the Ang II-induced STAT activation and c-Fos/c-Jun expression, then resulting in a more severe renal damage. These results suggest that SOCS proteins may act as negative regulators of Ang II signaling in renal cells and implicate SOCS as important modulators of renal damage.

PMID: 15829701 [PubMed - in process]

5.39 (Ro)accutane and pseudotumor cerebri :

To complete...

- References :

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf

5.40 (Ro)accutane and increased scar formation :

Isolating effects in the skin and disregarding severe effects in various parts of the brain, pituitary gland and organs such as the liver is current dermatological practise.

Hoffman la Roche itself has reported "abnormal wound healing, delayed healing of exuberant granulation tissue with crusting" in association with (Ro)accutane exposure in human subjects [0]. A dogmatic, non-scientifically proven view held as a truth is that Roaccutane decreases scar-formation in the skin, but does not necessarily have to be correct and needs clarification. Some evidence is pointing rather at the opposite direction. An increased scar-formation in subjects exposed to retinoic acid in doses over 1 microM is here suggested to be possible due to retinoic acid induced changes in the extracellular matrix affecting the cell migration in the healing process.

Integrin Alpha5 beta1 was found to be increased in various human cell lines exposed to

retinoic acid in low and high doses [1 and 4]. Integrins are cell membrane structures that link the cell membrane to the extracellular matrix [3].

Increased levels of the alpha5 beta1 integrin appears to have close relation to the formation and development of abnormal scars, and was found to be higher in hypertrophic scars than normal skin. To find a way to decrease the expression level of alpha5 beta1 integrin in fibroblasts may be a new approach to inhibit scar hypertrophy [2]. Furthermore, effects in the scar formation depending of the arrangement in the extracellular matrix during a continous proteolytic cleavage of integrin alpha5 beta1 induced by retinoic acid, as is suggested to be an early phase of the atra-induced apoptotic process, are not clarified. [5]

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5.41 (Ro)accutane and the skin :

To complete...

5.42 (Ro)accutane induced aging skin due to hormonal deficits :

Intrinsic skin aging is determined primarily by genetic factors and hormonal status. It reflects the same degenerative process seen in other organs. Skin function is one of the parameters most influenced by aging. The hormonal influences include reduced pituitary, adrenal and gonadal secretion. The hormonal changes of aging lead to the development of a specific body and skin phenotype. Individuals in developed lands spend up to a third of their life (women-post-menopausal) or perhaps 20 years (men-partial androgen deficiency of the aging man, PADAM) with oestrogen or androgen deficiency. Other hormones whose levels decrease with aging include melatonin, growth hormone (GH), dehydroepiandrosterone und insulin-like growth factor-I (IGF-I). Since the skin not only fulfils a protective function for the organism but is also an active peripheral endocrine

organ, which even releases effective hormones in the circulation, local hormone substitution could become interesting in the future [1].

5.43 (Ro)accutane induced skin fragility :

The origin and frequency of skin fragility, a frequent side effect of oral synthetic retinoids, was studied in ten patients receiving isotretinoin (13-cis-retinoic acid) for disorders of keratinization and in hairless mice treated with isotretinoin and the aromatic retinoid, etretinate (RO 10-9359). Clinical skin fragility occurred in eight of ten patients, and experimental friction blisters could be induced by pencil eraser abrasion in nine of nine patients and in the hairless mice. Light and electron microscopy of friction blisters showed fraying or loss of the stratum corneum and outer layers of the viable epidermis, loss of desmosomes and tonofilaments, and intracellular and intercellular deposits of amorphous material that did not stain with stains for mucin. The skin fragility produced by oral synthetic retinoids is epidermal in origin, since retinoids induce profound disruption of epidermal morphologic appearance [2].

To complete...

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5.44 (Ro)accutane and altered stress responses :

To complete...

- References :

To complete...

5.45 (Ro)accutane and suicide :

Hoffman la Roche itself includes "suicide suicide ideation suicide attempt and suicide" in associtation with (Ro)accutane exposure in human acne-subjects [0]. There are several case reports of human subjects that develop impulsive suicidal thoughts, ideations and completed suicides during (Ro)accutane exposure [1, 2, 3 and more].

Forced swim-tests in rat have shown alterations in behaviour, suggesting that (Ro)accutane may alter mechanisms important for the subjects will of survival [4].

There are no post market studies following up suicides that are likely to take place in the years following (Ro)accutane exposure, but it is here suggested that suicides may develop years after exposure as a direct consequence of continous suicidal thoughts, a progressively worsening condition, drastical loss of quality of life (QoL) or post-exposure development of suicidal thoughts, and therefore a (Ro)accutane induced suicide is likely to occur during exposure but also post exposure.

To complete...

- References :

[0] **No author** *Roche official complete US Accutane Product information*
www.rocheusa.com/products/accutane/pi.pdf

5.46 (Ro)accutane and altered thermoregulation :

- Thyroid hormones and thermoregulation :

In human acne-subjects exposed to (Ro)accutane, levels of thyroxine and triiodothyronine were significantly lower after exposure (p less than 0.05), indicating a (Ro)accutane induced clinical thyroid deficiency (hypothyroidism) [1].

Thyroid hormones (THs) have long been known to be involved in the control of thermoregulation in birds and mammals. In particular, they are reported to play a role in the regulation of heat production. The underlying mechanisms could be the stimulation of the nuclear and mitochondrial transcription of several genes involved in energy metabolism and/or a direct action on the activity of components of the mitochondrial respiratory chain. Attention has recently been focussed on a subfamily of mitochondrial anion carriers called uncoupling proteins (UCPs). These proteins are suspected to be involved in a partial dissipation of the mitochondrial proton electrochemical gradient that would uncouple phosphorylations from oxidations and hence produce heat. However, the involvement of uncoupling mechanisms in thermogenesis and particularly in the thermogenic effect of TH is still unclear. The thermogenic role of UCP1, specifically expressed in brown adipose tissue, and its regulation by TH in rodents is quite well recognised, but the involvement in heat production of its mammalian homologues UCP2, ubiquitously expressed, and UCP3, muscle and adipose tissue-specific, as well as the role of the muscular avian UCP (avUCP), are to be further investigated. The expression of the UCP2 and UCP3 genes was shown to be enhanced by TH in muscle of several rodent species, and to be increased in situations where thermogenesis is stimulated, whereas results are more contrasted in pig. There is now increasing evidence that the physiological role of the mammalian UCP3 and UCP2 is rather related to lipid oxidation and/or prevention of reactive oxygen species accumulation than to heat production by uncoupling. The expression of avUCP was also recently demonstrated to be strongly regulated by thyroid status in chicken, and overexpressed in experimental conditions favouring high triiodothyronine concentrations and thermogenesis. However, its real uncoupling activity and contribution to thermogenesis remain to be established [2].

- Disruptions in circadian rhytms through alterations in levels of melatonin and thyroid hormone :

Melatonin has shown to be significantly correlated with vitamin A status in mammals [3 and more]. Changes of TSH serum levels are smaller and those of FT4 are greater at night, when melatonin levels are higher, so that the response of anterior pituitary to hypothalamic TRH and of thyroid to hypophyseal TSH may be influenced by the pineal hormone that

may modulate the hypothalamic-pituitary-thyroid axis function and influence the circadian rhythm of body temperature [4].

- Conclusions :

Human acne-subjects exposed to (Ro)accutane are likely to experience an alteration in body heat throughout the day. This is likely partially depending on falling levels thyroid hormone and altered thyroid hormone receptor expression.

Unnormal fluctuations in body temperature during sleep, and physical excercise may be seen as an effect of (Ro)accutane exposure due to disturbance of melatonin-dependent circadian rhytms.

Other contributing factors involved in (Ro)accutane induced dysfunctional regulation of body heat are likely.

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Potential involvement of mammalian and avian uncoupling proteins in the thermogenic effect of thyroid hormones. (2005) Domest Anim Endocrinol. Jul;29(1):78-87.
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5.47 (Ro)accutane and premature vascular disease :

Statistically significant elevatation of plasma homocysteine levels have been seen in repeated independent studies of human subjects exposed to (Ro)accutane [1 and more]. It is suggested that the elevated Hcy levels in patients after 45 days on Iso therapy could be due either to the 'inhibition' of cystathionine-beta-synthase by the drug and/or their liver dysfunction [1]. Hoffman la Roche itself has admitted to these findings [0]. Significantly lower levels of thyroxine and triiodthyronine have been found in human acne-subjects exposed to the toxin after exposure, indicating a (Ro)accutane induced clinical hypothyroidism [2]. The relationship between hypothyroidism and a variety of cardiovascular risk factors is now well established [3 and more].

- Significantly elevated GGT (gamma-glutamyltranspeptidase) by retinoic acid, a recently clarified risk factor for CVD :

The relation of GGT to the risk of death from CVD was examined in a cohort of 163 944 Austrian adults that was monitored for up to 17 years. To evaluate GGT as an independent predictor, Cox proportional hazards models were calculated, which adjusted for established risk factors. In both men and women, high GGT was significantly (P<0.001) associated with total mortality from CVD, showing a clear dose-response relationship [4].

Retinoic acid increased the level of the GGT mRNA and this enhancement was progressive, depending on the duration of exposure and on the concentration of retinoic acid in the culture medium. When retinoic acid was removed from cultures which had been exposed to it for 4 days, the induced GGT activity remained unchanged. In contrast to transformed cell lines, retinoic acid did not induce the activity of GGT in normal/non-transformed rat liver epithelial cells [5].

To complete...

- References :

[1] **Schulpis KH, Karikas GA, Georgala S, Michas T, Tsakiris S.** *Elevated plasma homocysteine levels in patients on isotretinoin therapy for cystic acne.* (2001) Int J Dermatol. Jan;40(1):33-6.
[3] **Jublanc C, Bruckert E.** *Hypothyroidism and cardiovascular disease: role of new risk factors and coagulation parameters.* (2004) Semin Vasc Med. May;4(2):145-51.
[4] **Ruttmann E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H.** *{gamma}-Glutamyltransferase as a Risk Factor for Cardiovascular Disease Mortality. An Epidemiological Investigation in a Cohort of 163 944 Austrian Adults.* (2005) Circulation. Sep 26
[5] **Tsao MS, Batist G.** *Induction of gamma-glutamyl transpeptidase activity by all-trans retinoic acid in cultured rat liver epithelial cells.* (1988) Biochem Biophys Res Commun. Dec 30;157(3):1039-45.

5.48 (Ro)accutane and white matter vacuolation :

Post mortem examinations of a 13 month old child revealed white matter vacuolation in the brain stem tegmentum and cerebellar hemispheres similar to that seen in toxicity from hexachlorophene. (Ro)accutane had been given systemically during the subjects last week of life [1]. It remains to be answered, possibly by post-mortem examinations, how white matter in the brains of teenage and adult human subjects is affected by (Ro)accutane.

- References :

[1] **Sathingy N, Sternberg PE.**
Vacuolar myelinopathy of the brain and lamellar ichthyosis. (1986) Cutis. Jul;38(1):29-32.

5.49 (Ro)accutane and vitamin B deficiency :

Folate :

To complete...

Folate transport :

To complete...

Sp1 :

To complete...

Biochim Biophys Acta. 2005 Jan 21;1727(1):45-57. Epub 2004 Dec 31. Related Articles, Links
Transcriptional regulation of the human reduced folate carrier promoter C: synergistic transactivation by Sp1 and C/EBP beta and identification of a downstream repressor.
Payton SG, Whetstine JR, Ge Y, Matherly LH.
Department of Pharmacology, Wayne State University, School of Medicine, Detroit, MI, USA.

The human reduced folate carrier (hRFC) is ubiquitously but differentially expressed in human tissues and its levels are regulated by up to six alternatively spliced non-coding regions (designated A1/A2, A, B, C, D, and E) and by at least four promoters. By transient transfections of HepG2 human hepatoma cells with 5' and 3' deletion constructs spanning 2883 bp of upstream sequence, a transcriptionally important region was localized to within 177 bp flanking the transcriptional start sites for exon C. By gel shift and chromatin immunoprecipitation assays, Sp1 and C/EBP beta transcription factors were found to bind consensus elements (GC-box, CCAAT-box) within this region. The functional importance of these elements was confirmed by transient tranfections of HepG2 cells with hRFC-C reporter constructs in which these elements were mutated, and by co-transfections of Drosophila SL-2 cells with wild-type hRFC-C promoter and expression constructs for Sp1 and C/EBP beta. Whereas both Sp1 and C/EBP beta transactivated hRFC-C promoter activity, C/EBP alpha and gamma were transcriptionally inert. Sp1 combined with C/EBP beta resulted in a synergistic transactivation. In HepG2 cells, transfections with Sp1 and C/EBP beta both increased endogenous levels of hRFC-C transcripts. By 3' deletion analysis, a repressor sequence was localized to within 71 bp flanking the minimal promoter. On gel shifts, a novel transcriptional repressor was localized to within 30 bp. Collectively, these results identify transcriptionally important regions in the hRFC-C minimal promoter that include a GC-box and CCAAT-box, and suggest that cooperative interactions between Sp1 and C/EBP beta are essential for hRFC-C transactivation. Another possible factor in the tissue-specific regulation of the hRFC-C region involves the downstream repressor flanking the minimal promoter.

PMID: 15652157 [PubMed - indexed for MEDLINE]

- References :

Biochem J. 2004 Oct 15;383(Pt 2):249-57. Related Articles, Links
Roles of USF, Ikaros and Sp proteins in the transcriptional regulation of the human reduced folate carrier B promoter.
Liu M, Whetstine JR, Payton SG, Ge Y, Flatley RM, Matherly LH.

Experimental and Clinical Therapeutics Program, Barbara Ann Karmanos Cancer Institute, 110 E. Warren Ave., Detroit, MI 48201, USA.

The hRFC (human reduced folate carrier) is ubiquitously but differentially expressed in human tissues and its levels are regulated by up to seven non-coding regions (A1, A2, A, B, C, D and E) and at least four promoters. For the hRFC-B basal promoter, regulation involves binding of Sp (specificity protein) transcription factors to a critical GC-box. By transiently transfecting HT1080 cells with 5'- and 3'-deletion constructs spanning 1057 bp of upstream sequence, a transcriptionally important region was localized to 158 bp flanking the transcriptional start sites. By gel shift and chromatin immunoprecipitation assays, USF (upstream stimulatory factor), Sp1 and Ikaros-related proteins were bound to consensus elements (one E-box, two GC-box and three Ikaros) within this region. The functional importance of these elements was confirmed by transient tranfections of HT1080 cells with hRFC-B reporter constructs in which they were mutated, and by co-transfections of Drosophila Mel-2 cells with wild-type hRFC-B promoter and expression constructs for USF1, USF2a, Sp1 and Ikaros 2 and 8. Both USF1 and Sp1 proteins transactivated the hRFC-B promoter. Sp1 combined with USF1 resulted in a synergistic transactivation. Identical results were obtained with USF2a. Ikaros 2 was a repressor of hRFC-B promoter activity whose effects were partly reversed by the dominant-negative Ikaros 8. In HT1080 cells, transfection with Ikaros 2 decreased endogenous hRFC-B transcripts, whereas USF1 and Sp1 increased transcript levels. Ikaros 2 also decreased reporter gene activity and levels of acetylated chromatin associated with the endogenous promoter. Collectively, these results identify transcriptionally important regions in the hRFC-B promoter that include multiple GC-box, Ikaros and E-box elements. Our results also suggest that co-operative interactions between transcription factors Sp1 and USF are essential for high-level hRFC-B transactivation and imply that these effects are modulated by the family of Ikaros proteins and by histone acetylation.

PMID: 15214842 [PubMed - indexed for MEDLINE]

5.50 (Ro)accutane and other side-effects :

- Effects on salivary glands, salive secretion and salivary enzymes :

In rat submandibular salivary gland (SMSG) homogenates, the activity of (Na+ + K+)-ATPase was reduced after exposure to 13-cis retinoic acid. Fatty acid composition of total lipids in SMSG suggest that decrease in (Na+ + K+)-ATPase activity may be associated with changes in fatty acid composition of total lipids [1]. Mouth dryness and decreased flow rate of saliva is a common effect during exposure. Cases of irreversible xerostomia have been associated with previous exposure [2]. Activity of metaloproteinase 9 (MMP-9) has been found to be significantly altered in human subjects exposed to (Ro)accutane during exposure [3]. The long term effects on the function of salivary enzymes in subjects exposed to (Ro)accutane is unknown.

- References :

[1] **Alam SQ, Alam BS.**
Effect of 13-cis retinoic acid on fatty acid composition and membrane-associated enzymes

in rat submandibular salivary glands.
(1983) Arch Oral Biol. 28(6):507-11.
[2] **Bots CP, van Nieuw Amerongen A, Brand HS.**
[Enduring oral dryness after acne treatment] (2003) Ned Tijdschr Tandheelkd. Jul;110(7):295-7.
[3] **Oikarinen K, Salo T, Kylmaniemi M, Palatsi R, Karhunen T, Oikarinen A.** *Systemic oral isotretinoin therapy and flow rate, pH, and matrix metalloproteinase-9 activity of stimulated saliva.* (1995) Acta Odontol Scand. Dec;53(6):369-71.

Section 5b : Other long term effects

5.51 Elevated homocysteine :

High circulating concentrations of IL-1ra and IL-6 are independent correlates of hyperhomocysteinemia and may explain, at least in part, the association between homocysteine and atherosclerosis [1].

To complete...

"Homocysteine is an amino acid that can be generated in response to nutritionally deficient or nutritionally deficient diets. "

"When homocysteine levels increase in the blood, it is linked to massive inflammation and neurodegeneration!
In Alzheimer's disease and many other chronic diseases, we find a significant increase in homocysteine in blood tests. Since homocysteine is a potent excitotoxin and neurotoxin, high levels of homocysteine have been found to exacerbate the symptoms of Alzheimer's disease and other chronic diseases. Components of the metabolic degradation of homocysteine alter the NMDA (N-methyl-D-aspartate) receptor sites, resulting in multiple negative effects, including free radicals and a massive inflammatory cascade!
These free radicals and inflammation can trigger an autoimmune response in which the patient's immune system attacks the thyroid gland and / or other body systems. "

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5120102/

https://www.ncbi.nlm.nih.gov/pubmed/17852421

The administration of isotretinoin to humans has been shown to be associated with increased concentrations of homocysteine, which is a potential metabolic mechanism by which isotretinoin may promote depression :

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3276716/

- References :

[1] **Gori AM, Corsi AM, Fedi S, Gazzini A, Sofi F, Bartali B, Bandinelli S, Gensini GF, Abbate R, Ferrucci L** *A proinflammatory state is associated with hyperhomocysteinemia in the elderly.* (2005) Am J Clin Nutr. Aug;82(2):335-41.

J Biol Chem. 2001 Nov 23;276(47):43570-9. Epub 2001 Sep 18. Related Articles, Links
Transcriptional regulation of cell-specific expression of the human cystathionine beta -synthase gene by differential binding of Sp1/Sp3 to the -1b promoter.
Ge Y, Matherly LH, Taub JW.
Experimental and Clinical Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.

Cystathionine beta-synthase (CBS) catalyzes the condensation of serine and homocysteine to form cystathionine, an intermediate step in the synthesis of cysteine. We previously characterized the CBS -1b minimal promoter (-3792 to -3667) and found that Sp1/Sp3, nuclear factor Y, and USF-1 were involved in the regulation of basal promoter activity (Ge, Y., Konrad, M. A., Matherly, L. H., Taub, J. W. (2001) Biochem. J. 357, 97-105). In this study, the critical cis-elements and transcription factors in the CBS -1b upstream region (-4046 to -3792) were examined in HT1080 and HepG2 cells, which differ approximately 10-fold in levels of CBS transcripts transcribed from the CBS -1b promoter. In DNase I footprint and gel shift analyses and transient transfections of mutant CBS -1b promoter constructs into HT1080 and HepG2 cells, transcriptionally important roles for Sp1/Sp3 binding to three GC boxes and one GT box and for binding of myeloid zinc finger

1-like proteins to two myeloid zinc finger 1 elements were indicated. In gel shift assays, very low levels of Sp1/Sp3 DNA-protein complexes were detected in HT1080 cells compared with HepG2 cells despite comparable levels of nuclear factor Y and USF-1 binding and similar levels of Sp1 and Sp3 proteins on Western blots. Mixing of HT1080 and HepG2 nuclear extracts resulted in no difference in total Sp factor binding in gel shift assays, thus excluding a role for an unknown activator or inhibitor in the disparate Sp1/Sp3 binding between the lines. Increased Sp1/Sp3 binding in gel shift assays was observed upon treatment of HT1080 nuclear extracts with protein kinase A, and decreased Sp1/Sp3 binding resulted from treatment of HepG2 nuclear extracts with calf alkaline phosphatase, suggesting a role for changes in Sp1/Sp3 phosphorylation in transcription factor binding and transactivation of the CBS -1b promoter. Characterization of CBS promoter structure and function should clarify the molecular bases for variations in CBS gene expression in genetic diseases and the relationship between CBS and Down syndrome.

PMID: 11562358 [PubMed - indexed for MEDLINE]
Proc. Natl. Acad. Sci. USA, 10.1073/pnas.0504786102

Biochemistry
Regulation of human methylenetetrahydrofolate reductase by phosphorylation
(homocysteine | posttranslational modification)

Kazuhiro Yamada *, John R. Strahler , Philip C. Andrews , and Rowena G. Matthews *
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Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109-1066
Contributed by Rowena G. Matthews, June 9, 2005

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of methylenetetrahydrofolate to methyltetrahydrofolate, the methyl donor for the conversion of homocysteine to methionine. Regulation of MTHFR activity is crucial for maintaining cellular concentrations of methionine and S-adenosylmethionine (AdoMet). Purified recombinant human MTHFR expressed in insect cells is multiply phosphorylated on an N-

terminal extension of the protein that contains a highly conserved serine-rich region. Treatment by alkaline phosphatase removes seven phosphoryl groups from the enzyme. Thr-34 was identified as one of the seven phosphorylation sites by using a monoclonal antibody directed toward pThr-Pro. Mutation of Thr-34 to Ala completely blocks modification as judged by mass spectrometric analysis, suggesting that Thr-34 is the priming phosphorylation site. The Thr34Ala mutant was expressed in baculovirus-infected insect cells, and its enzymic properties were compared with wild-type enzyme. The mutant enzyme and alkaline phosphatase-treated wild-type enzyme are more active than untreated wild-type enzyme and less sensitive to inhibition by saturating AdoMet, indicating that phosphorylation at Thr-34 is critical for allosteric regulation of human MTHFR activity by AdoMet. The absence of methionine and the presence of adenosine in the cell culture medium, which lead to a low intracellular AdoMet/S-adenosylhomocysteine ratio, are associated with faster electrophoretic mobility of MTHFR, presumably because of less or no phosphorylation. Because the faster-mobility MTHFR is associated with the more active form of MTHFR, this response is likely to increase methionine production. Those observations suggest that AdoMet functions not only as an allosteric inhibitor but also to control phosphorylation of human MTHFR.

5.52 Vitamin A deficiency :

To complete...

5.53 Vitamin D deficiency :

To complete...

5.54 Life expectancy :

The statistical life-expectancy of subjects exposed to (Ro)accutane is not known. A statistically significant effect on life expectancy can not be excluded.

- Suggested loss of stem-cells due to apoptosis :

To complete...

- Significantly increased risk factors for premature aging induced by (Ro)accutane :

To complete...

- Possible adult mutations, high dose retinoic acid induced DNA instability and accelerated aging :

Laminopathies are a group of diseases due to mutations of type A-lamins, a group of proteins lining the inner aspect of cell nuclei [1]. Laminopathies have been found to accelerate aging due to defiecent DNA repair [3]. The lamin A promoter contains a responsive element for retinoic acid (L-RARE) and is thus affected during exposure of (Ro)accutane [2]. Recruitment of p53 binding protein 1 (53BP1) and Rad51 to sites of DNA

lesion is impaired in Zmpste24(-/-) MEFs and in HGPS fibroblasts, resulting in delayed checkpoint response and defective DNA repair [3]. High p53 protein expression (LI >/= 0.2) was detected in 25% of the lesions at baseline and in 18% of the lesions after isotretinoin exposure [4].

- Possible effect on telomeres :

Free Radic Biol Med. 2001 Dec 1;31(11):1287-312. Related Articles, Links
Reactive oxygen species, antioxidants, and the mammalian thioredoxin system.
Nordberg J, Arner ES.
Medical Nobel Institute for Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden.

Reactive oxygen species (ROS) are known mediators of intracellular signaling cascades. Excessive production of ROS may, however, lead to oxidative stress, loss of cell function, and ultimately apoptosis or necrosis. A balance between oxidant and antioxidant intracellular systems is hence vital for cell function, regulation, and adaptation to diverse growth conditions. Thioredoxin reductase (TrxR) in conjunction with thioredoxin (Trx) is a ubiquitous oxidoreductase system with antioxidant and redox regulatory roles. In mammals, extracellular forms of Trx also have cytokine-like effects. Mammalian TrxR has a highly reactive active site selenocysteine residue resulting in a profound reductive capacity, reducing several substrates in addition to Trx. Due to the reactivity of TrxR, the enzyme is inhibited by many clinically used electrophilic compounds including nitrosoureas, aurothioglucose, platinum compounds, and retinoic acid derivatives. The properties of TrxR in combination with the functions of Trx position this system at the core of cellular thiol redox control and antioxidant defense. In this review, we focus on the reactions of the Trx system with ROS molecules and different cellular antioxidant enzymes. We summarize the TrxR-catalyzed regeneration of several antioxidant compounds, including ascorbic acid (vitamin C), selenium-containing substances, lipoic acid, and ubiquinone (Q10). We also discuss the general cellular effects of TrxR inhibition. Dinitrohalobenzenes constitute a unique class of immunostimulatory TrxR inhibitors and we consider the immunomodulatory effects of dinitrohalobenzene compounds in view of their reactions with the Trx system.

Publication Types:
Review
Review, Tutorial

PMID: 11728801 [PubMed - indexed for MEDLINE]

Atherosclerosis. 2005 Jul 23; [Epub ahead of print] Related Articles, Links
Oxidized LDL induces a coordinated up-regulation of the glutathione and thioredoxin systems in human macrophages.
Hagg D, Englund MC, Jernas M, Schmidt C, Wiklund O, Hulten LM, Ohlsson BG, Carlsson LM, Carlsson B, Svensson PA.

Research Centre for Endocrinology and Metabolism, Division of Body Composition and Metabolism, Department of Internal Medicine, Vita straket 15, Sahlgrenska University Hospital, S-413 45 Goteborg, Sweden.

Using DNA microarray analysis, we found that human macrophages respond to oxidized

low-density lipoprotein (oxLDL) by activating the antioxidative glutathione and thioredoxin systems. Several genes of the glutathione and thioredoxin systems were expressed at high levels in macrophages when compared to 80 other human tissues and cell types, indicating that these systems may be of particular importance in macrophages. The up-regulation of three genes in these systems, thioredoxin ($P<0.005$), thioredoxin reductase 1 ($P<0.001$) and glutathione reductase ($P<0.001$) was verified with real-time RT-PCR, using human macrophages from 10 healthy donors. To investigate the possible role of these antioxidative systems in the development of atherosclerosis, expression levels in macrophages from 15 subjects with atherosclerosis (12 men, 3 women) and 15 matched controls (12 men, 3 women) were analyzed using DNA microarrays. Two genes in the glutathione system Mn superoxide dismutase ($P<0.05$) and catalase ($P<0.05$) differed in expression between the groups. We conclude that macrophage uptake of oxidized LDL induces a coordinated up-regulation of genes of the glutathione and thioredoxin systems, suggesting that these systems may participate in the cellular defense against oxidized LDL and possibly modulate the development of atherosclerosis.

PMID: 16046214 [PubMed - as supplied by publisher]

- References :

[1] **Vigouroux C.** *[Laminopathies: lipodystrophies, insulin resistance, syndromes of accelerated ageing... and others]* (2005) Ann Endocrinol (Paris). Jun;66(3):270-8.
[2] **Okumura K, Hosoe Y, Nakajima N.** *c-Jun and Sp1 family are critical for retinoic acid induction of the lamin A/C retinoic acid-responsive element.* (2004) Biochem Biophys Res Commun. Jul 23;320(2):487-92.
[3] **Liu B, Wang J, Chan KM, Tjia WM, Deng W, Guan X, Huang JD, Li KM, Chau PY, Chen DJ, Pei D, Pendas AM, Cadinanos J, Lopez-Otin C, Tse HF, Hutchison C, Chen J, Cao Y, Cheah KS, Tryggvason K, Zhou Z.**
Genomic instability in laminopathy-based premature aging. (2005) Nat Med. Jul;11(7):780-5.
[4] **Shin DM, Xu XC, Lippman SM, Lee JJ, Lee JS, Batsakis JG, Ro JY, Martin JW, Hittelman WN, Lotan R, Hong WK.** *Accumulation of p53 protein and retinoic acid receptor beta in retinoid chemoprevention.*
(1997) Clin Cancer Res. Jun;3(6):875-80.

5.55 Hypothyroidism :

In human acne-subjects exposed to (Ro)accutane, levels of thyroxine and triiodothyronine were significantly lower after exposure (p less than 0.05), indicating a (Ro)accutane induced clinical thyroid deficiency (hypothyroidism) [1].

- References :

[1] **Marsden JR, Trinick TR, Laker MF, Shuster S.** *Effects of isotretinoin on serum lipids and lipoproteins, liver and thyroid function.* (1984) Clin Chim Acta. Nov 30;143(3):243-51.

5.56 Elevated alkaline phosphatase :

To complete...

- References:

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf

5.57 Elevated triglycerides :

To complete...

- References:

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf

5.58 Elevated cholesterol :

To complete...

References:

[0] **No author** *Roche official complete US Accutane Product information* www.rocheusa.com/products/accutane/pi.pdf

5.59 Elevated TGF-beta :

The mRNA transcript of differentially expressed nucleolar TGF-beta1 target (DENTT) is expressed in several normal human tissues, with the highest level of expression in brain. Human brain cDNA library screening and 5' rapid amplification of cDNA ends yielded full-length DENTT cDNA containing an 1899-bp open reading frame encoding a predicted 633-amino-acid protein with four potential nuclear localization signals (NLSs) and two coiled-coil regions. DENTT contains a conserved 191-residue domain that shows significant identity to, and defines, the TSPY/TSPY-like/SET/NAP-1 superfamily [1].

To complete...

- References :

[1] **Ozbun LL, You L, Kiang S, Angdisen J, Martinez A, Jakowlew SB.** *Identification of differentially expressed nucleolar TGF-beta1 target (DENTT) in human lung cancer cells that is a new member of the TSPY/SET/NAP-1 superfamily.* (2001) Genomics. Apr 15;73(2):179-93.

5.60 (Ro)accutane and increased cancer susceptibility?

Introduction

A recently published study has identified a doubled risk of cancer reoccurence in a large population with testicular cancer exposed to distinct cancer-treatments due to effects from the exposure. Statistically significantly increased risks of solid cancers were observed among patients treated with radiotherapy alone (RR = 2.0, 95% CI = 1.9 to 2.2), chemotherapy alone (RR = 1.8, 95% CI = 1.3 to 2.5), and both (RR = 2.9, 95% CI = 1.9 to 4.2) [1]. *As of today, no study has evaluated the effects on cancer-susceptibility in subjects exposed to (Ro)accutane with no previous history of cancer*. Hoffman la Roche itself, the distributor of (Ro)accutane sees no increased susceptibility for any type of cancer in (Ro)accutane exposed human subjects, but states that "new information may be available" [0].

At the moment, there is no direct evidence that would point out a drastically increased cancer-susceptibility in humans, however, the issue needs proper evaluation, since some studies are showing a direct connection between certain factors recognizable as results from (Ro)accutane exposure, such as vitamin D deficiency, with higher susceptibility for cancer. However, cancer susceptibility is a complex parameter, dependent on multiple variables as prognostic factors, often in combinations, and also different depending on type of cancer.

The effects regarding risks of cancer susceptibility later in life in (Ro)accutane exposed acne-subjects, with no previous history of cancer, are not known either at an empirical or a theoretical level. Various altered parameters in exposed subjects are pointing in opposite directions. This section is aimed at discussing how a (Ro)accutane exposure in acne-subjects may affect cancer susceptibility based on a set of known and discussed risk factors.

Generally can be said, that with the knowledge of today, the major parameters that talks against a higher susceptibility for certain cancers are certain hormonal deficiencys detected post (Ro)accutane exposure in human subjects, including androgen deficiency and thyroid deficiency. However, those parameters do not provide a fully intact result.

The paramaters talking for a higher cancer susceptibility can be described as the alterations in immune-function discovered in human subjects exposed to (Ro)accutane, including altered cytokine production, possibly altered macrophage activation and receptor malfunctions.

- Vitamin A deficiency associated with higher incidence of cancer in experimental animals :

Vitamin A deficiency in experimental animals has been associated with a higher incidence of cancer and with increased susceptibility to chemical carcinogens. This is in agreement with the epidemiological studies indicating that individuals with a lower dietary vitamin A

intake are at a higher risk to develop cancer [2, 6 and more].

- Loss of retinoid signaling and retinoid resistance due to lessened retinoid receptor function suggested to increase cancer susceptibility :

Collections of data suggest that the RAR receptor acts as a tumour suppressor in several types of cancer, and confirm the role of retinoid signaling as important in cancer resistance [8 and more]. Tumour antigens such as PRAME may repress the retinoid receptor signaling and thus contribute to cancer development [10].

Exposure to retinoids in several types of cancer frequently lead to retinoid resistance and thus reoccurrence [9 and more]. These observations of retinoid resistance after heavy retinoid exposure may depend on a downregulation of retinoid receptor expression and coactivator function when supraphysiological doses of retinoids are administered to human subjects over time. After (Ro)accutane exposure in human acne-subjects, the expression of retinoid receptors, such as RAR receptors is surprisingly not either statistically or experimentally measured, but is likely to be significantly decreased.

"In situ hybridization was used to compare retinoid receptor expression profiles in head and neck squamous cell carcinomas, dysplastic lesions, adjacent normal tissues, and in tissues from normal volunteers (Xu et al., 1994). RAR, RAR, and RAR and RXR and RXR mRNAs were expressed in all samples from normal volunteers. The levels of RAR and RAR and RXR and RXR mRNAs were similar to that in most of the adjacent normal, hyperplastic, dysplastic, and malignant tissues. However, RAR mRNA levels were detected in only 70% of dysplastic and adjacent normal tissues, and were repressed further in dysplastic and malignant epithelium. RAR repression was also found in preneoplastic oral cavity lesions (Lotan et al., 1995), non-small-cell lung cancer (Castillo et al., 1997; Xu et al., 1997a; Picard et al., 1999), breast cancers (Widschwendter et al., 1997; Xu et al., 1997b), and esophageal cancer (Qiu et al., 1999). Other retinoid receptors were expressed in these tissues, but only RAR levels were significantly lower in the premalignant and tumor tissues. The correlation of RAR repression with epithelial carcinogenesis led to the hypothesis that RAR could act as a tumor suppressor. This view was supported by experiments where RAR was overexpressed in cell lines. In retinoid-sensitive human lung carcinoma cells, constitutive overexpression of RAR2 inhibited cellular proliferation (Houle et al., 1993)" [8].

5.60.1. Possible increased risk for skin cancer, ultraviolet radiation induced tumours in subjects exposed to (Ro)accutane :

Immunosuppressed patients are extremely susceptible to cutaneous squamous cell carcinoma, suggesting that immunosurveillance by T lymphocytes protects against this ultraviolet radiation-induced tumour. Both anti-CD8 and anti-CD4 treatment significantly enhanced the growth of transplanted tumours. In CD8-depleted animals, tumours grew rapidly in all animals. Tumour growth in CD4-depleted animals was slower, and 50% of these mice eventually rejected their tumours [3]. In transgenic mice local interruption of PML and RARalpha signaling in the skin, together with a systemic retinoid deficiency, initiates a tumor induction pathway that is independent of ras activation [4]. The major

reason propounded for an association of sun exposure with a protective effect in the development of cancer and improved survival is that vitamin D synthesis is a critical component of cellular networks that inhibit cellular proliferation and encourage apoptosis [14].

5.60.2. Possible attenuated effects on lung cancer from outdoor airborne particulate matter (APM) and smoking in subjects exposed to (Ro)accutane :

Findings of vitamin A status and possible attenuated effects from outdoor airborna particulate matter and smoking are pointing in different directions. The finnish ATBC study, involving a large population found that supplementation with supraphysiological doses of beta-carotene may modestly increase lung cancer incidence in cigarette smokers, and this effect may be associated with heavier smoking and higher alcohol intake [5].

However, the opposite, a pronounced vitamin A deficiency may be attenuated by APM. APM has the potency to deplete lung vitamin A in vivo and vitamin A might have a protective effect in the process of lung carcinogenesis, APM might increase the susceptibility for the development of lung cancer [6].

In mice, it was found that the the angiotensin II (AT2) receptor function in lung stromal fibroblasts may be a potential modulator of tumor susceptibility in chemical carcinogen-induced lung tumorigenesis. AT2 receptor null mice displayed higher susceptibility to lung cancer. The level of active TGF-beta in the conditioned medium was consistently higher with AT2-null fibroblasts than with wild-type fibroblasts [7]. The AT2 receptor is one of the receptors heavily affected from (Ro)accutane exposure, and its expression is suggested to be inhibited [8].

5.60.3. The metabolic syndrome, diabetes and cancer susceptibility :

To complete...

5.60.4. (Ro)accutane induced vitamin D deficiency and cancer susceptibility: suggested variations between type of cancer :

A significant fall in the level of 1,25-dihydroxyvitamin D, and a significant increase in the molar ratio of 24, 25-dihydroxyvitamin D to 25-hydroxyvitamin D was found in human subjects after exposure to (Ro)accutane, indicating a (Ro)accutane induced significant 1,25-dihydroxyvitamin D deficiency [11].

Colon cancer and vitamin D deficiency :

The relationship between vitamin D deficiency and colon cancer has been extensively shown [13, 15 and more]. In vitro and in vivo studies demonstrated that 1,25-dihydroxycholecalciferol [1,25(OH)(2)D(3)] and its analogs inhibit colon cancer cell proliferation. Vitamin D deficiency enhances the growth of colon cancer in mice. The tumor expression of VDR and 1alpha-OHase indicates possible autocrine/paracrine cell growth

regulation by vitamin D [13].

Prostate cancer and vitamin D deficiency :

Epidemiological evidence suggests that vitamin D deficiency also is associated with increased risk for prostate cancer. Results show that cholecalciferol, at physiological levels: (i) inhibits anchorage-dependent growth (ii) induces differentiation by increasing PSA expression and (iii) exerts its effects by up-regulating vitamin D receptor (VDR), retinoid-X receptors (RXRs), and androgen receptor (AR). Furthermore, it was discovered that human prostate epithelial cells constitutively express appreciable levels of 25-hydroxylase CYP27A1 protein, the enzyme which catalyzes the conversion of cholecalciferol to 25(OH)D(3), and that CYP27A1 is up-regulated by cholecalciferol. Recent studies show that human mitochondrial CYP27A1 can also catalyze 1alpha-hydroxylation of 25(OH)D(3) to calcitriol. The presence of 25-hydroxylase in human prostate epithelial cells has not previously been shown. Since human prostate epithelial cells have the necessary enzymes and the rare ability to locally convert cholecalciferol to the active hormone calcitriol, it is proposed that they are a prime target for chemoprevention of prostate cancer with cholecalciferol whose safety is well established as a supplement in vitamins and fortified foods [17].

1,25-dihydroxyvitamin D(3) (1,25(OH)(2)D(3)) and its analogues have been shown to inhibit proliferation of human cancer cells mediated by vitamin D receptor (VDR). CYP24A1 mRNA was significantly up-regulated in colon, ovary and lung tumors, but down-regulated in breast tumor relative to the analogous normal tissues. As a comparison, VDR mRNA was modestly down-regulated in colon, breast and lung tumors, but highly up-regulated in ovarian tumors [12].

Hepatocarcinogenesis and vitamin D deficiency :

In rats, labeling of oval cells, a cell compartment possibly associated with the repopulation of the liver parenchyma, was significantly reduced by vitamin D depletion. Control rat livers of both groups showed normal liver histology, and no foci, nodules or oval cells were detected in either group. The present data suggest that vitamin D depletion leads to increased in vivo susceptibility to chemicals known to induce hepatocarcinogenesis. Vitamin D depletion in rats caused changes on focus size, which was found to be significantly greater in vitamin D-depleted rat livers at weeks 2 to 6; focus area (volume fraction) was also found to be consistently larger in livers of vitamin D-depleted rats than in those of normal rats [16].

Breast cancer and vitamin D deficiency :

Reduction of epidermal growth factor receptor (EGFR) mRNA and protein by 1,25-dihydroxyvitamin D3 has been documented in MCF7, T47D, and BT549 breast cancer cells [20].

5.60.5. (Ro)accutane and irreversible androgen deprivation: the effects on cancer susceptibility :

"A reduced process of inactivation of retinoic acid (all trans) after treatment with 13-cis retinoic acid compared to treatment with (all trans) retinoic acid"

"The expression of de novo mRNA of cytochrome P450 1A1, a major enzyme metabolizing xenobiotics, in SZ95 sebocytes, was induced by (all trans) retinoic acid, but not by 13-cis retinoic acid. "

"Both 13-cis retinoic acid and (all trans-) retinoic acid suppressed the expression of cytochrome P450 1A2 mRNA. "

https://www.ncbi.nlm.nih.gov/pubmed/10951254

(Ro)accutane is in repeated studies found to cause a DHT deficiency post exposure partially through a significant inhibition of 5-alpha-r [18 and more].

It was previously assumed that androgen deficiency would have only positive effects on susceptbility of prostate cancer. Over 60 years ago, Huggins and Hodges discovered androgen deprivation as a first-line therapy for metastatic prostate cancer, which leads to remissions typically lasting 2 to 3 years, but in most men prostate cancer ultimately progresses to an androgen-independent state resulting in death due to widespread metastases. Multiple mechanisms of androgen independence have now been documented, including amplification of the androgen receptor as well as signal transduction pathways that bypass the androgen receptor completely [19].

- Conclusions :

To complete...

- References :

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Carcinogenesis. 2004 Jun;25(6):923-9. Epub 2004 Feb 19. Related Articles, Links

Low CYP1A2 activity associated with testicular cancer.
Vistisen K, Loft S, Olsen JH, Vallentin S, Ottesen S, Hirsch FR, Poulsen HE.
Department of Pharmacology, The Panum Institute, University of Copenhagen, Denmark.

The incidence rate of testicular cancer has increased during the last 50 years. An interplay between changing environmental factors and individual susceptibility, e.g. in foreign compound metabolizing enzymes, may have important influences on the risk of testicular cancer. The cytochrome P4501A2 (CYP1A2) enzyme and the bimodally expressed enzyme N-acetyltransferase2 (NAT2) metabolize many procarcinogens/carcinogens. The aim of this population-based case-control study was to investigate if CYP1A2 or NAT2 activity measured as a ratio of urinary metabolites of dietary caffeine is a risk factor in testicular cancer. 378 men participated (80 seminomas, 104 non-seminomas and 194 controls). The CYP1A2 activity was lower in the cases than in the controls [median and 30-70% percentiles: 4.7 (3.9-5.7) and 5.2 (4.4-6.4), respectively]. The subjects were classified in tertiles with low, medium or high CYP1A2 activity. A low CYP1A2 activity was associated with the highest risk of testicular cancer. Including all participants except men using drugs suspected to influence CYP1A2 activity (n = 15), medium and low activity conferred odds ratios (ORs) of 1.54 [confidence intervals of 95% (CI(95%)) 0.93-2.55] and 2.11; CI(95%) (1.23-3.62), respectively, of having testicular cancer. Excluding smokers (n = 157) the ORs of medium and low activity were 3.63; CI(95%) (1.53-8.60) and 4.70; CI(95%) (2.03-10.89), respectively. After further exclusion of cases that had received chemotherapy or radiation (n = 47), similar significant results were achieved. In the groups with the lowest CYP1A2 activity the ORs for seminoma and non-seminoma were 2.12; CI(95%) (0.93-4.81) and 2.10; CI(95%) (1.02-4.32). The phenotype of NAT2 was not associated with testicular cancer. In conclusion, we found no association of NAT2 phenotype to testicular cancer, whereas significant associations between CYP1A2 activity and testicular cancer were shown.

PMID: 14976127 [PubMed - indexed for MEDLINE]

Proc Natl Acad Sci U S A. 2005 Jun 14;102(24):8758-63. Epub 2005 Jun 6. Related Articles, Links
Nitric oxide synthase II suppresses the growth and metastasis of human cancer regardless of its up-regulation of protumor factors.
Le X, Wei D, Huang S, Lancaster JR Jr, Xie K.
Department of Gastrointestinal Medical Oncology, M. D. Anderson Cancer Center, University of Texas, 1515 Holcombe Boulevard, Houston, TX 77030, USA.

Inducible nitric oxide (NO) synthase (NOS) II has been implicated in macrophage-mediated antitumor activity. However, use of the NOS II gene in cancer therapy is problematic because of the double-edged nature of NO action. Herein we show that adenoviral vectors mediated effective NOS II gene transfer into various human tumors. Production of NO significantly up-regulated multiple angiogenic molecules. However, the NO-producing tumor cells did not form tumors or metastases in ectopic or orthotopic xenograft nude mouse models. The dramatic loss of malignancy was due to NO-mediated apoptosis. We also generated a series of adenoviral vectors harboring mutant NOS II genes that expressed mutant NOS II proteins with defined levels of enzymatic activity. Tumor cells transduced with these NOS II genes produced NO at different levels, which directly correlated with the antitumor activity in vitro and in vivo. This demonstration using a relevant biological system shows that NO produces dose-dependent antitumor activity in

vitro and in vivo, regardless of its up-regulation of protumor factors.

PMID: 15939886 [PubMed - indexed for MEDLINE]

Pathobiology. 2004;71(3):129-36. Related Articles, Links
Depletion of tumor-infiltrating macrophages is associated with amphoterin expression in colon cancer.
Kuniyasu H, Sasaki T, Sasahira T, Ohmori H, Takahashi T.
Department of Oncological Pathology, Nara Medical University Cancer Center, Kashihara, Japan. cooninh@zb4.so-net.ne.jp

Macrophage infiltration into colon cancer and amphoterin expression in cancer cells was examined in 42 human colon cancers invading the subserosa. The mean number of infiltrating macrophages was significantly higher in Dukes' B cases than in Dukes' C cases ($p = 0.0065$). Tumors with few infiltrating macrophages (macrophage depletion) were significantly more frequent in Dukes' C cases than in Dukes' B cases ($p = 0.0014$). No Dukes' C cases with relevant macrophage infiltration showed macrophage-cancer cell contact, whereas 5 Dukes' B cases showed such contact ($p < 0.0001$). In human colon cancer cells implanted in the cecum of nude mice, KM12SM (highly metastatic) tumors yielded less macrophage infiltration and more liver metastases than KM12C (low risk of metastasis) tumors (14 +/- 3 vs. 78 +/- 32 and 24 +/- 6 vs. 5 +/- 3 per liver, respectively). Amphoterin expression was detected at high frequency in both Dukes' B and C cases ($p = 0.0684$). In macrophage-depleted cases, amphoterin expression was significantly higher than that in non-depleted cases ($p = 0.0015$). To confirm biological effects of amphoterin on macrophages, an infiltration assay using the cell-layered Boyden chamber was done. Infiltration of PMA-treated U937 monocytes through the KM12SM cell layer was increased by pretreatment of KM12SM cells with amphoterin antisense S-oligodeoxynucleotide exposure. Moreover, extracted amphoterin inhibited PMA-U937 monocyte infiltration in a dose-dependent manner. Thus, amphoterin may play an important role in the inhibition of macrophage infiltration into colon cancer. Copyright 2004 S. Karger AG, Basel

PMID: 15051925 [PubMed - indexed for MEDLINE]

Proc Natl Acad Sci U S A. 2004 Jan 20;101(3):763-8. Epub 2004 Jan 12. Related Articles, Links
Increased primary tumor growth in mice null for beta3- or beta3/beta5-integrins or selectins.
Taverna D, Moher H, Crowley D, Borsig L, Varki A, Hynes RO.
Howard Hughes Medical Institute, Center for Cancer Research, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA.

Expression of alphavbeta3- or alphavbeta5-integrins and selectins is widespread on blood cells and endothelial cells. Here we report that human tumor cells injected s.c. into mice lacking beta3- or beta3/beta5-integrins or various selectins show enhanced tumor growth compared with growth in control mice. There was increased angiogenesis in mice lacking beta3-integrins, but no difference in structure of the vessels was observed by histology or by staining for NG2 and smooth muscle actin in pericytes. Bone marrow transplants suggest that the absence of beta3-integrins on bone marrow-derived host cells contributes to the enhanced tumor growth in beta3-null mice, although few, if any, bone marrow-

derived endothelial cells were found in the tumor vasculature. Tumor growth also was affected by bone marrow-derived cells in mice lacking any one or all three selectins, implicating both leukocyte and endothelial selectins in tumor suppression. Reduced infiltration of macrophages was observed in tumors grown in mice lacking either beta3-integrins or selectins. These results implicate cells of the innate immune system, macrophages or perhaps natural killer cells, in each case dependent on integrins and selectins, in tumor suppression.

PMID: 14718670 [PubMed - indexed for MEDLINE]

Yonsei Med J. 2005 Aug 31;46(4):449-55. Related Articles, Links
Obesity, insulin resistance and cancer risk.
Jee SH, Kim HJ, Lee J.
Department of Epidemiology and Health Promotion, Graduate School of Public Health, Yonsei University, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, Korea.
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Obesity is a known cause of metabolic syndrome which includes Type II diabetes, hypertension, and dyslipidemia. It is well documented that insulin resistance contributes to the mortality and the incidence of metabolic syndromes including central obesity, dyslipidemia, hyperglycemia and hypertension. Both obesity and diabetes are emerging topics for researchers to consider as having a possible causal association with cancer since the two factors have been viewed as risk factors for cancer. The present paper introduced the hypothesis of a possible causal relationship between obesity, insulin resistance and cancer and reviews relevant existing studies in this area. More efforts and studies are needed to clarify the mechanisms and the common risk factors which might be incorporated into interventions to prevent cancer and cardiovascular diseases as top causes of death.

PMID: 16127767 [PubMed - in process]

J Endocrinol Invest. 2005;28(5 Suppl):38-42. Related Articles, Links
The IGF system in childhood: physiology and clinical implications.
Pozo J, Martos-Moreno GA, Barrios V, Argente J.

Department of Pediatric Endocrinology, Universitad Autonoma de Madrid, Madrid, Spain.

Our understanding of the IGF-I system has increased dramatically in recent yr due in part to the advances in molecular and cellular biology. Not only can we now measure circulating levels of the members of this axis in order to address the possibly pathophysiological changes, but genetic alterations can now be identified as the underlying cause of specific clinical situations. In normal children, circulating levels of IGF-I and the IGF binding proteins (IGFBPs) change throughout development and in some cases are gender dependent. Children and adolescents with a variety of illnesses and metabolic disorders have altered circulating IGF-I and IGFBP levels. Hence, in children or adolescents with exogenous obesity, anorexia nervosa, coeliac disease, leukaemia and other types of cancer, as well as in cases of GH deficiency, this axis can be altered. These data may help us to understand the physiology and pathophysiology of this system, but the clinical or diagnostic utility of these measurements is still largely debated. Indeed, in most

of the above mentioned illnesses, circulating IGF and IGFBP levels overlap with normal values. Furthermore, these measurements do not provide data concerning levels of these factors at target tissues or of local synthesis and autocrine-paracrine effects. However, measurements of IGF-I and its binding proteins, as well as GH and its binding proteins, can help us to focus our analysis of patients suspected to have genetic abnormalities on the GH receptor, IGF-I, its receptor, IGFALS, or intracellular signalling proteins such as STAT5b or ERK. Possibly, the most clear clinical utility of circulating IGF-I measurements in children is in cases of GH deficiency or insensitivity or under GH treatment. However, the fact there are cases of children with non-detectable levels of circulating IGF-I that yet normal height and growth velocity, or with non-detectable levels of GH yet normal growth and IGF-I levels, raises many questions. Furthermore, circulating IGF-I levels may be within the normal control levels and the child may have a pathological growth pattern. Hence, just how useful are these measurements? Another clinically important question pertains to GH treatment in patients, such as in the Turner Syndrome, where supraphysiological levels of serum IGF-I are reached in order to induce growth. The interpretation and clinical utility of measurements of circulating IGF-I and its BPs are currently being widely discussed. As our knowledge of this system increases, with the identification of new members of this family and its intracellular mechanisms of action, as well as new genetic alterations in patients, the interpretation of laboratory results will also improve and help to better our diagnostic capability.

PMID: 16114274 [PubMed - in process]

Surgery. 1998 Dec;124(6):1094-8; discussion 1098-9. Related Articles, Links
Decreased expression of calcium-sensing receptor messenger ribonucleic acids in parathyroid adenomas.
Farnebo F, Hoog A, Sandelin K, Larsson C, Farnebo LO.
Department of Molecular Medicine, Karolinska Hospital, Stockholm, Sweden.

BACKGROUND: The set point for parathyroid hormone (PTH) secretion is increased in patients with primary hyperparathyroidism, possibly because of receptor defect(s). A decreased expression of calcium receptor (CaR) messenger ribonucleic acid (mRNA) and protein and a decreased expression of the putative calcium-sensing CAS (gp330/megalin) protein have been demonstrated in parathyroid adenomas. METHODS: Expression of CAS mRNA was studied in matched pairs of adenomas and adenoma-associated biopsy specimens from normal parathyroid glands from 15 patients with sporadic primary hyperparathyroidism. Cryostat sections were hybridized with an oligonucleotide complementary to CAS mRNA, rinsed, air dried, and exposed to x-ray film for semiquantification of radioactivity. RESULTS: Expression of CAS mRNA in the adenomas was lowered significantly to 25% (median; range 9% to 80%) of that of the corresponding biopsy specimens of normal parathyroid glands. No correlation was seen between CAS mRNA in the adenoma and preoperative serum calcium levels, PTH level, or weight of the adenoma. The levels of CAS mRNA were significantly lower than those observed previously for CaR mRNA. There was no significant correlation between the levels of CAS and CaR mRNA. CONCLUSIONS: Lowered levels of receptors sensing extracellular calcium (CaR and CAS) probably contribute to the increased set point for PTH secretion in primary hyperparathyroidism.

PMID: 9854589 [PubMed - indexed for MEDLINE]

Br J Cancer. 2005 Sep 5;93(5):602-6. Related Articles, Links
Angiotensin-converting enzyme gene insertion/deletion polymorphism is associated with risk of oral precancerous lesion in betel quid chewers.

Chung FM, Yang YH, Chen CH, Lin CC, Shieh TY.

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[2] 2Graduate Institute of Dental Sciences, College of Dental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

To investigate whether angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism is related to the risk of oral precancerous lesions (OPL) in Taiwanese subjects who chew betel quid, a total of 61 betel quid chewers having OPL were compared with 61 asymptomatic betel quid chewers matched for betel quid chewing duration and dosage. The frequency of homozygote for ACE D variant is significantly higher in the case subjects than that of the controls (44.3 vs 24.6%; P=0.0108). The adjusted odds ratio of the D homozygous for the risk of OPL is 8.10 (95% confidence interval (CI)=2.04-32.19, P=0.003). In the allelic base analysis, the D allele is also significantly associated with higher risk of OPL. When grouping the study subjects by smoking status, the association between ACE I/D polymorphism and risk of OPL was only observed in nonsmokers. Our results support the theory that genetic factors may contribute to the susceptibility of OPL and suggest that smoking and genetic factors may be differently involved in the development of OPL.British Journal of Cancer (2005) 93, 602-606. doi:10.1038/sj.bjc.6602746 www.bjcancer.com.

PMID: 16136034 [PubMed - in process]

Section 6 : (Ro)accutane effects suggested to worsen with age

6.1 Hormonal decline with age :

Levels of thyroxine and triiodothyronine were found to be lower *after* (Ro)accutane exposure in acne-subjects (p less than 0.05) [4]. Significant inhibition of thyroid receptor expression in various cell types has been observed in subjects treated with 5microM retinoic acid - plasma concentrations associated with the exposure to (Ro)accutane in acne-subjects. A decline that may be fortified with age.

In pituitary GH1 cells a maximal thyroid receptor beta-2 (TRbeta2) decrease of 50-70% in (Ro)accutane related doses in acne-subjects was observed [2]. TRbeta2 is found mainly in the pituitary gland and hypothalamus.

The amount of thyroid receptor (TR) mRNA, alpha as well as beta subtypes, was found to be significantly decreased in elderly, indicating an age-related sub-clinical hypothyroidism [1], and may mean that a worsening hypothyroidism with age in (Ro)accutane exposed subjects could be expected.

During and after a (Ro)accutane exposure a suppressive effect of the growth hormone stimulus is here suggested, by altered circulating GH/IGF-1 ratios, here suggested to be partially a result of inhibition of thyroid function [4] and inhibition of receptor TRbeta2 expression [2] (anterior pituitary cell-receptor inhibition and thyrotropes and somatropes increasingly undergoing apoptosis), 9-cis retinoic acid receptor-alpha (RXRalpha) interaction with IGFBP-3 [5]. The effects on GH/IGF-1 receptor expression during and after a (Ro)accutane exposure are not known.

A worsened effect of partial growth hormone deficiency is suggested to be worsened with age due to age-related further declining circulating GH-IGF levels, and with increasing age the GH/IGF-1 axis undergoes changes such as decreased GH and IGF-1 concentrations in the brain. This decline in hormone levels has been associated with changes of certain CNS functions such as age-related memory impairments [3].

Insulin-like growth factor-1 (IGF-1) and growth hormone (GH) have been suggested to promote memory and cognitive capabilities. Both GH and IGF-1 affect the size and morphology of the central nervous system (CNS) during development and alter differentiated cell functions like neural growth, myelination, and cognitive performance. [3]

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6.2 Retinoid receptor expression and age :

- Decreased retinoid receptor expression with age :

Inadequate retinoid status has often been described as occurring with aging. Several studies performed in animals have described the crucial incidence of age-related hypo-functioning of retinoid and thyroid signalling pathways, particularly in the brain. RARgamma expression was significantly decreased in elderly versus young subjects [1]. A significantly decreased retinoid receptor expression in human subjects after exposure to (Ro)accutane is here suggested.

In hypothyroid subjects, the concentration of TSH was elevated, and dramatically low T3 and T4 concentrations were associated with a decrease in the expression of TR beta. Expression of RAR alpha and RAR gamma significantly decreased in hypothyroid versus control subjects [2].

- Further decreased levels of retinoid metabolites with age in certain ares of the human brain :

In studied human brains, frontal lobes, but not occipital lobes, exhibited an age-related decline in retinol, total tocopherols, total xanthophylls and total carotenoids [3].

- References :

[1] **Feart C, Pallet V, Boucheron C, Higueret D, Alfos S, Letenneur L, Dartigues JF, Higueret P.** *Aging affects the retinoic acid and the triiodothyronine nuclear receptor mRNA expression in human peripheral blood mononuclear cells.* (2005) Eur J Endocrinol. Mar;152(3):449-58
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Mech Ageing Dev. 2005 Feb;126(2):333-9. Related Articles, Links
Candidate genes associated with ageing and life expectancy in the Jerusalem longitudinal study.
Stessman J, Maaravi Y, Hammerman-Rozenberg R, Cohen A, Nemanov L, Gritsenko I, Gruberman N, Ebstein RP.
Department of Geriatric Rehabilitation, Hadassah Hospital, Hebrew University Medical Center, Mt. Scopus, Jerusalem.

In an exploratory study, 11 common polymorphisms were examined for contributing to

longevity including: apolipoprotein E (apoE), methylenetetrahydrofolate reductase (MTHFR), cathepsin D (CAD), superoxide dismutase 2 (SOD2), angiotensinogen (AGT) and insulin-like growth factor 2 (IGF2), Leiden factor 7, p53 oncogene, dopamine D4 receptor (DRD4) and the serotonin transporter (SERT). Genotype and allele frequencies of these genes were compared in 224 older (75 years) Jewish Jerusalem residents of Ashkenazi ethnicity to a group of 441 younger subjects (22 years). Nominally significant results provide suggestive evidence in the Ashkenazi group that apoE, MHTFR, SOD2, IGF2 ApaI, and factor VII are risk factors for a single outcome, survival to 75. Overall, the more genetically homogenous Ashkenazi ethnic group showed evidence for association in five genes examined suggesting that future studies in this population would gainfully focus on this ethnic group.

PMID: 15621215 [PubMed - indexed for MEDLINE]

Neurobiol Aging. 2005 Jun 15; [Epub ahead of print] Related Articles, Links
Effects of intranasal insulin on cognition in memory-impaired older adults: Modulation by APOE genotype.

Reger MA, Watson GS, Frey WH 2nd, Baker LD, Cholerton B, Keeling ML, Belongia DA, Fishel MA, Plymate SR, Schellenberg GD, Cherrier MM, Craft S.

Geriatric Research, Education, and Clinical Center, Veterans Affairs Puget Sound Health Care System, 1660 S, Columbian Way, S182-GRECC, Seattle, WA 98108, USA; Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine, Seattle, WA 98195, USA.

Raising insulin acutely in the periphery and in brain improves verbal memory. Intranasal insulin administration, which raises insulin acutely in the CNS without raising plasma insulin levels, provides an opportunity to determine whether these effects are mediated by central insulin or peripheral processes. Based on prior research with intravenous insulin, we predicted that the treatment response would differ between subjects with (varepsilon4+) and without (varepsilon4-) the APOE-varepsilon4 allele. On separate mornings, 26 memory-impaired subjects (13 with early Alzheimer's disease and 13 with amnestic mild cognitive impairment) and 35 normal controls each underwent three intranasal treatment conditions consisting of saline (placebo) or insulin (20 or 40IU). Cognition was tested 15min post-treatment, and blood was acquired at baseline and 45min after treatment. Intranasal insulin treatment did not change plasma insulin or glucose levels. Insulin treatment facilitated recall on two measures of verbal memory in memory-impaired varepsilon4- adults. These effects were stronger for memory-impaired varepsilon4- subjects than for memory-impaired varepsilon4+ subjects and normal adults. Unexpectedly, memory-impaired varepsilon4+ subjects showed poorer recall following insulin administration on one test of memory. These findings suggest that intranasal insulin administration may have therapeutic benefit without the risk of peripheral hypoglycemia and provide further evidence for apolipoprotein E (APOE) related differences in insulin metabolism.

PMID: 15964100 [PubMed - as supplied by publisher]

J Neurochem. 2004 Feb;88(3):623-34. Related Articles, Links
A liver X receptor and retinoid X receptor heterodimer mediates apolipoprotein E expression, secretion and cholesterol homeostasis in astrocytes.

Liang Y, Lin S, Beyer TP, Zhang Y, Wu X, Bales KR, DeMattos RB, May PC, Li SD, Jiang XC, Eacho PI, Cao G, Paul SM.
Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, Indiana 46285, USA.

Apolipoprotein E (apoE) is an important protein involved in lipoprotein clearance and cholesterol redistribution. ApoE is abundantly expressed in astrocytes in the brain and is closely linked to the pathogenesis of Alzheimer's disease (AD). We report here that small molecule ligands that activate either liver X receptors (LXR) or retinoid X receptor (RXR) lead to a dramatic increase in apoE mRNA and protein expression as well as secretion of apoE in a human astrocytoma cell line (CCF-STTG1 cells). Examination of primary mouse astrocytes also revealed significant induction of apoE mRNA, and protein expression and secretion following incubation with LXR/RXR agonists. Moreover, treatment of mice with a specific synthetic LXR agonist T0901317 resulted in up-regulation of apoE mRNA and protein in both hippocampus and cerebral cortex, indicating that apoE expression in brain can be up-regulated by LXR agonists in vivo. Along with a dramatic induction of ABCA1 cholesterol transporter expression, these ligands effectively mediate cholesterol efflux in both CCF-STTG1 cells and mouse astrocytes in the presence or absence of apolipoprotein AI (apoAI). Our studies provide strong evidence that small molecule LXR/RXR agonists can effectively mediate apoE synthesis and secretion as well as cholesterol homeostasis in astrocytes. LXR/RXR agonists may have significant impact on the pathogenesis of multiple neurological diseases, including AD.

PMID: 14720212 [PubMed - indexed for MEDLINE]

J Biol Chem. 2004 Jan 30;279(5):3862-8. Epub 2003 Oct 29. Related Articles, Links
Astroglial regulation of apolipoprotein E expression in neuronal cells. Implications for Alzheimer's disease.

Harris FM, Tesseur I, Brecht WJ, Xu Q, Mullendorff K, Chang S, Wyss-Coray T, Mahley RW, Huang Y.
Gladstone Institute of Neurological Disease, University of California, San Francisco, California 94141-9100, USA.

Although apolipoprotein (apo) E is synthesized in the brain primarily by astrocytes, neurons in the central nervous system express apoE, albeit at lower levels than astrocytes, in response to various physiological and pathological conditions, including excitotoxic stress. To investigate how apoE expression is regulated in neurons, we transfected Neuro-2a cells with a 17-kilobase human apoE genomic DNA construct encoding apoE3 or apoE4 along with upstream and downstream regulatory elements. The baseline expression of apoE was low. However, conditioned medium from an astrocytic cell line (C6) or from apoE-null mouse primary astrocytes increased the expression of both isoforms by 3-4-fold at the mRNA level and by 4-10-fold at the protein level. These findings suggest that astrocytes secrete a factor or factors that regulate apoE expression in neuronal cells. The increased expression of apoE was almost completely abolished by incubating neurons with U0126, an inhibitor of extracellular signal-regulated kinase (Erk), suggesting that the Erk pathway controls astroglial regulation of apoE expression in neuronal cells. Human neuronal precursor NT2/D1 cells expressed apoE constitutively; however, after treatment of these cells with retinoic acid to induce differentiation, apoE expression diminished. Cultured mouse primary cortical and hippocampal neurons also expressed low levels of apoE. Astrocyte-conditioned medium rapidly up-regulated apoE expression in fully differentiated NT2 neurons and in cultured mouse primary cortical and

hippocampal neurons. Thus, neuronal expression of apoE is regulated by a diffusible factor or factors released from astrocytes, and this regulation depends on the activity of the Erk kinase pathway in neurons.

PMID: 14585838 [PubMed - indexed for MEDLINE]

Oncogene. 2004 Sep 16;23(42):7053-66. Related Articles, Links
Enhanced retinoid-induced apoptosis of MDA-MB-231 breast cancer cells by PKC inhibitors involves activation of ERK.
Pettersson F, Couture MC, Hanna N, Miller WH.
Lady Davis Institute for Medical Research, McGill University, 3755 Cote-Ste-Catherine Rd, Montreal, Quebec, Canada H3T 1E2.

Retinoids are vitamin A derivatives, which cause growth inhibition, differentiation and/or apoptosis in various cell types, including some breast cancer cells. In general, estrogen receptor (ER)-positive cells are retinoic acid (RA) sensitive, whereas ER-negative cells are resistant. In this report, we show that ER-negative MDA-MB-231 cells are strongly growth inhibited by retinoids in combination with a PKC inhibitor. While neither RA nor GF109203X (GF) has a significant growth inhibitory effect in these cells, RA+GF potently suppress proliferation. We found that RA+GF induce apoptosis, as shown by an increase in fragmented DNA, Annexin-V-positive cells and caspase-3 activation. Apoptosis was also induced by GF in combination with two synthetic retinoids. Expression of phosphorylated as well as total PKC was decreased by GF and this was potentiated by RA. In addition, treatment with GF caused a strong and sustained activation of ERK1/2 and p38-MAPK, as well as a weaker activation of JNK. Importantly, inhibition of ERK but not p38 or JNK suppressed apoptosis induced by RA+GF, indicating that activation of ERK is specifically required. In support of this novel finding, the ability of other PKC inhibitors to cause apoptosis in combination with RA correlates with ability to cause sustained activation of ERK.

PMID: 15273718 [PubMed - indexed for MEDLINE]

J Neurosci. 1996 Dec 1;16(23):7550-6. Related Articles, Links
Transcription factor AP-2 regulates human apolipoprotein E gene expression in astrocytoma cells.
Garcia MA, Vazquez J, Gimenez C, Valdivieso F, Zafra F.
Centro de Biologia Molecular Severo Ochoa, Facultad de Ciencias, Universidad Autonoma de Madrid, Spain.

Apolipoprotein E (apoE), one of the major plasma lipoproteins, also is expressed in a variety of cell types, including the glial cells of the nervous system. apoE is involved in processes of degeneration and regeneration after nerve lesions as well as in the pathogenesis of Alzheimer's disease (AD). Glial synthesis of apoE is activated in response to injury both in the peripheral and central nervous system. We now report that the activity of the proximal apoE promoter in astrocytes is upregulated by cAMP and retinoic acid, which act synergistically. Sequence analysis of the apoE promoter indicated the presence of several AP-2 consensus sequences that could mediate the stimulatory effect of cAMP and retinoic acid. The possible functional role of AP-2 was examined by cotransfection of AP-2-deficient HepG2 cells with an apoE promoter construct and a human AP-2 expression construct. Cotransfection with AP-2 significantly elevated apoE promoter

activity. DNase I footprinting technique revealed the existence of two binding sites for recombinant AP-2 in regions from -48 to -74 and from -107 to -135 of the apoE promoter. Mutations in these regions markedly impaired the trans-stimulatory effect of AP-2. These results indicate the existence of functional AP-2 sites in the promoter region of apoE that could contribute to the complex regulation of this gene in developmental, degenerative, and regenerative processess of the nervous system.

PMID: 8922411 [PubMed - indexed for MEDLINE]

J Clin Invest. 1997 Jul 15;100(2):310-20. Related Articles, Links
Neuronal cell death in Alzheimer's disease correlates with apoE uptake and intracellular Abeta stabilization.
LaFerla FM, Troncoso JC, Strickland DK, Kawas CH, Jay G.
Department of Virology, Jerome H. Holland Laboratory, Rockville, Maryland 20855, USA.

The brains of individuals with Alzheimer's disease (AD) are characterized by extracellular deposition of beta-amyloid protein (Abeta), intracellular neurofibrillary tangles, and loss of neurons. To study molecular markers associated with dying cells in the AD brain, in situ DNA labeling techniques were used to visualize cells with DNA fragmentation. We observed that intracellular accumulation of apolipoprotein E (apoE) is correlated with the detection of intracellular Abeta-like immunoreactivity within the same cytoplasmic granules, suggesting that uptake of lipids may have stabilized the hydrophobic Abeta protein within the cell. These apoE-containing neurons also exhibit high expression of a cell surface receptor, gp330, which is known to bind apoE. Cells containing significant nuclear DNA fragmentation express the highest level of cell surface gp330. Extracellular deposition of Abeta is detected only upon neuronal cell death, initially as halos of Abeta immunoreactivity around individual dying neurons, and subsequently as Abeta plaques containing numerous neuronal cell ghosts. Based on our in situ analysis of nuclear DNA fragmentation, we conclude that neuronal cell death likely occurs before the extracellular deposition of Abeta in AD brains.

PMID: 9218507 [PubMed - indexed for MEDLINE]

6.2 Cognitive decline, a possible role in Alzheimer´s :

(Ro)accutane and formation of beta-amyloid, a possible role in the pathology of alzheimers disease, elderly cognitive decline and dementia :

Introduction :

to complete

"Homocysteine is an amino acid that can be generated in response to nutritionally deficient or nutritionally deficient diets. "

"When homocysteine levels increase in the blood, it is linked to massive inflammation and neurodegeneration!
In Alzheimer's disease and many other chronic diseases, we find a significant increase in

homocysteine in blood tests. Since homocysteine is a potent excitotoxin and neurotoxin, high levels of homocysteine have been found to exacerbate the symptoms of Alzheimer's disease and other chronic diseases. Components of the metabolic degradation of homocysteine alter the NMDA (N-methyl-D-aspartate) receptor sites, resulting in multiple negative effects, including free radicals and a massive inflammatory cascade!
These free radicals and inflammation can trigger an autoimmune response in which the patient's immune system attacks the thyroid gland and / or other body systems. "

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5120102/

https://www.ncbi.nlm.nih.gov/pubmed/17852421

In the studies of (Ro)accutane induced degenerative pathologies, a distinction can be made between:

1) a suggested direct degenerative process during (Ro)accutane exposure as seen in mice [3 and 4]
2) a suggested possible slowly continous degenerative process post (Ro)accutane exposure and a possible role of retinoids in Alzheimer´s disease [2, 27 and more]

Retinoids are suggested to be of importance for the maintenance of the central nervous system, and a loss of retinoid signaling may contribute to the pathogenesis of Alzheimers disease [2 and more]. After 1 year of a vitamin-A dietary deficiency in adult rats, there was a deposition of amyloid beta in the cerebral blood vessels, suggesting a clear role of vitamin-A in the formation of beta amyloid. A downregulation of retinoic acid receptor alpha in the forebrain neurons of the retinoid-deficient rats and a loss of choline acetyl transferase expression, which precedes amyloid beta deposition was found. In neocortex of pathology samples of patients with Alzheimer's disease, the same retinoic acid receptor alpha deficit in the surviving neurons was observed [2]. Abnormal retinoid metabolism may be involved in the downstream transcriptional regulation of phospholipase A2-mediated signal transduction in Alzheimers disease (AD) [27].

- Suggested significant loss of hippocampal cells during exposure :

Results from (Ro)accutane exposure in adult mice are suggesting a significantly decreased hippocampal cell survival, and loss of cells during exposure [3 and 4]. Little is known about long-term effects of a (Ro)accutane exposure in humans. One of the principal theories of the pathology of Alzheimers disease is an increased formation of beta-amyloid [5 and more]. AD (Alzheimer's disease) is a neurodegenerative disease associated with progressive memory loss and leading to dementia. Except for cell-loss, two histological characteristics are observed in AD patients after autopsy: extracellular plaques and intracellular tangles [17]. Several enzymes and receptors of importance for the formation of beta-amyloid are distinctively altered in association with exposure of retinoic acid [6, 7 and more], as well as receptors, such as megalin, that likely are involved in the clearance of beta amyloid [26 and more], as well as disruption of a number of signaling pathways, that are suggested to be involved in the pathology of Alzheimers disease [27 and more].

- Formation of beta amyloid from APP :

The principal component of amyloid fibrils is beta/A4 amyloid protein, which can be generated by the aberrant processing of a large membrane-bound glycoprotein, the beta/A4 amyloid protein precursor (APP)3 [1]. The amyloid beta-peptide (approximately 4 kDa-M(r)) is generated by the proteolytic cleavage of a larger beta-amyloid precursor protein (beta APP) encoded by a gene on chromosome 21 [9].

- (Ro)accutane and disruptions in G-protein signaling; possible decreases in RGS protein expression :

RGS proteins :

A statistically significant downregulation of RGS4 was found in post-mortem examinations of brain samples from patients with Alzheimers Disease [31]. RGS proteins have been shown to control essential neurological functions by modulating G(i) and G(q) mediated signaling. More than 20 regulators of G-protein signaling, RGS proteins, have been identified, of which five are found to be expressed highly in the brain (RGS4, RGS7, RGS8, RGS11 and RGS17). During the G-protein activity cycle RGS reduces the GPCR signaling by accelerating the rate of GTP hydrolysis. RGS interacts specifically with the alpha subunit of the G-protein which leads to dimerization and an inactive heterotrimeric G-protein [31].

Retinoids and G-protein signaling

Significant interference between retinoids and G-protein signaling is well known. In the opthamologic field, retinoids are known to affect G(t) [32], but also a range of other G-proteins are found to be affected. In rat liver, during exposure RXR agonists were found to significantly decline mRNA levels of the G-protein subunit Galpha [33] - a subunit also known to be regulated by RGS proteins [31]. This observation and other observations are leading to the suggestion that (Ro)accutane alters RGS protein expression.

Transcription factor Phox2b :

In embryos deficient for Phox2b, RGS4 expression is downregulated in the locus coeruleus, sympathetic ganglia, and cranial motor and sensory neurons [35].
The importance of Phox2b in adult brain is not yet fully understood.

In the developing rat heart, Retinoic Acid disrupts the differentiation of cardiac neural crest cells into ganglionic cells destined to contribute to the parasympathetic innervation of the heart, by regulating the expression of Phox2a and Phox2b [34].

- (Ro)accutane induced increases in TGF-beta1 and significantly increased formation of beta amyloid from APP :

In acne subjects, six weeks of isotretinoin exposure caused a statistically significant 19% increase in suction blister fluid TGF-beta1 [18]. TGF-b (transforming growth factor-b), which is overexpressed in AD patients, is capable of enhancing the synthesis of APP by astrocytes by a transcriptional mechanism leading to the accumulation of Ab. TGF-b1

induces the binding of nuclear factors to the APPtre sequence. The APPtre sequence not only responds to the Smad3 transcription factor, but also the Sp1 (signal protein 1) transcription factor co-operates with Smads to potentiate the TGF-b-dependent activation of APP [17].

In small doses, retinoic acid is found to upregulate APP in neuronal cell lines [19, 20 and more]. To test whether overexpression of APP generates abnormally processed derivatives that affect the viability of neurons, full-length human APP complementary DNA was stably transfected into murine embryonal carcinoma P19 cells. These cells differentiate into post-mitotic neurons and astrocytes after exposure to retinoic acid. When differentiation of the APP cDNA-transfected P19 cells was induced, all neurons showed severe degenerative changes and disappeared within a few days [19].

- Binding and translocation of APP :

The binding of amyloid beta precursor protein may be affected, since APBB1 is located in a subcellular area heavily affected by retinoic acid, resulting in a translocation of APP (11p15).

1) Alteration of APP cleavage (Bace 1 and 2)
2) Alteration of beta amyloid binding
APPBP1 and 2
APBA2
APBA3
APBB1
APBB2
3) Alteration in formation of APP

APBB1 :

amyloid beta (A4) precursor protein-binding, family B,

member 1 (Fe65)
Location 11p15.5

- (Ro)accutane exposure and calpain redisribution :

It is well documented that activation of calpain, a calcium-sensitive cysteine protease, marks the pathology of naturally and experimentally occuring neurodegenerative conditions. Calpain-mediated proteolysis of major membrane-skeletal protein, alphaII-spectrin, results in the appearance of two unique and highly stable breakdown products, which is an early event in neural cell pathology [23]. The calcium-dependent protease, calpain, cleaves the cytoplasmic domain of the integrin beta3 subunit [22].

Calpastatin distribution is affected by the intracellular increase in free Ca(2+), which results in calpastatin progressively becoming a soluble protein. However, calpain is distributed in the soluble cell fraction and, in activating conditions, partially accumulates on the plasma

membrane. Similar behaviour has been observed in calpastatin localization in LAN-5 cells induced with retinoic acid, suggesting that the proteolytic system is activated during the differentiation process of these cells [24].

Alzheimer's beta-amyloid precursor protein (APP) is normally processed by an unidentified alpha-secretase. A unique feature of this protease is its high sensitivity to phorbol esters, yet the mechanism involved is unclear. We have previously reported that phorbol 12,13-dibutyrate (PDBu) activates calpain, a Ca2+-dependent protease, and PDBu-induced release of APPs (secreted APP) is sensitive to calpain inhibitors, suggesting that calpain is involved in APP alpha-processing. In the present study, we found that PDBu markedly promoted the expression of both mu- and m-calpains in cultured fibroblasts. Dose-response and time course studies revealed that mu-calpain was more sensitive to PDBu than m-calpain and the temporal course of the mu-calpain change coincides better with that of APPs release. Moreover, the stimulatory effect of PDBu on mu-calpain was selectively blocked by mu-calpain-specific siRNA (small interference RNA) and the blockage was accompanied by a concomitant decrease in APPs release. In contrast, m-calpain siRNA did not affect APPs release significantly. Measurement of amyloid beta protein (Abeta) release in the mu-calpain siRNA-treated cells indicated that Abeta40 and Abeta42 levels inversely changed in relation to APPs, and the changes in Abeta42 were more prominent than in Abeta40. Together, these data suggest that calpain, particularly mu-calpain, is a potential candidate for alpha-secretase in the regulated APP alpha-processing, and that changes in this protease can affect the outcome of the overall APP processing [21].

- During exposure: Inhibition of LRP-2 mediated beta-amyloid clearance and P-gp-mediated clearance - vitamin A deficiency after exposure, suggested decrease of both LRP-2 and P-gp :

The clearance mechanisms of beta amyloid are yet not fully understood, but based on observations in combinations with hypothesises. Two distinct patways of clearance are suggested: 1) beta amyloid couples to LRP-2 and 2) beta amyloid couples to P-gp. In addition PPARgamma has been suggested to, through an unknown pathway, influence beta amyloid clearance.

Critical in modulating beta-amyloid deposition in brain is the flux of Abeta across the blood brain barrier. The low-density lipoprotein receptor-related protein (LRP), is a large endocytic receptor that mediates the efflux of Abeta out of brain and into the periphery. The first step in the LRP-mediated clearance of Abeta involves the formation of a complex between Abeta and the LRP ligands apolipoprotein E (apoE) or alpha(2)-macroglobulin (alpha(2)M). The Abeta/chaperone complexes then bind to LRP via binding sites on apoE or alpha(2)M. The efflux of Abeta/chaperone complexes out of the neuropil and into the periphery may be attenuated by LRP-ligands that compete with apoE or alpha(2)M for LRP binding [26].

PPARgamma is suggested to be significantly affected during (Ro)accutane exposure. After exposure, when a vitamin A deficiency is present it may result in a downregulation of the PPARgamma receptor function.

- (Ro)accutane and formation of ceramide and a possible role in

Alzheimers disease, age-related cognitive decline and dementia :

Retinoic acid in pro-apoptotic doses (doses associated with exposure in acne-subjects) is currently used in research models that possibly may approach an understanding of the pathology of alzheimers disease and age-related cognitive decline [10 and more]. A 2.5-fold increase of ceramide mass in the supernatant was detected after 48 h of treatment with RA.

In retinoic acid (RA)-induced neuronal apoptosis, RA slightly increased de novo synthesis of ceramide, but interestingly, RA dramatically inhibited conversion of [14C] ceramide to glucosylceramide (GlcCer), suggesting that the increase of ceramide mass is mainly due to inhibition of the ceramide-metabolizing enzyme GlcCer synthase. In addition, a significant increase of the [14C] ceramide level in the culture medium was detected by chasing and turnover experiments without alteration of extracellular [14C] sphingomyelin levels [10].

- A possible role in (Ro)accutane induced memory alterations: The binding and disposition of beta-amyloid and low-density lipid receptor function - possible downregulation of the binding of apoJ/abeta1-40 complexes to LRP-2, through a downregulation of LRP-2 :

In F9 teratinoma cells, modest doses of retinoic acid is found to modulate the expression of megalin, a receptor that among other things is found to mediate uptake of ApoJ and ApoJ- abeta complexes into these cells [29]. Up to date, no study evaluates the effects on the megalin receptor in human acne-subjects exposed to massive doses of retinoic acid on various cell-lines, including cell lines in the brain. However, a significant suppression of the megalin receptor is likely in association with exposure, and likely also after exposure.

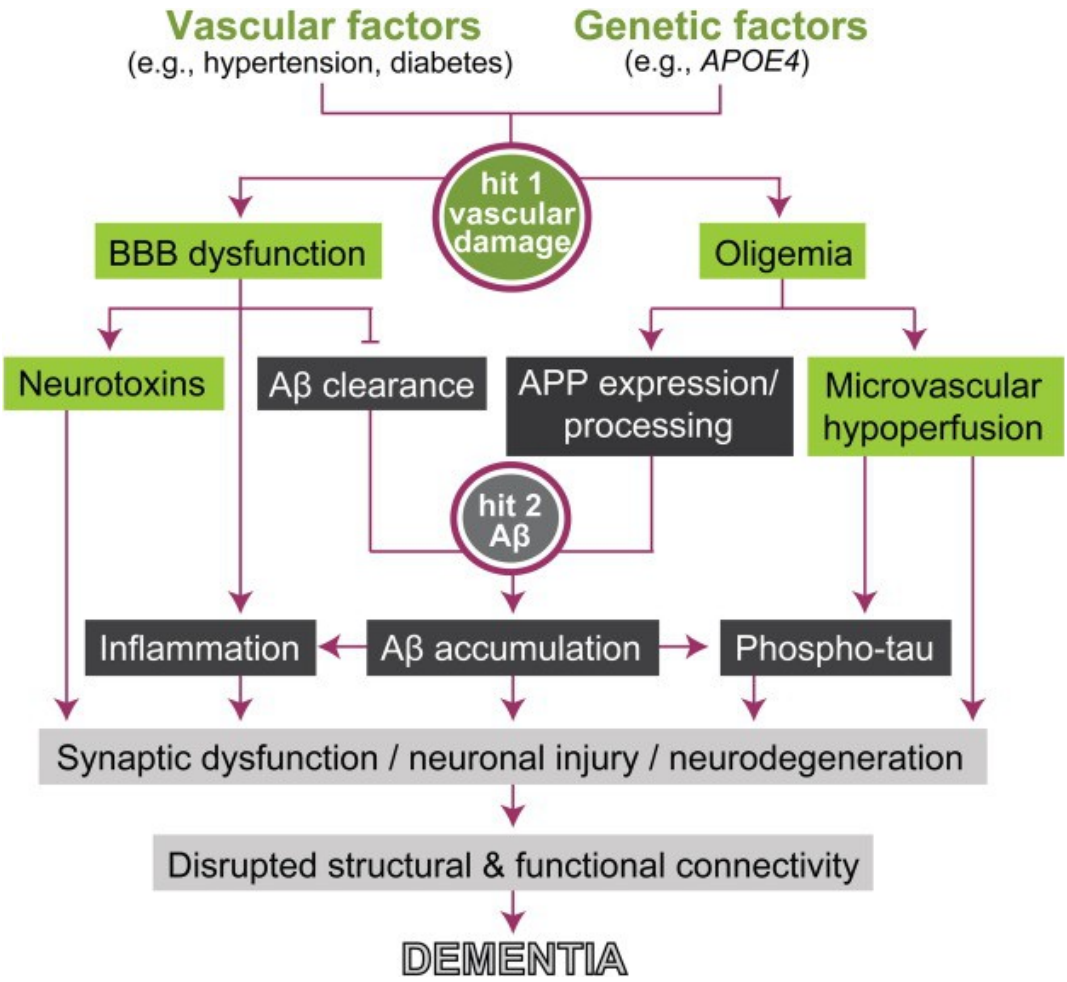

Figure 2 : ***Slokovich et al. (2005) Trends Neurosci. Apr;28(4):202-8.Neurovascular mechanisms of Alzheimer's neurodegeneration.***

Blood–brain barrier and blood–CSF barrier (inset) transport routes for Aβ. Aβ efflux across the BBB can predict brain amyloid burden in AD models and the development of plaques shifts the Aβ transport equilibrium. Double deletion of the genes encoding apoJ and apoE accelerates Aβ pathology in APP-overexpressing mice, raising a possibility that these apolipoproteins affect Aβ clearance and/or metabolism. Gp330/megalin (8), an apoJ receptor at the BBB and the choroid epithelium, could participate in clearance of Aβ–apoJ complexes from the brain across the BBB, and from CSF across the choroid epithelium of the blood–CSF barrier (inset) to maintain the sink action of CSF. P-glycoprotein at the luminal side of the BBB could reduce brain endothelial Aβ by promoting its efflux into blood. In addition to transport, interaction of Aβ with RAGE amplifies neurovascular stress and inflammation. LRP, an endocytotic and signaling receptor that has ligands including Aβ, apoE, α2M and APP, is linked genetically to AD and influences APP processing and Aβ clearance. APP-overexpressing mice overexpressing the LRP mini-receptor in neurons, however, accumulate soluble Aβ in brain. By contrast, LRP on brain capillary endothelium clears Aβ to blood with affinity inversely related to the content of β-sheets in Aβ, and lipoprotein receptors on astrocytes promote apoE-dependent degradation of Aβ deposits. Whether LRP on brain endothelium can also clear oligomeric Aβ, and whether chaperone proteins can assist clearance of aggregated Aβ across the BBB, is not known.

Apolipoprotein J (apoJ) has been shown to be the predominant amyloid beta-peptide

(Abeta)-binding protein in cerebrospinal fluid. The endocytic receptor low density lipoprotein receptor-related protein-2/megalin (LRP-2), which is expressed by choroid plexus epithelium and ependymal cells lining the brain ventricles and neural tube, binds and mediates cellular uptake of apoJ. Abeta alone did not bind directly to LRP-2; however, when Abeta1-40 was combined with apoJ to form a complex, binding to LRP-2 took place. The binding interaction could be blocked by inclusion of the receptor-associated protein, an antagonist of apoJ binding to LRP-2 [15]. A significant downregulation of megalin receptor function, is therefore likely to result in higher circulating levels of ApoJ and beta amyloid bound to ApoJ, because of the lack of binding and uptake to the megalin receptor.

Apolipoprotein J (apoJ), also known as clusterin and SP-40, binds soluble beta-amyloid and is up-regulated in the Alzheimer's disease (AD) brain [28 and more]. Abeta alone did not bind directly to LRP-2; however, when Abeta1-40 was combined with apoJ to form a complex, binding to LRP-2 took place [16]. Quantitative immunohistochemical analysis of five AD brains showed that 29% of Abeta deposited in the parenchyma was associated with apoJ. Of Abeta deposits with apoJ immunopositivity, 71% were associated with phospho-tau-positive dystrophic neurites in the surrounding tissue [28].

A possibility of a larger role of LRP-2 than what is yet known can not be excluded, due to its probable function in clearance of beta-amyloid complexes.

- (Ro)accutane and tau :

To complete...

6.3 Other factors that may contribute to the pathology of alzheimers, premature dementia and cognitive decline in association with (Ro)accutane exposure :

Formation and syntesis of ceramide is dependant of several key enzymes that by findings in mice are suggested to be altered by a (Ro)accutane exposure [10]. Ceramide is involved in the maintenance of cell regeneration and cell division [11]. Various hormonal deficits are found in Accutane exposed subjects, such as lessened thyroid function and conversion of dihydrotestosterone [12, 13]. Hormonal deficits may result in hypometabolism. Several alterations in glutamate transporters are found, meaning that cells are deprived of energy [xx].

Alzheimers disease is suggested to be characterized by hypometabolism, oxidative stress and adjustments of the glucose-fatty acid cycle. Cumulative evidence suggests that the brain in aging and AD actively adapts to the progressive fuel deprivation [14].

A possible pathology of dementia in Accutane-exposed subjects could therefore be related to the catabolic effects of a hypometabolism, suboptimal energy utilization, and suboptimal cell maintenance/ regeneration, suboptimal formation of ceramide, and accumulation of beta-amyloid.

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Dynamic expression of RGS4 in the developing nervous system and regulation by the neural type-specific transcription factor Phox2b. (2003) J Neurosci. Nov 19;23(33):10613-21.

J Cell Sci. 2004 Oct 1;117(Pt 21):5071-8. Epub 2004 Sep 21. Related Articles, Links

Efficient transfer of receptor-associated protein (RAP) across the blood-brain barrier.

Pan W, Kastin AJ, Zankel TC, van Kerkhof P, Terasaki T, Bu G.
Pennington Biomedical Research Center, Louisiana State University System, 6400 Perkins Road, Baton Rouge 70808, USA. weihong.pan@pbrc.edu

We have sought to identify a high-capacity transport system that mediates transcytosis of proteins from the blood to the brain. The 39 kDa receptor-associated protein (RAP) functions as a specialized endoplasmic reticulum chaperone assisting in the folding and trafficking of members of the low-density lipoprotein (LDL) receptor family. RAP efficiently binds to these receptors and antagonizes binding of other ligands. Previous studies have shown that two large members of the LDL receptor family, LDL receptor-related protein 1

(LRP1) and LDL receptor-related protein 2 (LRP2 or megalin), possess the ability to mediate transcytosis of ligands across the brain capillary endothelium. Here, we tested whether blood-borne RAP crosses the blood-brain barrier (BBB) by LRP1- or megalin-mediated transport by studying the pharmacokinetics of [125I]-RAP transport into the brain in intact mice and across cell monolayers in vitro. Our results show that [125I]-RAP is relatively stable in blood for 30 minutes and has a mean influx constant of 0.62+/-0.08 microl/g-minute from blood to brain. In situ brain perfusion in blood-free buffer shows that transport of [125I]-RAP across the BBB is a saturable process. Capillary depletion of brain homogenates indicates that 70% of [125I]-RAP is localized in the parenchyma rather than in the vasculature of the brain. Results of transport in stably transfected MDCK cells are consistent with the hypothesis that megalin mediates most of the apical-to-basolateral transport across polarized epithelial cells. The inhibition of [125I]-RAP influx by excess RAP and the involvement of megalin indicate the presence of a saturable transport system at the BBB. The higher permeability of RAP compared with that of melanotransferrin and transferrin show that the LRP receptor is a high capacity transport system. These studies suggest that RAP may provide a novel means of protein-based drug delivery to the brain.

PMID: 15383619 [PubMed - indexed for MEDLINE]

Hepatology. 2004 Jul;40(1):149-56. Related Articles, Links
Bile acid-induced negative feedback regulation of the human ileal bile acid transporter.
Neimark E, Chen F, Li X, Shneider BL.
Department of Pediatrics, Mount Sinai School of Medicine, New York, NY 10029, USA.

Ileal expression of the apical sodium-dependent bile acid transporter (ASBT) in the rat is unaffected by bile salts, yet in the mouse it is under negative-feedback regulation. The bile acid responsiveness of human ASBT is unknown. The human ASBT promoter linked to a luciferase reporter was studied in Caco-2 cells treated with chenodeoxycholic acid (CDCA) and transfected with expression plasmids for farnesoid X-receptor (FXR), short heterodimer partner (SHP), and retinoic acid receptor/retinoid X receptor (RAR/RXR). CDCA treatment of Caco-2 cells led to a 75% reduction in steady-state ASBT messenger RNA levels and a 78% reduction in human ASBT promoter activity. A dominant negative FXR abrogated the response to CDCA. Site-directed mutagenesis of an RAR/RXR cis element in the human ASBT promoter reduced its activity by 50% and eliminated the bile acid response. Retinoic acid activated the human ASBT promoter fourfold. SHP repressed the activity of the ASBT promoter and reduced activation by retinoic acid. Antisense mediated knock-down of SHP in Caco-2 cells partially offset the bile acid mediated repression of ASBT promoter activity. In conclusion, the human ASBT is positively regulated by retinoic acid. Bile acids induce a negative feedback regulation of human ASBT via an FXR-mediated, SHP-dependent effect upon RAR/RXR activation of ASBT.

PMID: 15239098 [PubMed - indexed for MEDLINE]

J Biol Chem. 2003 Mar 21;278(12):10028-32. Epub 2003 Jan 7. Related Articles, Links
Retinoid X receptor (RXR) agonist-induced antagonism of farnesoid X receptor (FXR) activity due to absence of coactivator recruitment and decreased DNA binding.
Kassam A, Miao B, Young PR, Mukherjee R.
Bristol-Myers Squibb Company, Experimental Station, Wilmington, Delaware 19880, USA.

The bile salt export pump (BSEP) plays an integral role in lipid homeostasis by regulating the canalicular excretion of bile acids. Induction of BSEP gene expression is mediated by

the farnesoid X receptor (FXR), which binds as a heterodimer with the retinoid X receptor (RXR) to the FXR response element (FXRE) located upstream of the BSEP gene. RXR ligands mimic several partner ligands and show additive effects upon coadministration. Using real-time quantitative PCR and cotransfection reporter assays, we demonstrate that the RXR agonist LG100268 antagonizes induction of BSEP expression mediated by endogenous and synthetic FXR ligands, CDCA and GW4064, respectively. Moreover, this antagonism is a general feature of RXR agonists and is attributed to a decrease in binding of FXR/RXR heterodimers to the BSEP-FXRE coupled with the inability of RXR agonists to recruit coactivators to FXR/RXR. Our data suggest that FXR/RXR is a conditionally permissive heterodimer and is the first example of RXR ligand-mediated antagonism of FXR activity. Because FXR agonists lower triglyceride levels, our results suggest a novel role for RXR-mediated antagonism of FXR activity in the development of hypertriglyceridemia observed with RXR agonists in rodents and humans.

PMID: 12519787 [PubMed - indexed for MEDLINE]

Cell Death Differ. 2005 May;12(5):512-22. Related Articles, Links
Ceramide triggers an NF-kappaB-dependent survival pathway through calpain.
Demarchi F, Bertoli C, Greer PA, Schneider C.
L.N.C.I.B. Laboratorio Nazionale Consorzio Interuniversitario Biotecnologie AREA Science Park, Padriciano 99, 34012 Trieste, Italy.

We have shown that C2 ceramide, a cell-permeable analog of this lipid second messenger, triggers an NF-kappaB dependent survival pathway that counteracts cell death. Activation of NF-kappaB and subsequent induction of prosurvival genes relies on calpain activity and is prevented on silencing of the calpain small subunit (Capn4) that is required for the function of ubiquitous calpains. We have demonstrated that p105 (NF-kappaB1) and its proteolytic product p50 can be targets of micro- and milli-calpain in vitro and that a p50 deletion mutant, lacking both the N- and the C-terminal ends, is resistant to calpain-mediated degradation. Capn4 silencing results in stabilization of endogenous p105 and p50 in diverse human cell lines. Furthermore, p105 processing and activation of NF-kappaB survival genes in response to C2 ceramide is impaired in Capn4-/- mouse embryonic fibroblasts defective in calpain activity. Altogether, these data argue for the existence of a ceramide-calpain-NF-kappaB axis with prosurvival functions.

PMID: 15933726 [PubMed - in process]

Acta Neurochir (Wien). 2005 Jun 9; [Epub ahead of print] Related Articles, Links
Spectrin breakdown products in the cerebrospinal fluid in severe head injury - preliminary observations.
O F, B PX, Szekeres-Barth X00f3 J, D X00f3 Czi T, Povlishock JT, B X00fc Ki A.
Department of Neurosurgery, Center for Medical and Health Sciences, Pecs University, Pecs, Hungary.

Background. Calcium-induced proteolytic processes are considered key players in the progressive pathobiology of traumatic brain injury (TBI). Activation of calpain and caspases after TBI leads to the cleavage of cytoskeletal proteins such as non-erythroid alpha II-spectrin. Recent reports demonstrate that the levels of spectrin and spectrin breakdown products (SBDPs) are elevated in vitro after mechanical injury, in the cerebrospinal fluid (CSF) and brain tissue following experimental TBI, and in human brain tissue after TBI.Methods. This study was initiated to detect spectrin and SBDP accumulation in the

ventricular CSF of 12 severe TBI-patients with raised intracranial pressure (ICP). Nine patients with non-traumatically elevated ICP and 5 undergoing diagnostic lumbar puncture (LP) served as controls. Intact spectrin and calpain and caspase specific SBDPs in CSF collected once a day over a several day period were assessed via Western blot analysis. Parameters of severity and outcome such as ICP, Glasgow Coma Scale and Glasgow Outcome Scale were also monitored in order to reveal a potential correlation between these CSF markers and clinical parameters.Results. In control patients undergone LP no immunoreactivity was detected. Non-erythroid alpha-II-spectrin and SBDP occurred more frequently and their level was significantly higher in the CSF of TBI patients than in other pathological conditions associated with raised ICP. Those TBI patients followed for several days post-injury revealed a consistent temporal pattern for protein accumulation with the highest level achieved on the 2(nd) -3(rd) days after TBI.Conclusion. Elevation of calpain and caspase specific SBDPs is a significant finding in TBI patients indicating that intact brain spectrin- and SBDP-levels are closely associated with the specific neurochemical processes evoked by TBI. The results strongly support the potential utility of these surrogate markers in the clinical monitoring of patients with severe TBI and provide further evidence of the role of calcium-induced, calpain- and caspase-mediated structural proteolysis in TBI.

PMID: 15924207 [PubMed - as supplied by publisher]

Biochem Biophys Res Commun. 2005 May 13;330(3):714-21. Related Articles, Links
Mu-calpain is functionally required for alpha-processing of Alzheimer's beta-amyloid precursor protein.
Chen M, Fernandez HL.
Neurobiology of Aging Research Laboratory, Bay Pines VA Medical Center, Bay Pines, FL 33744, USA. ming.chen@med.va.gov

Alzheimer's beta-amyloid precursor protein (APP) is normally processed by an unidentified alpha-secretase. A unique feature of this protease is its high sensitivity to phorbol esters, yet the mechanism involved is unclear. We have previously reported that phorbol 12,13-dibutyrate (PDBu) activates calpain, a Ca2+-dependent protease, and PDBu-induced release of APPs (secreted APP) is sensitive to calpain inhibitors, suggesting that calpain is involved in APP alpha-processing. In the present study, we found that PDBu markedly promoted the expression of both mu- and m-calpains in cultured fibroblasts. Dose-response and time course studies revealed that mu-calpain was more sensitive to PDBu than m-calpain and the temporal course of the mu-calpain change coincides better with that of APPs release. Moreover, the stimulatory effect of PDBu on mu-calpain was selectively blocked by mu-calpain-specific siRNA (small interference RNA) and the blockage was accompanied by a concomitant decrease in APPs release. In contrast, m-calpain siRNA did not affect APPs release significantly. Measurement of amyloid beta protein (Abeta) release in the mu-calpain siRNA-treated cells indicated that Abeta40 and Abeta42 levels inversely changed in relation to APPs, and the changes in Abeta42 were more prominent than in Abeta40. Together, these data suggest that calpain, particularly mu-calpain, is a potential candidate for alpha-secretase in the regulated APP alpha-processing, and that changes in this protease can affect the outcome of the overall APP processing.

PMID: 15809056 [PubMed - indexed for MEDLINE]

J Biol Chem. 2003 Oct 31;278(44):43245-53. Epub 2003 Aug 12. Related Articles, Links

In vivo calpain/caspase cross-talk during 3-nitropropionic acid-induced striatal degeneration: implication of a calpain-mediated cleavage of active caspase-3.
Bizat N, Hermel JM, Humbert S, Jacquard C, Creminon C, Escartin C, Saudou F, Krajewski S, Hantraye P, Brouillet E.

Unite de Recherche Associee Commissariat a l'Energie Atomique (CEA)-CNRS 2210, Service Hospitalier Frederic Joliot, Departement de Recherches Medicales (DRM), Direction des Sciences du Vivant (DSV), Commissariat a l'Energie Atomique, Orsay Cedex, France.

The role of caspases and calpains in neurodegeneration remains unclear. In this study, we focused on these proteases in a rat model of Huntington's disease using the mitochondrial toxin 3-nitropropionic acid (3NP). Results showed that 3NP-induced death of striatal neurons was preceded by cytochrome c redistribution, transient caspase-9 processing, and activation of calpain, whereas levels of the active/processed form of caspase-3 remained low and were even reduced as compared with control animals. We evidenced here that this decrease in active caspase-3 levels could be attributed to calpain activation. Several observations supported this conclusion. 1) Pharmacological blockade of calpain in 3NP-treated rats increased the levels of endogenous processed caspase-9 and caspase-3. 2) Cell-free extracts prepared from the striatum of 3NP-treated rats degraded in vitro the p34 and p20 subunits of active recombinant caspase-9 and caspase-3, respectively. 3) This degradation of p34 and p20 could be mimicked by purified mu-calpain and was prevented by calpain inhibitors. 4) mu-Calpain produced a loss of the DEVDase (Asp-Glu-Val-Asp) activity of active caspase-3. 5) Western blot analysis and experiments with 35S-radiolabeled caspase-3 showed that mu-calpain cleaved the p20 subunit of active caspase-3 near its catalytic site. 6) mu-Calpain activity was selectively inhibited (IC50 of 100 mum) by a 12 amino acid peptide corresponding to the C terminus of p20. Our results showed that calpain can down-regulate the caspase-9/caspase-3 cell death pathway during neurodegeneration due to chronic mitochondrial defects in vivo and that this effect may involve, at least in part, direct cleavage of the caspase-3 p20 subunit.

PMID: 12917435 [PubMed - indexed for MEDLINE]

Proteomics. 2004 Nov;4(11):3359-68. Related Articles, Links
Profiling proteins related to amyloid deposited brain of Tg2576 mice.
Shin SJ, Lee SE, Boo JH, Kim M, Yoon YD, Kim SI, Mook-Jung I.
Department of Biochemistry and Cancer Research Institute, College of Medicine, Seou; National University, Korea.

Alzheimer's disease (AD) is an age-related neurodegenerative disorder that is characterized by the extracellular deposition of beta-amyloid and intracellular hyperphosphorylation of tau in the cortex and hippocampus of the brain. These characterizations are caused by abnormal expression, modification and deposition of certain proteins. Post-translational modifications of proteins including oxidation and nitration might be involved in the pathogenesis of AD. In this study, AD-related proteins were identified in the cortex of Tg2576 mice used as a model for studying AD. Tg2576 mice express high levels of the Swedish mutated form of human beta-amyloid precursor protein (APP) and generated high levels of beta-amyloid in the brains. Using Western blotting and two-dimensional electrophoresis, proteins with differences in expression, oxidation and nitration in the cortex of Tg2576 mice brains were compared to littermate mice brains used as a control. The proteins with different expression levels were identified

using matrix-assisted laser desorption/ionization-time of flight and liquid chromatography-tandem mass spectrometry analyses. As a result, 12 proteins were identified among 37 different proteins using the PDQuest program. Furthermore, two proteins, laminin receptor and alpha-enolase, were more susceptible to oxidative modification in the brains of Tg2576 mice compared to those of littermates. Similarly, alpha-enolase, calpain 12, and Atp5b were more modified by nitration in brains of Tg2576 mice than those of littermates. Taken together, these proteins and their modifications may play an important role in the plaque deposition of Tg2576 mice brains.

PMID: 15378736 [PubMed - indexed for MEDLINE]

Eur J Neurosci. 2003 Dec;18(12):3305-10. Related Articles, Links
Erratum in:
Eur J Neurosci. 2004 Sep;20(5):1424.
Glutamate activates NF-kappaB through calpain in neurons.
Scholzke MN, Potrovita I, Subramaniam S, Prinz S, Schwaninger M.
Department of Neurology, University of Heidelberg, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany.

Glutamate induces gene transcription in numerous physiological and pathological conditions. Among the glutamate-responsive transcription factors, NF-kappaB has been mainly implicated in neuronal survival and death. Recent data also suggest a role of NF-kappaB in neural development and memory formation. In non-neuronal cells, degradation of the inhibitor IkappaBalpha represents a key step in NF-kappaB activation. However, little is known of how glutamate activates NF-kappaB in neurons. To investigate the signalling cascade involved we used primary murine cerebellar granule cells. Glutamate induced a rapid reduction of IkappaBalpha levels and nuclear translocation of the NF-kappaB subunit p65. The glutamate-induced reduction of IkappaBalpha levels was blocked by the N-methyl-d-aspartate inhibitor MK801. Specific inhibitors of the proteasome, caspase 3, and the phosphoinositide 3-kinase had no effect on glutamate-induced IkappaBalpha degradation. However, inhibition of the glutamate-activated Ca2+-dependent protease calpain by calpeptin completely blocked IkappaBalpha degradation and reduced the nuclear translocation of p65. Calpeptin also partially blocked glutamate-induced cell death. Our data indicate that the Ca2+-dependent protease calpain is involved in the NF-kappaB activation in neurons in response to N-methyl-d-aspartate receptor occupancy by glutamate. NF-kappaB activation by calpain may mediate the long-term effects of glutamate on neuron survival or memory formation.

PMID: 14686903 [PubMed - indexed for MEDLINE]

Ann Neurol. 2005 May;57(5):695-703. Related Articles, Links
Mitochondrial abnormalities in Alzheimer brain: mechanistic implications.
Bubber P, Haroutunian V, Fisch G, Blass JP, Gibson GE.
Weill Medical College of Cornell University at Burke Medical Research Institute, White Plains, NY 10605, USA.

Reductions in cerebral metabolism sufficient to impair cognition in normal individuals also occur in Alzheimer's disease (AD). The degree of clinical disability in AD correlates closely to the magnitude of the reduction in brain metabolism. Therefore, we tested whether impairments in tricarboxylic acid (TCA) cycle enzymes of mitochondria correlate with disability. Brains were from patients with autopsy-confirmed AD and clinical dementia

ratings (CDRs) before death. Significant ($p < 0.01$) decreases occurred in the activities of the pyruvate dehydrogenase complex (-41%), isocitrate dehydrogenase (-27%), and the alpha-ketoglutarate dehydrogenase complex (-57%). Activities of succinate dehydrogenase (complex II) (+44%) and malate dehydrogenase (+54%) were increased ($p < 0.01$). Activities of the other four TCA cycle enzymes were unchanged. All of the changes in TCA cycle activities correlated with the clinical state ($p < 0.01$), suggesting a coordinated mitochondrial alteration. The highest correlation was with pyruvate dehydrogenase complex ($r = 0.77$, $r2 = 0.59$). Measures to improve TCA cycle metabolism might benefit AD patients.

PMID: 15852400 [PubMed - indexed for MEDLINE]

Prog Neuropsychopharmacol Biol Psychiatry. 2005 Mar;29(3):407-10. Related Articles, Links
Mitochondrial aging and dysfunction in Alzheimer's disease.
Sullivan PG, Brown MR.
University of Kentucky, Spinal Cord and Brain Injury Research Center (SCoBIRC), Lexington, KY 40536-0305, USA. PatSull@uky.edu

Disruptions in energy metabolism have been suggested to be a prominent feature, perhaps even a fundamental component, of Alzheimer's disease (AD). These abnormalities in cerebral metabolism precede the onset of neurological dysfunction as well as gross neuropathology of AD. These changes may stem from inhibition of mitochondrial enzymes including pyruvate dehydrogenase, cytochrome c oxidase, and alpha-ketoglutarate dehydrogenase. Several lines of evidence also suggest a role for oxidative stress in the neuropathology associated with the disease state. Because mitochondria are the major site of free radical production in cells, they are also a primary target for oxidative damage and subsequent dysfunction. This link between mitochondrial dysfunction and the pathophysiology of AD is supported by several lines of evidence.

Publication Types:
Review
Review, Tutorial

PMID: 15795049 [PubMed - indexed for MEDLINE]

Anal Biochem. 1984 Jun;139(2):353-8. Related Articles, Links
Nonenzymatic decarboxylation of pyruvate.
Constantopoulos G, Barranger JA.
Triton X-100, retinol, retinoic acid, retinal, hexane, dithiothreitol, mercaptoethanol, and some other commercially available chemicals caused nonenzymatic decarboxylation of pyruvate and alpha-ketoglutarate. "Lipids" obtained from human or pigeon liver homogenates using isopropanol/hexane also had very high nonenzymatic decarboxylating activity on these two alpha-ketoacids; most of this activity could be traced to the hexane (Eastman) used in the extraction. Optimum pH of the reaction with dithiothreitol and mercaptoethanol was 7-8 and with the other chemicals around 10, but considerable activity was present at pH 7-8. Liver homogenates had a scavenger effect on the decarboxylating activity of Triton X-100 and of dithiothreitol. Dithiothreitol and mercaptoethanol at high concentrations (greater than 1 mM) also had a scavenger effect on the decarboxylating activity of the "lipids." Pretreatment of Triton X-100, dithiothreitol, retinol, and the "lipids" with catalase markedly decreased the decarboxylating activity,

while treatment with boiled catalase failed to do so. The results suggest that these compounds contain oxidizing contaminants, perhaps peroxide derivatives. Powerful oxidizing impurities have been reported in Triton X-100 from various sources by Y. Ashani and G. N. Catravas (1980, Anal. Biochem 109, 55-62). Such peroxide derivatives may cause nonenzymatic decarboxylation of pyruvate and alpha-ketoglutarate, presumably by a mechanism similar to the well-known nonenzymatic decarboxylation of alpha-ketoacids by hydrogen peroxide. In the absence of catalase and/or other protective agents against reactive oxygen derivatives, these chemicals would interfere in the assays of pyruvate dehydrogenase, pyruvate dehydrogenase complex, and alpha-ketoglutarate dehydrogenase complex which depend on the release of 14CO2 from alpha[1-14C]ketoacids.

PMID: 6476373 [PubMed - indexed for MEDLINE]

J Biol Chem. 2005 Jul 15; [Epub ahead of print] Related Articles, Links
Beta-amyloid-induced dynamin 1 depletion in hippocammpal neurons: A potential mechanism for early cognitive decline in Alzheimer's disease.
Kelly BL, Vassar R, Ferreira A.

Institute for Neuroscience, Northwestern University, Chicago, IL 60611.

Synaptic dysfunction is one of the earliest events in the pathogenesis of Alzheimer's disease. However, the molecular mechanisms underlying synaptic defects in AD are largely unknown. We report here that Abetathe main component of senile plaques, induced a significant decrease in dynamin 1, a protein that is essential for synaptic vesicle recycling, and hence, for memory formation and information processing. The Abetainduced dynamin 1 decrease occurred in the absence of overt synaptic loss and was also observed in the Tg2576 mouse model of Alzheimer's disease. In addition, our results provided evidence that the Abeta-induced decrease in dynamin 1 was likely the result of a calpain-mediated cleavage of dynamin 1 protein and possibly the down-regulation of dynamin 1 gene expression. These data suggest a mechanism to explain the early cognitive loss without a major decline in synapse number observed in Alzheimer's disease, and propose a novel therapeutic target for Alzheimer's disease intervention.

PMID: 16002400 [PubMed - as supplied by publisher]

Curr Alzheimer Res. 2004 Feb;1(1):33-8. Related Articles, Links
Recent evidence regarding a role for Cdk5 dysregulation in Alzheimer's disease.
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Based on a growing literature, cyclin-dependent kinase 5 (Cdk5) has been implicated in the pathological processes that contribute to neurodegeneration in Alzheimer's disease (AD). Cdk5 is ubiquitously expressed, but its activity is largely localized to post-mitotic neurons due to neuron-specific expression of its activators p35 and p39. Sufficient Cdk5 activity is critical to normal central nervous system development, as in its absence, neuronal migration and axonal path finding are deranged. Conversely, excessive and mislocalized Cdk5 activity appears to be detrimental to neuronal function. In fact, the pathological hallmarks of AD, beta-amyloid aggregates and neurofibrillary tangles, have been linked to Cdk5-mediated neuronal death. In this model, beta-amyloid is the toxic

stimulus that disrupts intracellular calcium homeostasis, leading to activation of calpains, a family of calcium-dependent proteases. Calpain-mediated cleavage of p35, yields a truncated p25 fragment that possesses a longer half-life, lacks the necessary sequence targeting it to membranes, but retains the capacity to activate Cdk5. The resulting excessive and mislocalized Cdk5 activity targets tau as a substrate for hyperphosphorylation, which is a prerequisite of paired helical filament (PHF) formation. A number of recent reports, utilizing diverse methods, lend further support to this model of AD neurodegeneration, and several strategies for combating Cdk5 dysregulation have even been devised. However, the study of Cdk5 in AD is not without controversy, and questions remain regarding its role in the pathology. Herein, the most recent findings regarding this model are reviewed.

PMID: 15975083 [PubMed - in process]

Br J Pharmacol. 2005 May 23; [Epub ahead of print] Related Articles, Links
Inhibition of the cdk5/MEF2 pathway is involved in the antiapoptotic properties of calpain inhibitors in cerebellar neurons.
Verdaguer E, Alvira D, Jimenez A, Rimbau V, Camins A, Pallas M.
1Rudolf-Boehm-Institut fur Pharmakologie und Toxikologie, Universitat Leipzig, Hartelstrasse, 16-18, 04107 Leipzig, Germany.

Experimental data implicate calpain activation in the pathways involved in neuronal apoptosis. Indeed, calpain inhibitors confer neuroprotection in response to various neurotoxic stimuli. However, the pathways involved in calpain activation-induced apoptosis are not well known.We demonstrate that apoptosis (40%) induced by serum/potassium (S/K) withdrawal on cerebellar granule cells (CGNs) is inhibited by selective calpain inhibitors PD150606 (up to 15%) and PD151746 (up to 29%), but not PD145305 in CGNs. zVAD-fmk, a broad spectrum inhibitor of caspases, attenuates apoptosis (up to 20%) mediated by S/K deprivation and protects against cell death, as measured by MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium]) assay.PD150606 and PD151746 prevented apoptosis mediated by S/K withdrawal through inhibition of calpain. Furthermore, PD151746 was able to inhibit caspase-3 activity.After S/K withdrawal, we observed an increase in cdk5/p25 formation and MEF2 phosphorylation that was prevented by 40 muM PD150606 and PD151746. This indicates that calpain inhibition may be an upstream molecular target that prevents neuronal apoptosis in vitro.Taken together, these data suggest an apoptotic route in S/K withdrawal in CGNs mediated by calpain activation, cdk5/p25 formation and MEF2 inhibition. Calpain inhibitors may attenuate S/K withdrawal-induced apoptosis and may provide a potential therapeutic target for drug treatment in a neurodegenerative process.British Journal of Pharmacology advance online publication, 23 May 2005; doi:10.1038/sj.bjp.0706280.

PMID: 15912127 [PubMed - as supplied by publisher]

Biochem J. 1999 Oct 15;343 Pt 2:467-72. Related Articles, Links
Changes in intracellular localization of calpastatin during calpain activation.
Tullio RD, Passalacqua M, Averna M, Salamino F, Melloni E, Pontremoli S.
Department of Experimental Medicine, Biochemistry Section, University of Genoa, Viale Benedetto XV 1, 16132 Genoa, Italy.

Localization of the two main components of the Ca(2+)-dependent proteolytic system has been investigated in human neuroblastoma LAN-5 cells. Using a monoclonal antibody

which recognizes the N-terminal calpastatin domain, it has been shown that this inhibitory protein is almost completely confined in two granule-like structures not surrounded by membranes. Similar calpastatin distribution has been found in other human and in murine cell types, indicating that aggregation of calpastatin is a general property and not an exclusive characteristic of neuronal-like cells. The existence of such calpastatin aggregates is confirmed by the kinetics of calpastatin-activity release during rat liver homogenization, which does not correspond to the rate of appearance of cytosolic proteins or to the disruption of membrane-surrounded organelles. Calpastatin distribution is affected by the intracellular increase in free Ca(2+), which results in calpastatin progressively becoming a soluble protein. However, calpain is distributed in the soluble cell fraction and, in activating conditions, partially accumulates on the plasma membrane. Similar behaviour has been observed in calpastatin localization in LAN-5 cells induced with retinoic acid, suggesting that the proteolytic system is activated during the differentiation process of these cells. The involvement of calpastatin in controlling calpain activity, rather than its activation process, and the utilization of changes in calpastatin localization as a marker of activation of the system is discussed.

PMID: 10510315 [PubMed - indexed for MEDLINE]

Curr Alzheimer Res. 2004 May;1(2):121-5. Related Articles, Links
The role of P-glycoprotein in cerebral amyloid angiopathy; implications for the early pathogenesis of Alzheimer's disease.
Vogelgesang S, Warzok RW, Cascorbi I, Kunert-Keil C, Schroeder E, Kroemer HK, Siegmund W, Walker LC, Pahnke J.
Department of Neuropathology, University of Greifswald, Germany.

It has been shown in vitro that beta-amyloid (Abeta) is transported by P-glycoprotein (P-gp). Previously, we demonstrated that Abeta immunoreactivity is significantly elevated in brain tissue of individuals with low expression of P-gp in vascular endothelial cells. These findings led us to hypothesize that P-gp might be involved in the clearance of Abeta in normal aging and particularly in Alzheimer's disease (AD). As we were interested in the early pathogenesis of Abeta deposition, we studied the correlation between cerebral amyloid angiopathy (CAA) and P-gp expression in brain tissue samples from 243 non-demented elderly cases (aged 50 to 91 years). We found that endothelial P-gp and vascular Abeta were never colocalized, i.e., vessels with high P-gp expression showed no Abeta deposition in their walls, and vice versa. Abeta deposition occurred first in arterioles where P-gp expression was primarily low, and disappeared completely with the accumulation of Abeta. At this early stage, P-gp was upregulated in capillaries, suggesting a compensatory mechanism to increase Abeta clearance from the brain. Capillaries were usually affected only at later stages of CAA, at which point P-gp was lost even in these vessels. We hypothesize that Abeta clearance may be altered in individuals with diminished P-gp expression due, e.g., to genetic or environmental effects (such as drug administration). The impairment of Abeta clearance could lead to the accumulation and earlier deposition of Abeta, both in the walls of blood vessels and in the brain parenchyma, thus elevating the risk of CAA and AD.

PMID: 15975076 [PubMed - indexed for MEDLINE]

Trends Endocrinol Metab. 2005 Mar;16(2):59-65. Related Articles, Links
The role of insulin receptor signaling in the brain.
Plum L, Schubert M, Bruning JC.

Institute for Genetics and Center for Molecular Medicine Cologne, Department of Mouse Genetics and Metabolism, University of Cologne, Weyertal 121, 50931 Cologne, Germany.

The insulin receptor (IR) is expressed in various regions of the developing and adult brain, and its functions have become the focus of recent research. Insulin enters the central nervous system (CNS) through the blood-brain barrier by receptor-mediated transport to regulate food intake, sympathetic activity and peripheral insulin action through the inhibition of hepatic gluconeogenesis and reproductive endocrinology. On a molecular level, some of the effects of insulin converge with those of the leptin signaling machinery at the point of activation of phosphatidylinositol 3-kinase (PI3K), resulting in the regulation of ATP-dependent potassium channels. Furthermore, insulin inhibits neuronal apoptosis via activation of protein kinase B in vitro, and it regulates phosphorylation of tau, metabolism of the amyloid precursor protein and clearance of beta-amyloid from the brain in vivo. These findings indicate that neuronal IR signaling has a direct role in the link between energy homeostasis, reproduction and the development of neurodegenerative diseases.

Neuroscience. 2001;105(3):651-61. Related Articles, Links

Regulation of apolipoprotein E secretion in rat primary hippocampal astrocyte cultures.
Cedazo-Minguez A, Hamker U, Meske V, Veh RW, Hellweg R, Jacobi C, Albert F, Cowburn RF, Ohm TG.
Karolinska Institutet, NEUROTEC, Section for Experimental Genetics, Huddinge, Sweden.

Apolipoprotein E isoforms may have differential effects on a number of pathological processes underlying Alzheimer's disease. Recent studies suggest that the amount, rather than the type, of apolipoprotein E may also be an important determinant for Alzheimer's disease. Therefore, understanding the regulated synthesis of apolipoprotein E is important for determining its role in Alzheimer's disease.We show here that in rat primary hippocampal astrocyte cultures, dibutyryl-cAMP increased apolipoprotein E secretion with time in a dose-dependent manner (to 177% at 48 h) and that retinoic acid potentiated this effect (to 298% at 48 h). Dibutyryl-cAMP also gave a rapid, albeit transient, increase of apolipoprotein E mRNA expression (to 200% at 1 h). In contrast, the protein kinase C activator phorbol 12-myristate 13-acetate decreased both apolipoprotein E secretion (to 59% at 48 h) and mRNA expression (to 22% at 1 h). Phorbol 12-myristate 13-acetate also reversed the effects of dibutyryl-cAMP. Apolipoprotein E secretion was also modulated by receptor agonists for the adenylyl cyclase/cAMP pathway. Isoproterenol (50 nM, a beta-adrenoceptor agonist) enhanced, while clonidine (250 nM, an alpha2-adrenoceptor agonist) decreased, secreted apolipoprotein E. We also analysed the effects of agonists for the phospholipase C/protein kinase C pathway. Arterenol (1 microM, an alpha1-adrenoceptor agonist) and serotonin (2.5 microM) enhanced, whereas carbachol (10 microM, an acetylcholine muscarinic receptor agonist) decreased secreted apolipoprotein E. The effects of these non-selective receptor agonists were modest, probably due to effects on different signalling pathways. Arterenol also potentiated the isoproterenol-mediated increase. We also show that phorbol 12-myristate 13-acetate and dibutyryl-cAMP have opposite effects on nerve growth factor, as compared to apolipoprotein E, secretion, suggesting that the results obtained were unlikely to be due to a general effect on protein synthesis.We conclude that astrocyte apolipoprotein E production can be regulated by factors that affect cAMP intracellular concentration or activate protein kinase C. Alterations in these signalling pathways in Alzheimer's disease brain may have consequences for apolipoprotein E secretion in this disorder.

PMID: 11516830 [PubMed - indexed for MEDLINE]

J Neurochem. 2005 Aug;94(3):828-38. Related Articles, Links
Age-associated changes in mRNA levels of Phox2, norepinephrine transporter and dopamine beta-hydroxylase in the locus coeruleus and adrenal glands of rats.
Zhu MY, Wang WP, Iyo AH, Ordway GA, Kim KS.
Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, Mississippi 39216, USA. mzhu@psychiatry.umsmed.ed

Age-related changes in the gene expression of the transcription factors, Phox2a and 2b, and two marker proteins, norepinephrine transporter (NET) and dopamine beta-hydroxylase (DBH), of noradrenergic neurons were characterized in the locus coeruleus (LC) and adrenal glands using in situ hybridization. Analysis of changes was performed in rats that were 1-23 months of age. Compared to 1-month-old rats, there was a 62% increase of Phox2a messenger RNA (mRNA) in the LC of 3-month-old rats, and a decline of 37% in 23-month-old rats. In contrast, levels of Phox2b mRNA in the LC remained unchanged in 3-month-old rats, but declined to a 30% reduction in 23-month-old rats. Interestingly, mRNA levels of NET in the LC decreased with increasing age to a reduction of 29%, 30% and 43% in 3-, 8- and 23-month-old rats, respectively. Similarly, DBH mRNA in the LC declined with increasing age to a 56% reduction in 23-month-old rats. mRNA levels of Phox2a, Phox2b, NET and DBH in the adrenal medulla of 23-month-old rats were significantly lower than those of 1-month-old rats. Semi-quantitative reverse transcription assays of the same genes yielded data similar to in situ hybridization experiments, with beta-actin mRNA levels being unchanged across the ages. Taken together, these data reveal that reduced Phox2 mRNAs in the LC and adrenal medulla of aging rats are accompanied by a coincidental decline in mRNA levels of NET and DBH and suggest a possible relationship between Phox2 genes and the marker genes in noradrenergic neurons after birth.

PMID: 16033425 [PubMed - indexed for MEDLINE]

6.4 Serotonin transport and age :

Arch Gen Psychiatry. 2005 May;62(5):537-44. Related Articles, Links
Influence of serotonin transporter promoter region polymorphisms on hippocampal volumes in late-life depression.
Taylor WD, Steffens DC, Payne ME, MacFall JR, Marchuk DA, Svenson IK, Krishnan KR.
Department of Psychiatry, Duke University Medical Center, Durham, NC 27710, USA. taylo006@mc.duke.edu

CONTEXT: Polymorphisms in the promoter region of the serotonin transporter gene (5-HTTLPR) influence transcription and may play a role in the pathogenesis and course of depression. Recent research demonstrates that specific polymorphisms may be associated with differences in hippocampal volumes in subjects with depression. OBJECTIVE: To examine associations between 5-HTTLPR genotype and hippocampal volumes in elderly control subjects and elderly subjects classified as having early or late onset of depression. DESIGN: Cohort study examining baseline characteristics. PARTICIPANTS: Subjects were community dwelling and 60 years or older. Using a definition of early-onset depression as depression first occurring at 50 years or younger, we examined 72 subjects with early-onset depression, 63 subjects with late-onset

depression, and 83 healthy control subjects. MAIN OUTCOME MEASURES: All subjects underwent genotyping for the 5-HTTLPR and underwent brain magnetic resonance imaging. Analyses of hippocampal volumes were controlled for total cerebral volume, age, and sex. RESULTS: The interaction between diagnosis and 5-HTTLPR genotype was statistically significant for the right hippocampus (P = .04). Subjects with late-onset depression who were homozygous for the long (L) allele (L /L genotype) had significantly smaller right hippocampal volumes than did L /L subjects with early-onset depression (P = .046) or L /L control subjects (P = .01). Post hoc analyses showed that later age of depression onset was associated with smaller hippocampal volumes in subjects with the L /L genotype, but earlier age of onset was associated with smaller hippocampal volumes in subjects who were homozygous for the short (S) allele (S/S genotype). CONCLUSIONS: Subjects with late-onset depression who were homozygous for the L allele exhibited smaller hippocampal volumes than other groups. Genotype also mediated the effect of age of onset on hippocampal volumes. Our findings differ from previous work; however, we examined an older and larger cohort of subjects than previous studies. Possible explanations for these findings include interactions between the serotonergic system and neurotrophic factors or cortisol response to stresses, each of which may affect hippocampal volumes.

PMID: 15867107 [PubMed - indexed for MEDLINE]

Section 7 : Gene-expression

Introduction :

As a general overview retinoic acid is suggested to insert its function through two major pathways that are not always possible to distinguish:

1) Through changes in the composition of the extracellular matrix, leading to alteration of membrane GPI-anchor proteins via integrin activation and cleavage, and downstream reactions such as modulation of g-protein coupled receptors, glutamate transporters, insulin receptors and IP(3) receptors. Leading to TGF-beta activation. Increased alkaline phosphatase and reduced cytochrome c complexes.

2) Through modulation of the RXR/RAR/PPAR/TR complex located in the nucleus, leading to among other things activation of the caspases, generation of ROS, alterations in the succinate dehydrogenase. Reduced mitochondrial membrane potential. The release of cytochrome c.

- **References :**

To complete...

7.1 TGF-beta/GPI-pathway :

Six weeks of isotretinoin treatment caused a statistically significant 19% increase in suction blister fluid TGF-beta1. [2] Glycosylphosphatidylinositol (GPI)-anchored proteins have been demonstrated to bind transforming growth factor-beta (TGF-beta) in certain cell lines. GPI-anchored protein(s) are suggested to inhibit TGF-beta signaling and implicate r150 as the GPI-anchored protein responsible for this inhibition in human keratinocytes [1]. TGF-beta1 was found to significantly down-regulate IP3R protein expression in mesanglial cells[5].

mRNA levels of the type 1 IP3 receptors were found to be decreased significantly in cerebellum and hypothalamus, but not in the brain stem of rats treated with retinoic acid, compared to untreated littermates. The mRNA levels of the type 2 IP3 receptor were significantly decreased in all tested tissues, cerebellum, hypothalamus, and also in brain stem after the treatment with retinoic acid. These results show that gene expression of both type 1 and 2 IP3 receptors is regulated by retinoic acid, although the effect of retinoic acid on mRNA levels of the type 1 IP3 receptors is dependent on brain area. [4]

IP(3) receptor channel clusters control Ca(2+) intracellular release from the endoplasmic reticulum (ER) to the cytosol in various cells and species. [3]

To complete...

- References :

[1] **Tam BY, Finnson KW, Philip A.** *Glycosyl-phosphatidylinositol-anchored proteins regulate transforming growth factor-beta signaling in human keratinocytes.* (2003) J Biol Chem. Dec 5;278(49):49610-7.

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7.2 Significant inhibition of NF-Y phosphorylation :

- Inhibition of transcription factor NF-Y through decreased phosphorylation due to CDK inhibition :

The transcriptional activator NF-Y is a heterotrimeric complex composed of NF-YA, NF-YB, and NF-YC, which specifically binds the CCAAT consensus present in about 30% of eukaryotic promoters. All three subunits contain evolutionarily conserved core regions, which comprise a histone fold motif (HFM) in the case of NF-YB and NF-YC. In vitro binding studies and nuclear import assays reveal two different transport mechanisms for NF-Y subunits. While NF-YA is imported by an importin beta-mediated pathway, the NF-YB/NF-YC heterodimer is translocated into the nucleus in an importin 13-dependent manner. Importin beta binding is restricted to the monomeric, uncomplexed NF-YA subunit.

In contrast, the nuclear import of NF-YB and NF-YC requires dimer formation. Only the NF-YB/NF-YC dimer, but not the monomeric components, are recognized by importin 13 and are imported into the nucleus. Importin 13 competes with NF-YA for binding to the NF-YB/NF-YC dimer. Our data suggest that a distinct binding platform derived from the HFM of both subunits, NF-YB/NF-YC, mediates those interactions [1]. C/EBP homologous protein (CHOP) is retinoid-responsive gene [2].

NF-Y transcription through phosphorylation is CDK dependant [3], in a similiar manner to transcription of Sp1 via phosphorylation. Sp1 and NF-Y are ubiquitously expressed. In retinoic acid regulation of several genes, the NF-Y binding sites are located near a Sp1 binding site [4].

- Conclusion :

Transcription factory NF-Y is significantly downregulated in high doses of retinoic acid. This due to lessened binding potential due to inhibition of CDK mediated phosphorylation of NF-Y.

The effects on C/EBK with high dose RA exposure are unknown. Possible effects on the importins are unknown.

- References :

[1] **Kahle J, Baake M, Doenecke D, Albig W.**
Subunits of the heterotrimeric transcription factor NF-Y are imported into the nucleus by distinct pathways involving importin beta and importin 13.
(2005) Mol Cell Biol. Jul;25(13):5339-54.
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[3] **Yun J, Chae HD, Choi TS, Kim EH, Bang YJ, Chung J, Choi KS, Mantovani R, Shin DY.** *Cdk2-dependent phosphorylation of the NF-Y transcription factor and its involvement in the p53-p21 signaling pathway.* (2003) J Biol Chem. Sep 19;278(38):36966-72.
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Interaction between the two ubiquitously expressed transcription factors NF-Y and Sp1.
(1999) Gene. Jun 24;234(1):61-9.

7.3 HIF-1 and ATF-2 with coactivator CBP :

To complete...

7.4 Transcriptor factor AP1 and AP2 :

- AP1 :

To complete...

- AP2 :

Retinoids activate AP2 transcription factors [1 and more]. The AP2 transcription factor family is a set of developmentally regulated, retinoic acid inducible genes composed of four related factors, AP2alpha, AP2beta, AP2gamma, and AP2delta. AP2 factors orchestrate a variety of cell processes including apoptosis, cell growth, and tissue differentiation. AP2alpha targets the p53 tumor suppressor protein. Studies with chromatin immunoprecipitation demonstrate that AP2alpha is brought to p53 binding sites in p53-regulated promoters. The interaction between AP2alpha and p53 augments p53-mediated transcriptional activation, which results in up-regulation of the cyclin-dependent kinase inhibitor p21(WAF1/CIP1) [2].

Alkaline phosphatase causes redistribution of AP-2 subunits to cytosol due to dephosphorylation and detachment from membranes

In rat brain cell membranes, AP-2 á subunits are redistributed to the cytosol by exposure to alkaline phosphatase due to dephosphorylation of these proteins. The phosphorylation status of á1 and á2, before and after the exposure was measured using specific antibodies against either phosphorylated serine or tyrosine residue. Proteins phosphorylated in serine overlapped with both á subtypes, and underwent partial dephosphorylation by exposure to alkaline phosphatase. The rate of dephosphorylation may be related more to á2 than á1 detachment from the membranes. When samples were incubated with inhibitors of alkaline phosphatase, both subtypes were overphosphorylated.

- Conclusion :

(Ro)accutane upregulates AP2, constituting one pathway to inhibition of cyclin dependent kinases and translocation of p53.

- References :

[1] **Stefanik P, Macejova D, Mravec B, Brtko J, Krizanova O.** *Distinct modulation of a gene expression of the type 1 and 2 IP(3) receptors by retinoic acid in brain areas.* (2005) Neurochem Int. Jun;46(7):559-64.
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Mol Cell Biol. 2002 Jul;22(13):4522-34. Related Articles, Links

Retinoic acid receptors inhibit AP1 activation by regulating extracellular signal-regulated kinase and CBP recruitment to an AP1-responsive promoter.

Benkoussa M, Brand C, Delmotte MH, Formstecher P, Lefebvre P.
INSERM U 459 and Ligue Nationale Contre le Cancer, Faculte de Medecine Henri Warembourg, 59045 Lille Cedex, France.

Retinoids exhibit antineoplastic activities that may be linked to retinoid receptor-mediated transrepression of activating protein 1 (AP1), a heterodimeric transcription factor composed of fos- and jun-related proteins. Here we show that transcriptional activation of an AP1-regulated gene through the mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) pathway (MAPK(ERK)) is characterized, in intact cells, by a switch from a fra2-junD dimer to a junD-fosB dimer loading on its promoter and by simultaneous recruitment of ERKs, CREB-binding protein (CBP), and RNA polymerase II. All-trans-retinoic acid (atRA) receptor (RAR) was tethered constitutively to the AP1 promoter. AP1 transrepression by retinoic acid was concomitant to glycogen synthase kinase 3 activation, negative regulation of junD hyperphosphorylation, and to decreased RNA polymerase II recruitment. Under these conditions, fra1 loading to the AP1 response element was strongly increased. Importantly, CBP and ERKs were excluded from the promoter in the presence of atRA. AP1 transrepression by retinoids was RAR and ligand dependent, but none of the functions required for RAR-mediated transactivation was necessary for AP1 transrepression. These results indicate that transrepressive effects of retinoids are mediated through a mechanism unrelated to transcriptional activation, involving the RAR-dependent control of transcription factors and cofactor assembly on AP1-regulated promoters.

PMID: 12052862 [PubMed - indexed for MEDLINE]

Biochem Biophys Res Commun. 2005 May 13;330(3):695-700. Related Articles, Links
Binding of AP-2 adaptor complex to brain membrane is regulated by phosphorylation of proteins.

Alberdi A, Sartor T, Sosa MA.
Instituto de Histologia y Embriologia, Facultad de Ciencias Medicas, Universidad Nacional de Cuyo, Mendoza (5500), Argentina. aalberdi@fcm.uncu.edu.ar

Phosphorylation of proteins appears as a key process in early steps of clathrin coated vesicle formation. Here, we report that treatment of post-nuclear fraction with alkaline phosphatase induced redistribution of alpha subunits of AP-2 adaptor complex to cytosol and this effect was higher in the alpha2 subunit. A high serine phosphorylation status of alpha subunits correlated with the higher affinity of AP-2 to membranes. Using a simple binding assay, where membranes were incubated with either purified adaptors or cytosols, we observed an inhibitory effect of tyrphostin, a tyrosine kinase inhibitor, on the binding of AP-2 to membranes, but also an unexpected decrease induced by the phosphatase inhibitor cyclosporine. We also show an inhibitory effect of ATP mediated by cytosolic proteins, although it could not be related to the phosphorylation of AP-2, suggesting an action upstream a cascade of phosphorylations that participate in the regulation of the assembly of AP-2 to membranes.

PMID: 15809053 [PubMed - indexed for MEDLINE]

Science. 1998 Aug 7;281(5378):821-4. Related Articles, Links
Role of phosphorylation in regulation of the assembly of endocytic coat complexes.
Slepnev VI, Ochoa GC, Butler MH, Grabs D, De Camilli P.
Howard Hughes Medical Institute and Department of Cell Biology, Yale University School of Medicine, 295 Congress Avenue, New Haven, CT 06510, USA.

Clathrin-mediated endocytosis involves cycles of assembly and disassembly of clathrin coat components and their accessory proteins. Dephosphorylation of rat brain extract was shown to promote the assembly of dynamin 1, synaptojanin 1, and amphiphysin into complexes that also included clathrin and AP-2. Phosphorylation of dynamin 1 and synaptojanin 1 inhibited their binding to amphiphysin, whereas phosphorylation of amphiphysin inhibited its binding to AP-2 and clathrin. Thus, phosphorylation regulates the association and dissociation cycle of the clathrin-based endocytic machinery, and calcium-dependent dephosphorylation of endocytic proteins could prepare nerve terminals for a burst of endocytosis.

PMID: 9694653 [PubMed - indexed for MEDLINE]

7.5 Significant inhibition of Sp1 phosphorylation :

- Gene-expression through Sp1 :

Modulation of Sp1 activity by nuclear receptors is a novel mechanism by which fat-soluble hormones regulate gene expression. RARs/RXRs physically interact with Sp1, potentiate Sp1 binding to the GC box motifs, and thus enhance transactivation of the urokinase promoter, which lacks a canonical RAR-responsive element/RXR-responsive element [1]. Nuclear receptors [retinoic acid receptor (RAR), thyroid hormone receptor (TR), vitamin D(3) receptor, peroxisome-proliferator-activated receptor and retinoic X receptor] induce an electrophoretic mobility increase of Sp1-GC-rich DNA complexes. Concomitantly, binding of Sp1 to the GC-box is enhanced [2]. In Ehrlich tumor cells, Sp1-DNA binding was inhibited by phosphatase exposure, demonstrating that *phosphorylation of Sp1 is critical for its DNA binding capacity*. Immunoprecipitation experiments revealed that Sp1 is mostly phosphorylated on serine residues [3, 7] with less than 5% on threonine and none on tyrosine residues [7].

- (Ro)accutane induced significantly decreases of phosphorylation of Sp1 through multiple pathways, leading to a decreased transcription :

A list of kinases and phosphatases are known to be involved in Sp1 phosphorylation: DNA-PK, CDK2, ERK, PKC, PI3 kinase, PP1, PP2a and more, depending on cell line and function [7]. Experimental observations have found (Ro)accutane induced significant inhibition of at least three of these, however, it is here suggested that (Ro)accutane can inhibit ERK and possibly even PP1, PP2a and DNA-PK as well:

1) Inhibition of CDK2 :

(Ro)accutane is found to inhibit multiple of these kinaseses and phosphatases: In LCLs, RA-induced LCL accumulation in the G0/G1 phases correlated with the loss of the catalytic activity of all three G1-associated CDKs (CDK2, CDK4 and CDK6) and with increased levels of underphosphorylated pRb and, in some LCLs, p130. LCLs arrested in G0/G1 by RA also showed a significant decrease in the protein levels of cyclins D2, D3 and A, together with a reduction in the amount of cyclin D associated with CDK4 and CDK6, probably accounting for the inhibition of the relative kinase activity [5]. Cyclin-dependent kinases (CDKs) is essential for cell cycle transversal. CDKs mediate phosphorylation of Sp1 [6].

2) Inhibition of PKC :

Protein kinase C from 10T1/2 cells can be eluted by linear gradient of NaCl in two fractions. Following treatment with 10(-5) M 13-cis-retinoic acid a decrease of total PKC activity was observed [8].

3) Inhibition of PI3K/Akt pathway :

In MTSV1-7 breast epithelial cells, CRBP-I inhibits, in a retinoic acid receptor-dependent manner, the PI3K/Akt survival pathway. Inhibition of PI3K/Akt was necessary and sufficient to explain the antitumor effects of CRBP-I and was mediated by decreased p85 regulatory and p110 catalytic subunit heterodimerization. We present evidence consistent with the idea that this effect is due to CRBP-I inhibition of p85 phosphorylation at Y688 [9].

Phosphatidylinositol 3-kinase (PI3K) is a critical signaling node that is regulated in response to the activation of growth factor receptors, G protein-coupled receptors, integrins and other cell signals that impact on cell survival and apoptosis. Class Ia PI3Ks consist of a regulatory (most commonly p85) and a catalytic (p110) subunit and catalyze the phosphorylation of phosphatidylinositol at the 3-hydroxy position of the inositide ring. The formation of phosphatidylinositol 3,4,5-trisphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate is of particular relevance because PIP3 recruits downstream effectors to the plasma membrane. One such effector is Akt, which is activated by phosphoinositide-dependent kinase 1 which is likewise recruited to PIP3 sites. Akt is a serine−threonine protein kinase that once activated acts to inhibit apoptosis by phosphorylation of several key substrates. CRBP-I markedly inhibits Akt activity in cells in collagen I or in suspension relative to adherent monolayers, suggesting that Akt inhibition is a critical path through which CRBP-I promotes anoikis [9].

4) Inhibition of ERK :

In HSC-1 cells, suppression of ERK1/2 and Akt activation is presumed to be involved in the RA-induced suppression of hTERT [10].

- PPARgamma ligands :

In NSCLS cells, PPARgamma ligands also diminished the phosphorylation of cyclic adenosine monophosphate response element binding protein (CREB), diminished Sp1 nuclear protein expression, and prevented the binding of these transcription factors to CRE and Sp1 sites, respectively, within the Fn promoter. In summary, our results

demonstrate that PPARgamma ligands inhibit Fn gene expression in NSCLC cells through PPARgamma-dependent and -independent pathways that affect both CREB and Sp1 [4].

- Conclusion :

In small doses retinoic acid facilitate Sp1 binding to GC boxes (promoter regions). In toxic, supraphysiological doses retinoic acid is suggested to inhibit binding of Sp1 through decreased phosphorylation of Sp1 with at least two signal pathways involved, probably more. The inhibition of Sp1 phosphorylation is here suggested to span over all cell lines and regions where Sp1 promoter activity is essential.

- References :

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Cellular retinol-binding protein-I inhibits PI3K/Akt signaling through a retinoic acid receptor-dependent mechanism that regulates p85-p110 heterodimerization.
(2005) Oncogene. Feb 24;24(9):1598-606.
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Blood. 2005 Jul 1;106(1):304-10. Epub 2005 Mar 10. Related Articles, Links

Arsenic suppresses gene expression in promyelocytic leukemia cells partly through Sp1 oxidation.

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The mechanism by which arsenic dramatically affects gene expression remains poorly understood. Here we report that prolonged exposure of acute promyelocytic leukemia NB4 cells to low levels of arsenic trioxide increased the expression of a set of genes responsible for reactive oxygen species (ROS) production. We hypothesize that arsenic-induced ROS in turn contribute partially to altered gene expression. To identify genes responsive to arsenic-induced ROS, we used microarray gene expression analysis and identified genes that responded to arsenic and hydrogen peroxide but whose response to arsenic was reversed by an ROS scavenger, N-acetyl-L-cysteine. We found that 26% of the genes significantly responsive to arsenic might have been directly altered by ROS. We further explored the mechanisms by which ROS affects gene regulation and found that the Sp1 transcription factor was oxidized by arsenic treatment, with a corresponding decrease in its in situ binding on the promoters of 3 genes, hTERT, C17, and c-Myc, whose expressions were significantly suppressed. We conclude that ROS contributed partly to arsenic-mediated gene regulation and that Sp1 oxidation contributed to gene suppression by arsenic-induced ROS.

PMID: 15761015 [PubMed - indexed for MEDLINE]

Brain Res Mol Brain Res. 2003 Apr 10;112(1-2):153-62. Related Articles, Links
Role of an AP-2-like element in transcriptional regulation of mouse micro-opioid receptor gene.
Ko JL, Liu HC, Loh HH.
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Previously, several important cis-elements and trans-factors have been shown to play a functional role in the proximal promoter of mouse micro-opioid receptor (MOR) gene. In this study, we defined another functional element located the in -450 to -400 bp (translational start site designated as +1) region of the proximal promoter, which is also essential for the full promoter activity. It is designated as the morAP-2-like element for its sequence homologous to the consensus AP-2 element. Surprisingly, electrophoretic mobility shift analysis (EMSA) revealed that Sp1 and Sp3, but not AP-2 proteins, were specifically bound to the morAP-2-like element. Mutation of the morAP-2-like element, resulting in a loss of Sp binding, led to an approximately 35% decrease in activity, further confirming the positive role of the morAP-2-like element in MOR gene expression. Dephosphorylation of Sp proteins with alkaline phosphatase also decreased Sp binding to the morAP-2-like element in EMSA, suggesting phosphorylation of Sp is essential for its binding to this element. However, direct or indirect activation of PKA, a classical G-protein coupled signaling pathway, resulted in no significant change of Sp binding to the morAP-2-like element, nor of the promoter activity the SH-SY5Y cells, MOR expressing cells, suggesting that phosphorylation of Sp does not involve PKA. These results suggest that the binding of different phosphorylated forms of Sp proteins to the morAP-2-like element may contribute to the fine tuning of MOR expression in different cells.

PMID: 12670713 [PubMed - indexed for MEDLINE]

Section 8 : (Ro)accutane and its effects explained - for the beginner

8.1 Hormones and gene-expression :

- Introduction for the beginner :

Please see the other sections for references and more detailed information!

- What is gene-expression?

(Ro)accutane influences gene-expression. Cells contain DNA that encode what characteristics a cell shall have, but the cells genes are also controlled by influence from among other things mRNA, which combined with the DNA instructs the cell. This is why steroids can change the characteristics of several body functions, because they influence mRNA, and thus gene-expression. (Ro)accutane, retinoic acid, is a form of vitamin A, and is classified as a steroid.

- Hormones and their rôle :

Unlike many other steroids that tend to increase hormonal levels, the high doses of vitamin A that (Ro)accutane contains is found to *lower* many different hormonal levels permanently. The doses of vitamin A in its chemical derivative form that (Ro)accutane contains and that are given to acne-subjects are up to 100 times higher than the daily recommended intake. The effect of lower hormonal levels can be said to be a slower metabolism, or lower energy utilization, cell division and conversion rates in the body. This is likely the reason as to why (Ro)accutane, except for currently in acne-subjects, is used for many cancers such as prostate cancer, thyroid cancer, glioblastoma (a form of brain cancer) and more.

- Which hormones are affected?

The hormonal effects from (Ro)accutane are very wide. Measurments of acne-subjects that have received (Ro)accutane for several months have shown clearly lower levels of thyroid hormone, vitamin D (which also is a hormone), dihydrotestosterone (which is the most active form of testosterone). A relation between vitamin A levels and other hormones is also found, and these include growth hormone, insulin (which could be classified as a hormone), parathyroid hormone and other hormones. There is evidence indicating that acne-subjects that have taken (Ro)accutane are developing a serious vitamin A deficiency.

- What does the actual hormonal activity depend on?

The hormonal activity in the human body and brain not only depend on the actual measured levels of hormones. It also depends on the capacity of transporting hormones, the capacity of metabolizing hormones to their most active forms, the capacity of the kidney to recycle the hormones, liver function, the capacity of the cells to take up the hormone, receptor expression and the sensitivity of these receptors. (Ro)accutane is found to influence all of these different factors. The effect is thus not only altered levels of circulating hormones, but also consists of other more complicated effects. Not all of the effects that (Ro)accutane has are known.

- Receptor expression and sensitivity :

(Ro)accutane is found to affect many different kinds of receptors distributed in nearly every single organ in the human body. The receptors are found in various parts of the cell. Some in the nucleus of the cell, like many of the hormonal receptors, and some receptors have other locations. The receptor couples to the hormone, and through a complicated process initiates gene-transcription.

In experimental cell lines, (Ro)accutane is found to suppress several of these receptors. This means that when the hormone reaches the cell, there are less receptors that are to respond to the hormone. This in turn may lead to a partial resistance of the hormone. Many factors are also indicating that (Ro)accutane through complex chemical interactions may change the affinity of the hormonal receptors, which means that the hormones bind less efficiently to them.

- Why are homones so important?

Hormones control the renewal of cells. They control energy expenditure in the cell. They control conversion rates of fat. They are largely involved in the immune-system. They play a large role in the human brain. Hormones are also found to be involved in acne. Deficiencys of different hormones are likely to cause unwanted effects, also called side-effects, because their large role in many areas and many functions all over the body. Also, the levels of hormones are found to decline with age, so that a hormonal deficiency at an early age is likely to get worse over time.

- Hormones and the brain :

In the brain receptors are found for several hormones that are affected by (Ro)accutane. These steroids in the brain are called neurosteroids. Vitamin A is found to be of major importance in the human brain, regulating among other things cell survival and renewal of cells. Neurosteroids are suggested to influence mood, sexual interest, energy levels, ability to concentrate, memory and many more functions, of which all not are known.

8.2 Gene Databases - information about genes :

- Introduction for the beginner: Gene Databases

When studying the (side)-effects of (Ro)accutane, there are many similarities between at a first glance very different effects. One of the causes to this is the gene-expression. One gene that is expressed in the skin can also be widely expressed in cells in the brain, liver, kidneys, heart and many more cells. This is the case with the retinoid X receptor, RXR - that is shown to be heavily affected by the retinoid 13-cis-retinoic acid (Roaccutane). This means that when the substance is used in a very high dose, all cells that carry these receptors are going to be affected. As an example, let us take a look at this retinoid receptor by looking in a Gene Database.

Genecards - RXRA

RXR exists in three forms. We are going to take a look at RXRalpha (RXRA).

- Where is the gene expressed?

The genecard for RXRA contains a box where the expression of the receptor is listed in different parts of the body. (Says "tissue" "clones per gene" "total clones"). A high expression means that there are many copies of the receptor.

- Related genes :

This is a very useful section, because if a gene is related in structure (has a sequence that reminds about the gene that is searched for), in this case RXRA, we find that these genes also are very likely to be affected. In the case of RXRA, since we know it is affected, we can find out that other receptors within these families with high certainity also are affected. In this case it turns out that RXRA has many related genes, and also is found to affect many genes secondarily:

InterPro Domains and Families:

IPR008946 Str_ncl_receptor
IPR000003 RtnoidX_receptor
IPR001628 Znf_C4steroid
IPR001723 Stdhrmn_receptor
IPR000536 Hrmon_recept_lig

- Sequences :

Generally the sequences (of "small amino-acids" - nucleic acids, of which the gene consists) do not tell a whole lot, if not analysed with powerful computerized tools. However, some short sequences may reveal a bit about binding of residues and transcription.

- Size :

Different genes have different sizes.

- Other information :

The gene-card contains much more good information, such as references to studies done on the gene, similarities in different species and more. Genecards are powerful tools in biochemical research, and many databases are available to the public.

Annexes :

Annexe 1 : links

To complete...

Annexe 2 : Summarizing studies of retinoids with links

http://www.ncbi.nlm.nih.gov/entrez/query.fcgicmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15882777&query_hl=2

[1] **Lane MA, Bailey SJ.** *Role of retinoid signalling in the adult brain.* (2005) Prog Neurobiol. Mar;75(4):275-93. **[Abstract Pubmed]**

Comments: Contains tables of areas in the brain where retinoid signaling is present, and tables of metabolic enzymes affected. Summarizes metabolic pathways of retinoids. Discusses affected neurotransmitters.

[2] **Mey J, McCaffery P.** *Retinoic acid signaling in the nervous system of adult vertebrates.* (2004) Neuroscientist. Oct;10(5):409-21. **[Abstract Pubmed]**

Comments: Summary of retinoid signaling in the brain. Retinoid receptor mediated transcription.

Annexe 3 :Research links

Publicly available gene databases (click for direct link):

Entrez gene database

Genecards database

Omim database

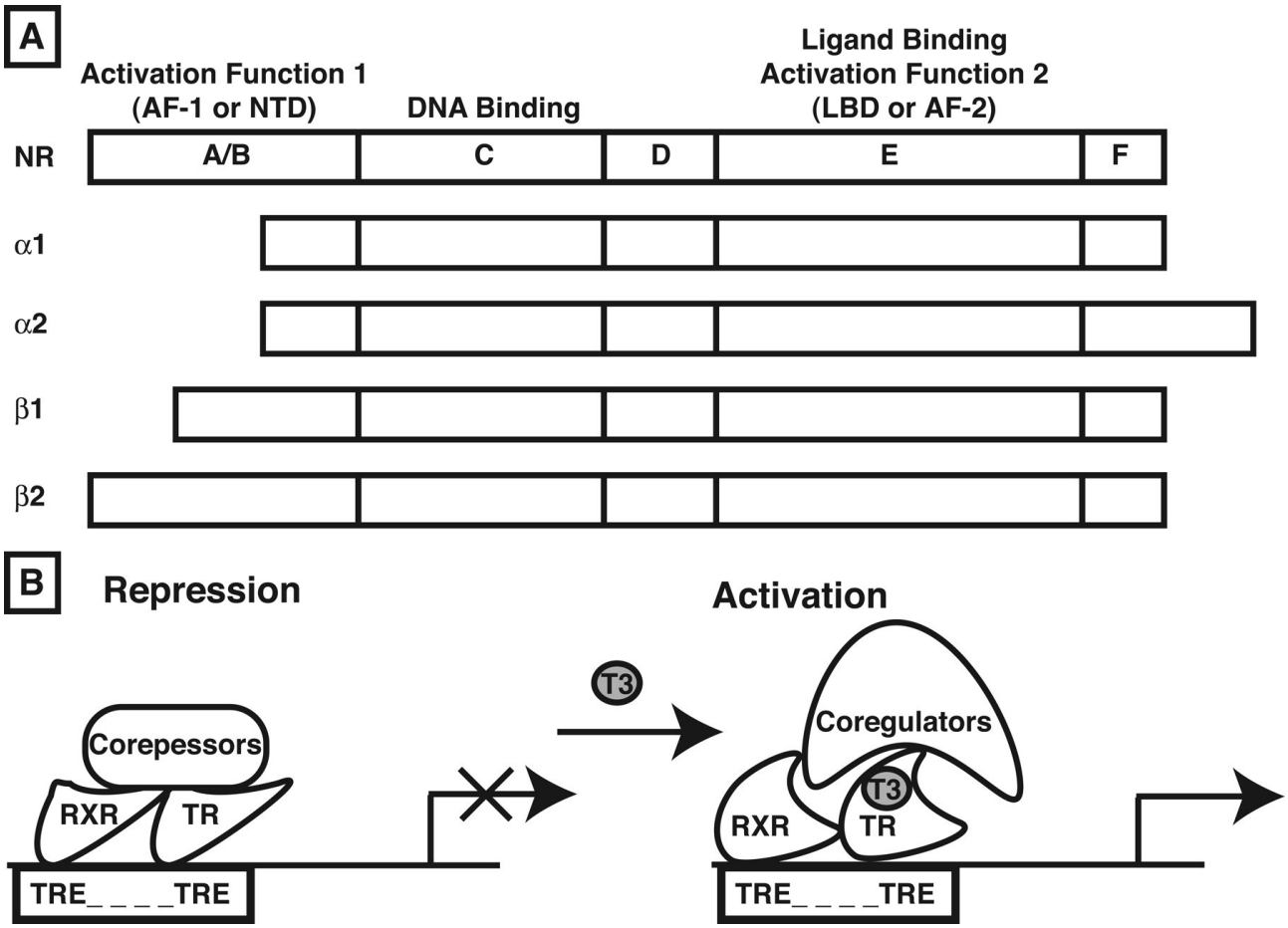
Unigene database

UniprotKB/Swissprot database

Université René Descartes database, France

Annexe 4 : Pictures and graphs

- Section 1 :



www3.interscience.wiley.com/cgi-bin/fulltext/110539009/nfig001

https://thesis.library.caltech.edu/2250/8/Chapter2.pdf

- Section 2.1 : Roaccutane and brain degeneration

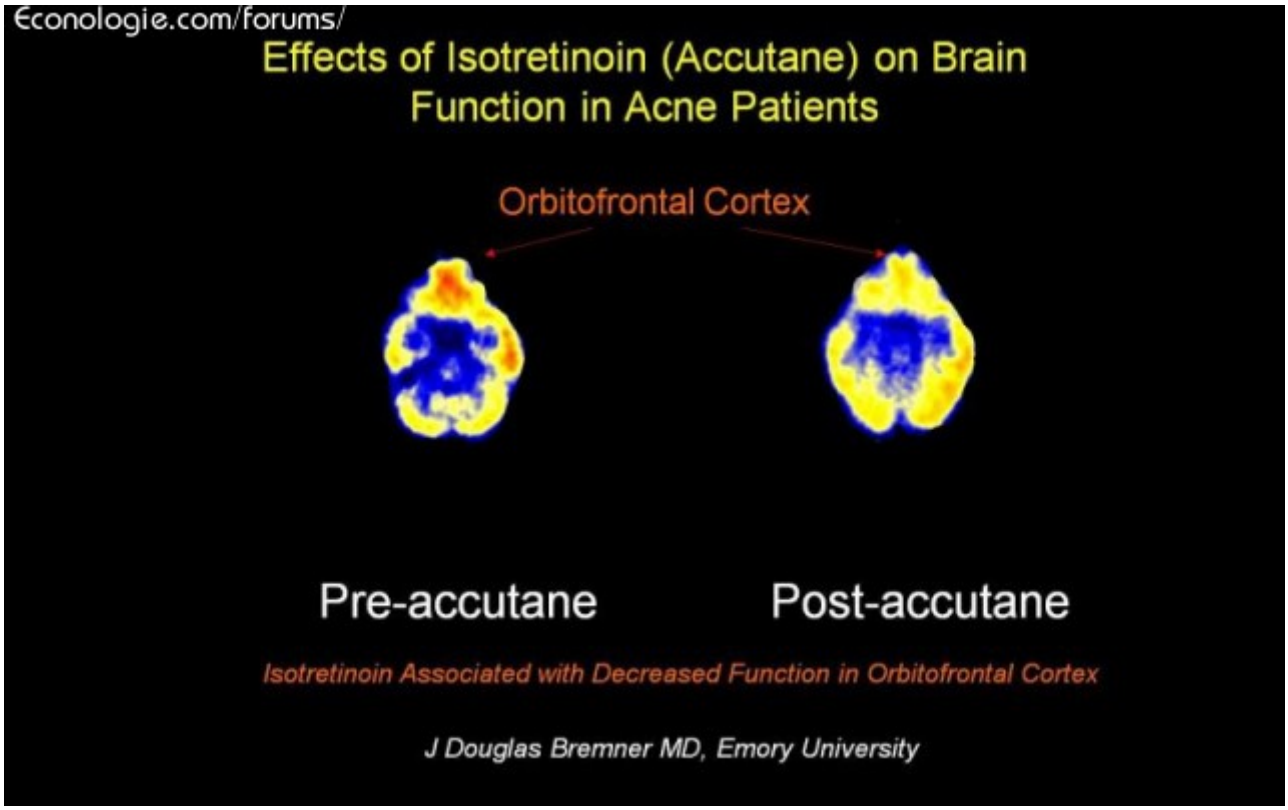


Figure 1. Bremner JD et al. *Functional brain imaging alterations in acne patients treated with isotretinoin.* (2005) Am J Psychiatry. May;162(5):983-91.

- Section 5b : Other long term effects

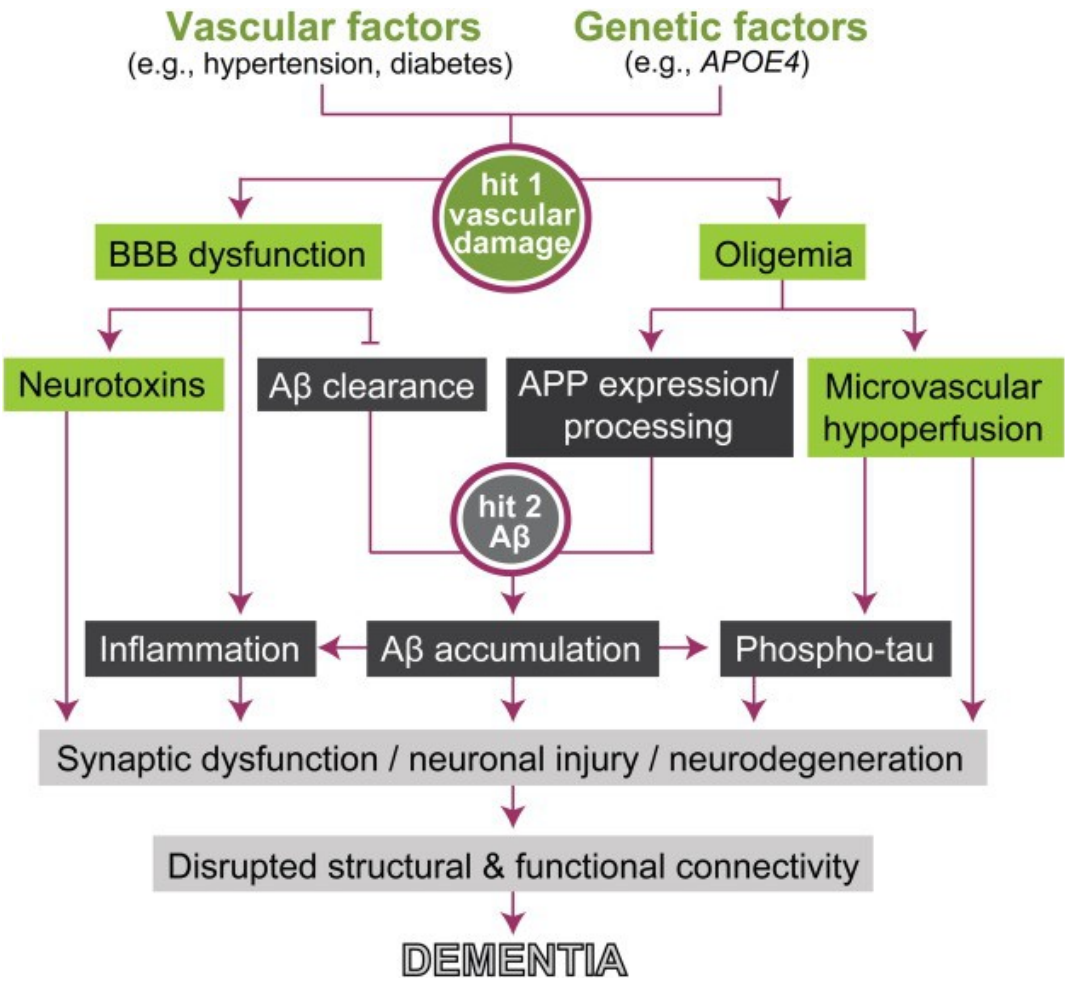


Figure 2 : ***Slokovich et al. (2005) Trends Neurosci. Apr;28(4):202-8.Neurovascular mechanisms of Alzheimer's neurodegeneration.***
Blood–brain barrier and blood–CSF barrier (inset) transport routes for Aβ. Aβ efflux across the BBB can predict brain amyloid burden in AD models and the development of plaques shifts the Aβ transport equilibrium. Double deletion of the genes encoding apoJ and apoE accelerates Aβ pathology in APP-overexpressing mice, raising a possibility that these apolipoproteins affect Aβ clearance and/or metabolism. Gp330/megalin (8), an apoJ receptor at the BBB and the choroid epithelium, could participate in clearance of Aβ–apoJ complexes from the brain across the BBB, and from CSF across the choroid epithelium of the blood–CSF barrier (inset) to maintain the sink action of CSF. P-glycoprotein at the luminal side of the BBB could reduce brain endothelial Aβ by promoting its efflux into blood. In addition to transport, interaction of Aβ with RAGE amplifies neurovascular stress and inflammation. LRP, an endocytotic and signaling receptor that has ligands including Aβ, apoE, α2M and APP, is linked genetically to AD and influences APP processing and Aβ

clearance. APP-overexpressing mice overexpressing the LRP mini-receptor in neurons, however, accumulate soluble Aβ in brain. By contrast, LRP on brain capillary endothelium clears Aβ to blood with affinity inversely related to the content of β-sheets in Aβ, and lipoprotein receptors on astrocytes promote apoE-dependent degradation of Aβ deposits. Whether LRP on brain endothelium can also clear oligomeric Aβ, and whether chaperone proteins can assist clearance of aggregated Aβ across the BBB, is not known.

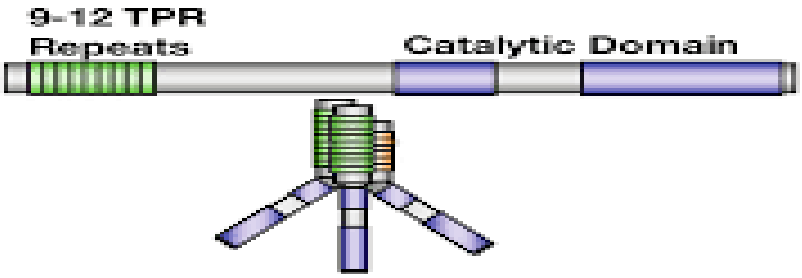


Figure 2-2. O-GlcNAc Transferase Domains and Functionality. OGT exists as both 110 and 78 kDa isoforms that interact to form a hetero-trimer of two 110 kDa and one 78 kDa subunits *in vivo*. The *N*-terminal tetratricortico peptide repeats of OGT are organized into a suprahelical structure responsible for protein:protein interactions that help specify the activity of OGT. The C-terminal transferase domain is characterized by a relatively low K_m (500 nM) for UDP-GlcNAc, which allows it to compete with endoplasmic reticulum-based transferases that use UDP-GlcNAc to synthesize glycosaminoglycans.

Figure 3 : ***Slawson et al. (2005) O-GlcNAc cycling: How a single sugar post-translational modification is changing the way we think about signaling networks***

To complete

Figure 4 ***: Lissamine green corneal staining of a dry eye patient taking isotretinoin. Photo courtesy of Eric Donnenfeld, M.D.***

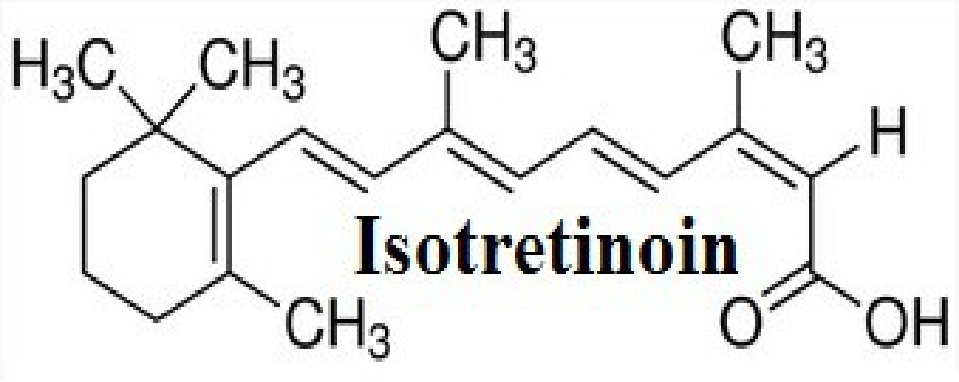


Figure 5 : isotretinoin molecular schema

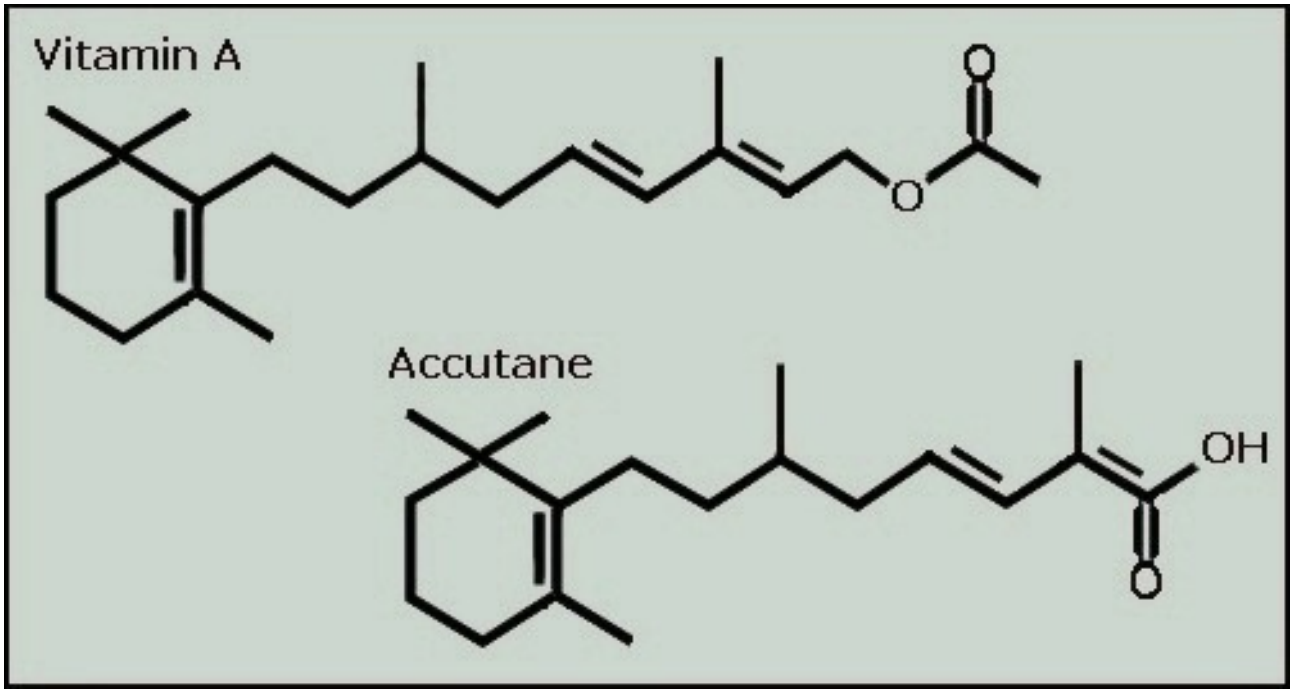


Figure 6 : Vitamine A, and Accutane, molecular schema

Annexe 5 : Abbreviations used in the forum

List of abbreviations used, genes/receptors/enzymes significantly affected by (Ro)accutane. Alphabetical order. Hyperlinks to Genecards Database.

List under construction.

- Metabolites, general terms :

1,25(OH)(2)D(3) 1alpha,25-dihydroxyvitamin D(3)
9-cis-RA 9-cis-retinoic acid; a metabolite of (Ro)accutane
13-cis-RA 13-cis retinoic acid; the active compound in (Ro)accutane
ATRA all-trans-retinoic acid
AD Alzheimer´s disease
CHF Congestive heart failure
CVD Cardiovascular disease
GH growth hormone
IGF-1 insulin like growth factor 1
PD Parkinson´s disease

- Receptors :

AR androgen receptor
FXR farnesoid X receptor
GHR Growth hormone receptor
gp330 megalin
IGF1R Insulin like growth factor I receptor
IGF2R Insulin like growth factor II receptor
IL1R1 Interleukin 1 receptor type 1
IR insulin receptor
IRS1 insulin receptor substrate 1
LXR liver X receptor; isoforms
PPAR peroxisome proliferator-activated receptor; isoforms
RA retinoic acid
RAR retinoid acid receptor; isoforms
ROR retinoic acid related orphan receptor; isoforms
RXR retinoid X receptor; isoforms
TLR toll-like receptor
TR thyroid receptor; isoforms
TRK tyrosine kinase; isoforms

- Enzymes/binding proteins/genes :

5-alpha-r 5-alpha-reductase
CRABP cellular retinoic acid binding protein
ERK
GGP gamma-glutamyltransferase
IGFBP1 Insulin like growth factor binding protein 1
IGFBP3 Insulin like growth factor binding protein 3

L-FABP liver type fatty acid binding protein
LPL lipoprotein lipase
FAS fatty acid synthase
PKA protein kinase A
PKC protein kinase C
RBP retinol binding protein
RoDH-4 retinol dehydrogenase-4
SERT
SOCS-1
SREBP-1 sterol regulatory element binding protein-1
TRK tyrosine kinases, isoforms

- Transcription factors :

AP-1
AP-2
NF-kappaB nuclear factor kappa B
NF-Y nuclear factor Y
SP-1 stimulatory protein 1
SP-3
STAT5A Signal transducer and activator of transcription 5A
STAT5B Signal transducer and activator of transcription 5B
TNF-alpha tumour necrosis factor alpha

Annexe 6 :Hoffman la Roche´s product line

- (Ro)accutane induced effects related to effects from other substances from Hoffman la Roche´s product line :

Some mechanisms in hoffman la Roche´s product line may be directly recognizable as partial effects that are induced from (Ro)accutane. A relation to research done on (Ro)accutane induced effects can not be excluded. Therefore, research on (Ro)accutane induced effects may be beneficial in clarifying effects of other products marketed by Hoffman la Roche and vice versa. However, other common characteristics include that the mechanism of action seldom is fully known, if known at all, and that the selectivity is limited. For some substances there is even a lack of declared metabolic pathways.

- (Ro)accutane (isotreinoin) :

Non-selective, highly toxic retinoid. Non-defined general and wide extensive effects.

- Xenical (orlistat) :

Non-selective lipoprotein lipase inhibitor. Inserts its action by forming covalent bonds with serine residues [0]. The lipoprotein lipase is found to be inhibited by 13-cis-RA [x].

- Naprosyn (naproxen) :

NSAID, non-selective prostaglandin syntethase (PGHS) inhibitor [0]. PGHS is involved in the catabolic pathways of 13-cis-RA [1].

PGHS-catalyzed oxidation of RA and (13Z)-RA was shown to form free radical adducts, using electron spin resonance (ESR) spin trapping techniques and 5-phenyl-4-penten-1-yl hydroperoxide (PPHP) or 13-hydroperoxy-9-cis-11-trans-octadecadienoic acid (13-OOH-18:2) as hydroperoxide substrates [1].

- Herceptin : (trastuzumab)

Anti-Her2 monoclonal antibody. Her2 positive breast cancer. Under evaluation.

Lack of markers

Although many different anticancer drugs appear to mediate tumor regression by inducing apoptosis, there is currently no consistent evidence that any of the molecules implicated in this process can be used as predictive markers [4].

It is not known when and in which patients Herceptin may be beneficial, due to a lack of accurate predictive markers.

Resistance to Herceptin common; higher rates of metastases found in patients exposed to Herceptin; possibly due to EGF-R receptor malfunction over time

The majority of patients who achieve an initial response to Herceptin generally acquire resistance within one year [3]. Recently, patients with Her-2-positive breast tumors who were treated with trastuzumab have been reported to develop CNS metastases at higher

rates, often while responding favorably to treatment [2].

Contradictions exist. It is not known why a resistance to Herceptin occurs frequently. There is a lack of data showing what aquired resistance means in terms of survival and increased/decreased development of metastases. Herceptin treatment causes c-erbB2 phosphorylation of Y877 and Y1248 [6]. The role of overexpression of the EGF-R receptor family in cancer development is not fully understood. The role of lack of EGF-R expression/aquired resistance is not fully understood.

Questions to be answered in the future include if there is an optimal rate of HER2 expression and optimal rate of EGFR function (neither overexpressed, nor underexpressed) for the most beneficial cancer treatment?

- Activase (alteplase) :

- Bondronat (ibandronate) :
Biphosphonate [0].

- Cardene (nicardipine hydrochloride) :
Non-selective calcium channel blocker/ calcium ion influx inhibitor [0].

- CellCept (mycophenolate mofetil) :
Non-selective inosine monophosphate dehydrogenase (IMPDH) inhibitor. 2-morpholieothyl ester of mycophenolic acid (MPA). Immunosuppressive [0].

- Cytovene (ganciclovir) :
Synthetic guanine derivative [0].

- Demadex (torsemide) :
Non-selective diuretic of the pyridine-sulfonylurea class. Inhibition of the Na+/K+/2Cl- carrier system. Increased urinary secretion of sodium, cloride and water [0].

- Fortovase (saquinavir) :
Non-selective inhibition of HIV-protease [0].

- Fuzeon (enfuvirtide) :
Non-selective inhibition of HIV-1 fusion with CD4 cells [0].

- Gantrisin (acetyl sulfisoxazole) :
Non-selective antibacterial sulfonamide [0].

- Hivid (zalcitabine) :

- Invirase (squinavir) :
Inhibitor of HIV protease. Binds to protease active site and inhibits cleavage of of viral polyproteins [0].

- Kytril (granisetron hydrochloride) :
Partially selective 5-HT3 antagonist. Blocks serotonin stimulation. May show affinity for 5HT1, 5HT1a, 5HT1b/c, 5-HT2, alpha1 alpha2 and beta adrenoreceptors, dopamine D2, histamine H1, benzodiazepine, picrotoxin or opioid receptors [0].

- Lariam (mefloquine hydrochloride) :
Non-selective anti-malaria agent. Acts on blood schizonticide. Unclear mechanism of action. Produces carboxylic acid metabolites [0].

- Rocaltrol (calcitriol) :
Synthetic vitamin D analogue. 1,25 dihydroxyvitamin D3. D3 is catalyzed by 25-OHase present in the liver, with end product 25-Hydroxyvitamin D3. Hydroxylation of 25-Hydroxyvitamin D3 occurs in the mitochondria of kidney tissue by renal alpha hydroxylase to produce 1,25 dihydroxyvitamin D. Resultant hypocalcemia and hyperparathyroidism are major cause of the metabolic bone disease and renal failure. In acutely uremic rats, calcitriol has been shown to stimulate intestinal calcium absorbtion. Calcitriol and other vitamin D metabolites are transported in blood by an alpha -globulin vitamin D binding protein [0].

- Recephin (ceftriaxone sodium) :
Broad spectrum cephalosporin antibiotic [0].

- Referon (interferon alfa-2a recombinant) :
Non defined antiproliferative general and wide effects [0].

- Romazicon (flumazenil) :
Non-selective benzodiazepine receptor antagonist. GABA/benzodiazepine inhibition. Does not, according to the manufacturer affect the opioid signaling. Albumin counts for 2/3 of binding [0].

- Tamiflu (oseltamivir phosphate) :
Converted to oseltamivir carboxylate by ester hydrolysis. Possible inhibition of influenza virus neuraminidase by possible alteration of virus particle aggregation and release [0].

- Ticlid (ticlopidine hydrochloride) :
Non-selective platelet aggregation ihibitor. Irreversible inhibition of ADP-induced platelet fibrinogen binding [0].

- Toradol (ketorolac tromethamine) :
Non-selective NSAID. Pyrrolo-Pyrrole group. Inhibition of prostaglandin synthesis [0].

- alcyte (valganciclovir) :
Non-selective antiviral agent. Phosphorylation via cellular kinases to produce ganciclovir triphosphate. Phosphorylation partially dependent on viral kinases. Action through inhibition of DNA-synthesis [0].

- Valium (diazepam) :
Non-selective benzodiazepine [0].

- Vesanoid (tretinoin) :
Non-selective retinoid. Highly toxic [0].

- Xeloda (capecitabine) :
5-FU precursor. Flouropyrimidine carbamate. Metabolized through carboxylesterase - cyd deaminase - thymidine phosphorylase to 5-flouracil (5-FU) [0].

- Zenapax (daclizumab) :
IgG1 monoclonal antibody, binds to p55 alpha, CD25 or Tac subunit of the high affinity human IL-2 receptor [0].

- References :

[0]
[1] **Freyaldenhoven MA, Lloyd RV, Samokyszyn VM.**
Prostaglandin H synthase-catalyzed oxidation of all-trans- and 13-cis-retinoic acid to carbon-centered and peroxyl radical intermediates. (1996) Chem Res Toxicol. Jun;9(4):677-81.
[2] **Weil RJ, Palmieri DC, Bronder JL, Stark AM, Steeg PS.** *Breast cancer metastasis to the central nervous system.* (2005) Am J Pathol. Oct;167(4):913-20.
[3] **Menendez JA, Vellon L, Lupu R.** *Targeting fatty acid synthase-driven lipid rafts: a novel strategy to overcome trastuzumab resistance in breast cancer cells.* (2005) Med Hypotheses. 64(5):997-1001.
[4] **Duffy MJ.** *Predictive markers in breast and other cancers: a review.* (2005) Clin Chem. Mar;51(3):494-503.
[5]
[6] **Diermeier S, Horvath G, Knuechel-Clarke R, Hofstaedter F, Szollosi J, Brockhoff G.** *Epidermal growth factor receptor coexpression modulates susceptibility to Herceptin in HER2/neu overexpressing breast cancer cells via specific erbB-receptor interaction and activation.* (2005) Exp Cell Res. Apr 1;304(2):604-19.

Annexe 7 : discutions on specialized forums

- Are we missing the point? Inhibiting 5AR...(discution on the forum http://max001.proboards.com/) :

Posted the Feb 6, 2010 at 4:49pm by finresearch :

« Hi to all Accutane Sufferers

I have news for you that may help bring this problem to the next level.

Accutane sufferers: You have soulmates!

I am a moderator at www.propeciahelp.com, a site for men which have experienced **persistent side effects** from finasteride (Propecia, Proscar, generics). Our men typically took these medications to combat hair loss. We also have a number of men which have taken dutasteride (Avodart) or saw palmetto based preparations instead of finasteride. Our member count is currently over 1500 and increasing daily.

The reason I am posting here is because we had a guy sign up at our site the other day who reported the exact same side effect profile like we have, **from having taken Accutane**. I since have been researching what Accutane sufferers had to say and checked out what has been published from a science/medical point of view. Today, I can say two things for sure:

[li]You people have exactly the same symptom profile like “we” do

[/li][li]Science has so far miserably failed in understanding your problem.[/b]
[/li][/ul]
I am 99.9% sure that we are dealing with exactly the same problem and can mutually benefit from each other. Before going any further, let me give you a rundown of our list of symptoms (some, of course, apply only to men):

Frequent:
* Unexplainable, out-of-the-blue depression (from light to suicidal)
* Emotional Blunting / Emotionally Flat
* Difficulty Concentrating
* Forgetfulness (can't recall names, places etc) / Losing Train of Thought
* Stumbling over Words / Getting Tongue Tied
* Slowdown of mental processes
* Lack of Motivation
* Anxiety / Panic Attacks
* Social Phobia
* Persistent or Unexplainable Fatigue
* Sleep Disturbances
* Loss of Libido/Sex Drive
* thingy Feels "disconnected" from head or body
* Erectile Dysfunction
* Loss of Morning / Spontaneous / Nocturnal Erections
* Penile Tissue Changes (narrowing, shrinkage, curving, numbness, wrinkled)

* Watery Ejaculate
* Reduced Ejaculate
* Inability to Ejaculate / Orgasm
* Testicular Pain
* Testicular Shrinkage / Loss of Fullness
* Stomach Pains / Digestion Problems / Bloating
* Changes in Fat Distribution / Weight Gain
* Gynecomastia (male breasts)
* Muscle wasting
* Muscle pain
* Muscle weakness
* Dry skin, dry eyes
* Prostate problems (difficulty to urinate or frequent urination)
* Various Hormones are out of whack
* No or bad reaction to androgen replacement (TRT, DHT)

Less frequent:
* Blurry vision/acuity decrease
* Tinnitus (ringing/high pitched sound in ears)
* Lowered body temperature / decreased metabolism
* Lack of blood flow to the thingy (cold thingy)
* Dry/Dark circles under eyes
* Elevated Liver Values (AST/ALT)
* Increased hair loss, thinning of hair (often accompanied by itchy scalp)
* Chest pain
* Joint pain
* Oversensitivity to light
* Burning sensation in hands (inner side) and fingers, sometimes in feet

Like with Accutane, we have unfortunately also had a number of suicides because of the severe depression that often comes with our “crash”. It is important to note that these side effects typically surface to the full extent around two weeks (on average) AFTER quitting finasteride/saw palmetto/dutasteride (sound familiar?). This time span can vary strongly anywhere from 2-3 days up to 6 months. The affected men are typically in their early twenties and often in very good health before taking these preparations.

Some of these symptoms can be attributed to any garden variety depression you may say. True, but you don’t get penile shrinkage, muscle wasting, complete penile numbness and severe gynecomastia from depression. So what is the common denominator between isotretinoin (**Accutane**), finasteride (**Propecia, Proscar**, etc.), dutasteride (**Avodart**) and **saw palmetto**? If you agree that “our” list of symptoms pretty well matches “your” list (I have since seen numerous confirmations that it does), then logically these four substances must have something in common that does this to us.

The answer is: ***ALL FOUR substances inhibit an enzyme called 5alpha-reductase (5AR)***. 5AR is what converts the hormone testosterone to the more potent 5alpha-dihydrotestosterone (DHT).

ALL FOUR MEDICATIONS SEEM TO CAUSE THE SAME PROBLEMS!

<u>This cannot be a coincidence!!</u>

This is Accutane's main mode of action, **that almost everybody seems to be missing**:

"In accordance with recent findings, no change in serum testosterone and **significant decreases in 5 alpha- dihydrotestosterone (DHT)**, 5 alpha-androstane-3 alpha,17 beta-diol glucosiduronate, and androsterone glucosiduronate levels were observed after treatment."

jcem.endojournals.org/cgi/content/abstract/80/4/1158

"the ratios of the 5 alpha/5 beta metabolites (androsterone:aetiocholanolone and allo-THF:THF) were at the upper limit of the reference range and were lowered after treatment, suggesting that **5 alpha-reductase activity is sensitive to isotretinoin**."

www.ncbi.nlm.nih.gov/pubmed/1827343?itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum&ordinalpos=3

DHT plays a MAJOR role in hair loss and **ACNE** (also in women!). Women have male hormones as well, only in smaller quantities than men. Accutane works by reducing DHT through inhibition of 5AR (mainly type 1).

"These results support the concept that target tissue **androgen production plays an important hormonal role in the pathogenesis of acne in women** and that plasma 3α-diol G may be the most sensitive marker of this process."

www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WM8-4SSR30H-9&_user=10&_coverDate=03%2F31%2F1985&_rdoc=1&_fmt=high&_orig=search&_sort=d&_docanchor=&view=c&_searchStrId=1196903230&_rerunOrigin=google&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=e487fc5e5e13c34a4ebb3675f8332bc3

"Acne is one of the most common skin disorders. **Androgens** are known to play an important and **<u>possibly central role</u>**."

www.ncbi.nlm.nih.gov/pubmed/8252744?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_SingleItemSupl.Pubmed_Discovery_RA&linkpos=3&log$=relatedarticles&logdbfrom=pubmed

"This study suggests that DHT may not only be involved in sebum production but also in production of proinflammatory cytokines in acne."

www.ncbi.nlm.nih.gov/pubmed/20043171?itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum&ordinalpos=1

Androgens play a central role in Acne AND Hair Loss. Both Accutane and hair loss medications work by reducing the androgen hormone DHT. The result is a common persistent side effect profile. **I don't know how much clearer it can get**.

Many men at www.propeciahelp.com have unsuccessfully tried supplementing and

adjusting hormones together with some of the best specialists on this planet. Most have had no success with this; few guys had limited success at best. We are getting pretty close to establishing that the fact of inhibiting 5AR has somehow made us partially androgen resistant. How this can be and exactly what (other?) pathways may be involved is still a mystery – but we are working on it and getting science involved wherever and however we can. Incidentally, my personal focus is on exactly on this area. I have been working relentlessly on getting scientists on board and have been in contact with many of them at a global level. My areas of interest are molecular biology and genetics/epigenetics.

I am now very interested to see how Accutane sufferers will react to my post. If we can establish together that we are really talking about the same problem, this would give our "joint cause" an incredible boost in power. **The bigger we can make this problem, the more relevant it will become**. This will make it much easier to get the dozens/hundreds of millions of dollars funding that are needed to get the basic research in this area to the next level. Science is just starting to really realize that chemicals can have persistent side effects:

« ...it is becoming increasingly apparent that chemicals can cause changes in gene expression that persist long after exposure has ceased. Here we present the hypothesis that commonly-used pharmaceutical drugs can cause such persistent epigenetic changes. »

www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WN2-4WFPPMM-1&_user=10&_coverDate=11%2F30%2F2009&_rdoc=1&_fmt=high&_orig=search&_sort=d&_docanchor=&view=c&_searchStrId=1194155102&_rerunOrigin=google&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=24dcac7a47354d1e76a8d7ee88858cc6

Look at the table of contents - did you see this??

Teratogens with epigenetic effects: thalidomide and isotretinoin

This was published in November 2009!

It would be great if we could take a crack at this together.
Wishing everybody a soon recovery!

Finresearch »

Posted on Apr 27, 2010 at 9:49am by Taned

« This information is 100% correct, and these problems are very hard to treat, if not impossible, as examined from my own situation. My suggestion is, if you have suffered a collection of side-effects on that list, you should seek a very knowledgeable endocrinologist doctor in the United States. However when the drug is taken for a long period of time, usually these drugs do not only lower the level of your hormones, but killed off/blocked the effects of hormone receptors. I personally have had very minimal long term success treating these problems so far.

This is what to do :

1) Find a doctor who believes you and is willing to work with you in your area, if that doesn't work out then consider seeing the two doctors who have some knowledge about

these problems (Dr.Crisler and Shippen) explain to them your situation in detail, as everyones case is different.

2) Get a FULL endocrine work up, including everything, from testosterone, to prolactin, to adiol g levels, to estrogen, iron levels, and so on, if things come back abnormal then you have something to treat. This might be hard to convince your doctor to test for EVERYTHING, but bring him some studies to help your case.

3) Consider getting a stool test done to check for dybosis or bad bacteria, you can order them online it's easy.

4) Definitely get some urine neurotransmitter testing, Accutane and Finasteride can both block the effect of neurohormones in the brain which can obviously cause many serious problems. However they say urine testing isin't that accurate, but better than nothing.

5) I'd say the main thing is to keep an eye on the symptoms you suffer with. Pay close attention always, too see if anything is improving, or getting worse. The way that these drugs work are so unique and labwork doesn't seem to mean as much. It's all about symptoms. Don't get me wrong, lab tests are important obviously, but these drug effects are not understood properly so lab tests are just a vague parameter. Of course if you are on any replacement therapys then blood work is a must. Many people have full blown hypogonadism and andropause symptoms with testosterone levels in the mid range after Propecia/Accutane therefore like I said before, symptoms is much more important.

Again we must collaborate and continue to stay on top of the latest research and technology, as one day they may be able to reverse this. But it is so difficult with the mental side-effects and neurological damage, I wish I could be put in a coma, and then woken up when the cure is here. Atleast if I only had physical side effects, I could stay at home and play games all day, you don't need your health to play games, however you DO need a functioning brain, energy, a fight or flight response, emotions, and pleasure too. It breaks my heart to enjoy nothing, and have all my powerful emotions blunted to the point life isin't worth living anymore.

I will report back in the next year or two, stress has a very bad effect on my health and I can't handle it, along with everything else. I understand why Max dissapeared, he couldn't handle the stress. Good luck to everyone who suffer from these silent, but life changing conditions »

- Discution on http://www.acne.org/messageboard/topic/295030-repairing-the-long-term-damage-from-accutane/page-3

Firstly as i say, all these symptoms are based around retinoic acid (accutane) and it's effect in the body. Retinoic acid cannot be stored in the liver like normal vitamin A. It only works in the skin cells. All these people thinking Accutane is still stored in their livers are wrong, it's not. The reason in my mind why Accutane causes side effects is because it is a glucuronidated drug. Ie as it comes into the body, the liver binds the retinoic acid to glucuronic acid molecule which makes it more water soluble. The only problem is over time these glucurides build up and slow down bile flow, or bile acid transport to be precise. Just like anabolic steroid's Accutane causes a similar Liver toxicity. **If your suffering from accutane side effects, check your stools....**i know i sounds gross but how dark are your stools? if they are a light brown / clay colour you ain't got enough bile flow, chances are your bile has slowed to a snails pace. […]

Accutane basically shuts down the bile acid transport in people **with severe accutane side effects, joint cracking, hair loss, seb derm, psoriasis, impotence**. All these side effects are caused by the accutane still being in your body, because once the bile has stopped, how is the retinoic acid going to be removed? it isn't? the bodys going to go into hibernation mode and it'll try and protect the organs by storing the accutane in the fat cells. A lack of bile means a lack of fat absorption hence inability to gain weight.

Annexe 8 : Declaration of mutagenesis; a future must

- The future use of substances that significantly affect gene-expression at multiple levels :

Substances used for cancer such as (Ro)accutane affect gene-expression at several levels. There are today good methods such as RT-PCR and Western blotting, for determining factors such as what genes are affected, which binding affinity to the substance they have and what role they play in the cell.

There is a lesser use of wide and well functioning determination rates of site directed mutagenesis/deletion of transcriptional promoter sites (through point mutagenesis) in association with the exposure of substances highly likely to result in mutagenesis, even though studies involving the induction of point mutagenesis exist.

- Novel wide and accepted methods needed for determining and declaring the statistical rate of mutagenesis :

A general problem is to determine and declare the prevalence of point mutagenesis. Some methods are discussed in Bajaj et al (2005) [1]. To declare the statistical rate of point mutagenesis is of highest importance, not only in the case of (Ro)accutane and other cancer treatments, but for all substances that significantly influence gene-expression. The rate of point mutagenesis will without doubt have an outcome on the patients long-term health. Factors such as a downregulation of the function of certain receptor types due to deletions of promoter binding sites may significantly affect further cancer reoccurrence, the development of diabetes, CVD and other diseases.

I am convinced that the future of medicine therefore holds:

1) A statistical method wide for determinination of overall probability of significant point mutagenesis of various critally important genes given any used substance and dose.

2) In the case of found significant point mutagenesis which could be expected in several areas for example with toxic exposure, significant receptor inhibition or growth factor inhibition, a *statistical wide and accepted method* for determination and declaration of the rate in percent and in intervals of mutagenesis given determined for the subjects critically important *genes*, *dose* of the substance used and *time* it is used. This declared data may in clinical practise serve as a very adequate additional information in the decision of the accuracy of the clinical use of any substance.

- Simplified example of declaration of mutagenesis :

An example of declaration may look as the following:

x Name of substance: Cancer substance X

x Clinically relevant rate of mutagenesis/deletion of transcriptional promoter sites: Yes

x Determination of critical areas for point mutagenesis

x Affected receptor subtypes: Receptor family Q

x Targeted transcription factors: Sp1, Sp3

x Estimated reduction of residue/protein function with deleted transcriptional promoter sites: 80%

x Active/inactive phenotype: Active at low and high initial expression

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x Dose/plasma concentration 0,1A
Rate of average mutagenesis of sites for receptor/receptor family Q with time 1, 2 and 3

Deletion of functioning Q receptor transcriptional promoter sites with time 1: interval: 3% to 5% time 2: 8-12% time 3: 12-16%

x Dose/plasma concentration 0,5A
Rate of average mutagenesis of sites for receptor/receptor family Q with time 1, 2 and 3

Deletion of functioning Q receptor transcriptional promoter sites with time 1: interval: 15% to 25% time 2: 30-40% time 3: 40-55%

x Dose/plasma concentration 1,0A
Rate of average mutagenesis of sites for receptor/receptor family Q with time 1, 2 and 3

Deletion of functioning Q receptor transcriptional promoter sites with time 1: interval: 45% to 60% time 2: 70% to 85% time 3: >95%

---

x Conclusions, discussion of outcome and prevalence of cancer reoccurence given statistical rate of downregulation of receptor function due to mutagenesis.

- References :

[1] **Bajaj, K., Chakrabarti, P., and Varadarajan, R.** *Mutagenesis-based definitions and probes of residue burial in proteins* (2005) Proc. Natl. Acad. Sci. USA, 10.1073/pnas.0505089102

Annexe 9 : Max's advice

«Thank you for your comments.
Please scan all sections of the forum if you want to find out what studies that are publicly available and are performed on retinoids and the brain.

You suggest a more reader friendly forum. I might very well add a section that is written in a simplified language. However, there is a purpose with showing a greater spectra of complexity, first of all the communication with people within these represented scientifical fields, and secondly, to show any person with limited biochemical knowledge, that RoAccutane is a substance with very wide and complicated effects.

Please bring any information to a person with biochemical/specialist competence in the field where your symptoms are most pronounced for clarification and correct interpretation.

Kind regards,

Max »

Annexe 10 : pharmaco-épidémiologic studies

- Statistical study on the risk of depression related to isotretinoin:

[Isotretinoin and the risk of depression in patients with acne vulgaris, Lawrence Azoulay, University of Montreal, 2007]
https://www.ncbi.nlm.nih.gov/pubmed/18363422

Open access study link:
https://papyrus.bib.umontreal.ca/xmlui/bitstream/handle/1866/15672/Azoulay_Laurent_2007_these.pdf?sequence=1

In fact, in a significant sample drawn from a database of 30,496 patients in Quebec who took isotretinoin from 1984 to 2003, the gross, short-term risk is 2.00(95% CI = 1.03 to 3.89), and the ***relative risk*** in the medium or long term is from ***2.68*** (IC 95% = 1,10 à 6,48)...
By analogy, this risk approximates the risk for a woman to develop lung cancer when she consumes more than ten cigarettes a day, or even 20 according to the statistical samples **[1]**.

[1] Table 1 (page 5). Risk rates for lung cancer deaths observed in men and women in six prospective studies, based on the amount of cigarettes smoked.

From the study: Scientific evidence linking tobacco use to lung cancer, Dr. Norman L. Jones, MD, FRCP, FRCP (C), Michael G. DeGroote School of Medicine McMaster University Hamilton, Ontario, June 2008 .

http://www.wsiat.on.ca/tracitdocuments/mlodocuments/discussions/fsmoking.pdf

- thank you for reading page 9 of the following link, taken from the UK Drug Regulatory Authority:
2.5 Association between acne and depression :

https://assets.publishing.service.gov.uk/media/5492db7ce5274a42900002f2/DSU2.pdf

"***There is a lack of robust population-based studies*** comparing the frequency of suicide and suicidal ideation in adolescents with and without acne. **Such a study would be beneficial in helping to understand the role of acne, *as well as treatments such as isotretinoin, in suicide.* "**

Therefore, an impartial and independent reproduction of an epidemiological and statistical study similar to that of Dr. Azoulay of 2007, in other countries, would have a major interest in the anticipation of public health problems concerning iatrogenic depressions due to isotretinoin, in long term health.

Annexe 11 : épigenetic

A theory states that brain receptors are destroyed, but this is partially reversible because they can be reconstructed by brain plasticity. [3] The main problem is at the level of neurotransmitters (intestines, adrenals), parathyroid and toxicity / global congestion, and particularly cerebral.

"Changes in the epigenetic chromatin may be short-term, lasting a few minutes, or lasting for months or even years in examples of chronic abuse of addictive substances in man or the animal. "
http://www.aaem.pl/Epigenetic-regulation-in-drug-addiction,71809,0,2.html

[3] Again, epigenetics, strictly defined, concerns heritable changes in gene expression. What is described here is any change in gene expression that can be induced by outside influences. These are two different things. Again, epigenetic changes are long-term changes that are potentially heritable and, as I pointed out above, most epigenetic changes are not passed on to the offspring, certainly not to the point where they have an effect detectable on evolution. The rest is the regulation of genes, which is often transient but which, depending on the process, can continue in the long term as long as the stimulus causing the regulatory change is present. As is often noted, the quickest way to get an organ back to normal is to stop doing the bad things that caused it to malfunction in the first place. Like P.Z. Myers said it

[...] "In part, the root of the problem here is that we are falling into an artificial dichotomy, on the one hand defining embarrassment as a countable and distinct character that can be torn off and mapped as a fixed sequence bits in a computer database, and on the other hand putting in a different plane all these disordered cellular processes that affect what the gene does in the cell, and we try with difficulty to classify them as separate. This is very similar to the nature-culture controversy, where the real problem is that biology does not fall into these simple conceptual lockers and we strive to distinguish the indiscernible. You are the product of genes and cellular and environmental interactions. "
http://scienceblogs.com/insolence/2013/02/11/epigenetics-you-keep-using-that-word-i-do-not-think-it-means-what-you-think-it-means/

"Epigenetic factors, including DNA methylation, histone modifications, and impaired expression of microRNAs play a key role in controlling changes in gene expression and genomic instability." throughout human life. Epigenetic changes are finely balanced and highly reversible in normal tissues. "

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4651002/

"Chromatin modifications and DNA methylation are potentially reversible in response to particular environmental conditions. The food, social, behavioral, and physiological environment can alter the epigenome, with long-term consequences for gene expression, cell signaling, and thus phenotype. "

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2951010/

"Epigenetic changes have been associated with exposure to environmental pollutants. Exposure to toxic metals, including arsenic, cadmium, lead, nickel, chromium and methylmercury, has been associated with aberrant changes in DNA methylation and histone modifications [4] . Metals are known to increase the production of reactive forms of oxygen, and oxidative damage to DNA can alter the ability of methyltransferases to interact with DNA, resulting in changes in DNA methylation [5].]. Exposure to atmospheric pollutants, such as particulate matter, black carbon, and benzene, has also been associated with global and / or genomic DNA methylation alterations [4, 6]. These alterations are similar to epigenetic changes in diseases related to exposure to air pollution. , such as cardiovascular parameters and hematological malignancies. Endocrine-disrupting chemicals that are toxic to reproduction (bisphenol A, dioxin, diethylstilbestrol and persistent organic pollutants), pesticides, and chemicals in drinking water are also being studied in association with epigenetic alterations in animal studies, in vitro and human [4, 6] Most epigenetic alterations are reversible [...]. "
http://www2.keelpno.gr/blog/?p=2955

"Unlike genes, which can only be modified by complex gene therapies, epigenetic markers are reversible and can therefore be the subject of environmental approaches or drug treatments. First, avoidance of certain chemicals and toxic agents, or better lifestyle and diet choices, can prevent alterations of the epigenome. "

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3574773/

Many of these epigenetic modifications are reversible by selected dietary intakes. The genome is programmed by the epigenome and the epigenome in turn is largely programmed by the social and physical environment. "
https://www.ncbi.nlm.nih.gov/pubmed/180953304

https://www.ncbi.nlm.nih.gov/pubmed/17988634

"Isotretinoin has three levels of presence in the body:
a) in the short term: 46-48h half-life (50% decrease in plasma level)
b) in the medium term: about one month at the intracellular detection limit (excluding RNA) - then the "good" effects begin to dissipate and acne and sebum can potentially return
c) long-term: up to one year to eliminate RNA and stop the regulation of gene transcription

Because of c) (and the massive impact it has on HOX gene transcription), you should not get pregnant for a full year after stopping the drug. Doctors say 6 months, but the safe choice is one year. "
Reference: https://www.ncbi.nlm.nih.gov/pubmed/9427083

Annexe 12 : studies to integrate in this PDF

https://www.ncbi.nlm.nih.gov/pubmed/14996403

"Phosphatidylcholine, in the form of CPAP, or DLPC.

Vitamin E can reduce hypervitaminosis A.

https://www.ncbi.nlm.nih.gov/pubmed/9093712

https://en.wikipedia.org/wiki/Hypervitaminosis_A

http://s2.e-monsite.com/2010/02/12/07/resize_550_550//Sans-titre58.jpg

http://www.ncbi.nlm.nih.gov/pubmed/2333716

http://www.ncbi.nlm.nih.gov/pubmed/2870901

http://www.ncbi.nlm.nih.gov/pubmed/15116059

http://www.ncbi.nlm.nih.gov/pubmed/1677495

http://dmd.aspetjournals.org/content/38/7/1211.abstract

« Isotretinoin and its metabolites are conjugated, possibly with glucuronic acid, before being excreted in urine and feces. Excretion of unchanged isotretinoin in urine appears to be negligible. Isotretinoin appears to be excreted in feces, mainly as unchanged drug. Limited data suggest that isotretinoin and its metabolites are excreted in feces via biliary elimination and that the drug and its metabolites also undergo enterohepatic circulation. In adults with normal renal and hepatic function, 65-85% of a single, radiolabeled 80 mg oral dose of isotretinoin is excreted in urine and feces in approximately equal proportions. »

http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5282379

http://www.ncbi.nlm.nih.gov/pubmed/1676630

http://dmd.aspetjournals.org/content/38/7/1211.full

http://www.ncbi.nlm.nih.gov/pubmed/1677495

http://www.ncbi.nlm.nih.gov/pubmed/8117316

http://www.sciencedirect.com/science/article/pii/S0006291X03003322
http://press.endocrine.org/doi/abs/10.1210/jcem.80.4.7714084
http://www.ncbi.nlm.nih.gov/pubmed/9298137

http://www.ncbi.nlm.nih.gov/pubmed/10951254

http://www.readcube.com/articles/10.1111/j.1753-4887.1984.tb02378.x

http://agence-prd.ansm.sante.fr/php/ecodex/frames.php?specid=67952249&typedoc=R&ref=R0194594.htm
http://www.medsafe.govt.nz/profs/datasheet/o/oratanecap.pdf
http://www.ncbi.nlm.nih.gov/pubmed/9390712

« A balance of RA synthesis and catabolism in the cell maintains the correct levels of RA regulated transcription. Exposure of the cells to isotretinoin, which is isomerized in tissue to the active all-trans RA10, 11, will destabilize this balance and result in inappropriate gene transcription. »

« Brain regions that are endogenously regulated by RA, and which may be disrupted by isotretinoin to potentially promote depression, have been described in our previous review and include the striatum, hippocampus and frontal cortex.27 An area of the brain, however, that has been little considered for retinoid action is the hypothalamus. The hypothalamus is the hormone regulatory center of the brain and, as part of the hypothalamus/pituitary/adrenal (HPA) axis, is a central component in the response to stress. Hyperactivity of this system is a reproducible finding in depression.

« It was found in this same study that photoperiod regulates RALDH in tanycytes as well as RAR/RXR's in the hypothalamus, therefore showing changes in RA signaling driven by seasonal changes. Given that seasonal affective disorder is a form of depression driven by seasonality there is the potential for RA to influence this disorder. »

« One particular RA regulated gene in the hypothalamus that may provide a link between RA and depression is corticotrophin-releasing hormone (CRH)134 a key regulatory factor in the HPA axis135 which may contribute to HPA axis hyperactivity in depression.136, 137 »

« Isotretinoin administration to human subjects is associated with a decrease of biotinidase,152 and the presumed decrease in biotin that would result from this may contribute to depression. »

« Headache was the most common side effect of isotretinoin after dry skin62 while a number of other reports have associated isotretinoin with pseudotumor cerebri.54, 169–171 The occurrence of headache with isotretinoin usage has been linked to depression172 suggesting that patients who show a CNS side effect such as headache may also be more susceptible to isotretinoin-induced depression. »

"The administration of isotretinoin to human subjects has been shown to be associated with increased homocysteine 166 concentrations, as well as decreases in 5-methyl-tetrahydrofolate 167, which is a potential metabolic mechanism by which isotretinoin may promote depression. "

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3276716/

"Headache was the most common side effect of isotretinoin after dry skin (62), while a

number of other reports associated isotretinoin with brain pseudotumors. (54, 169-171). The occurrence of headache with the use of isotretinoin has been associated with depression (172) suggesting that patients have CNS side effects such as headaches may also be more sensitive to isotretinoin-induced depression. "
"This study found that four months of treatment with 13-cis RA led to a significant reduction in cerebral metabolism in the orbito-frontal cortex (Figure 3), (174) a region that was associated with depression. In the case of patients reported to the Norwegian Medicines Agency, single brain photon emission computed tomography (SPECT) was performed in 15 cases that reported lasting neurological symptoms. Impairment of brain function was observed in all cases where frontal lobe blood flow was altered or reduced (173). Ten of these patients were assessed as having organic brain lesions. "
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3276716/

Roaccutane greatly reduces the functioning of the pituitary gland:
http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1013&html=1

Article about all the supposed effects of roaccutane:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3276716/

https://www.landesbioscience.com/journals/dermatoendocrinology/MelnikDE3-3.pdf

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http://www.uiowa.edu/~c046138/tut-elim.htm
