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Mechanisms behind the lipid-lowering effects of ACTH

Studies in different experimental models

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Mechanisms behind the lipid-lowering action of ACTH

Studies in different experimental models

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Institutionen för Laboriemedicin

Klinisk Kemi och Farmakologi

Akademisk avhandling som med vederbörligt tillstånd av Medicinska fakulteten vid Lunds Universitet för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentligen försvaras i Föreläsningssal 2, Universitetssjukhuset i Lund, fredagen den 16 november 2007, kl. 9.00



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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. M. Skoog, N. Xu, M. Berggren-Söderlund, J. A. Lovegrove, P. Nilsson-Ehle. ACTH reduces the rise in ApoB-48 levels after fat intake.
Atherosclerosis 2007: 191;433-439.
- II. M. Skoog, N. Xu, P. Nilsson-Ehle. Lipid synthesis and secretion in HepG2 cells is not affected by ACTH.
In manuskript.
- III. N. Xu, M. Hurtig, U. Ekström, P. Nilsson-Ehle. Adrenocorticotrophic hormone retarded metabolism of low-density lipoprotein in rats.
Scand J Clin Lab Invest 2004: 64;217-22.
This article is available at: <http://www.informaworld.com>
- IV. X. He, H. Xu, X. Xu, M. Skoog, G. Luo, L. Zhen, J. Zhu, Q. Mu, P. Nilsson-Ehle, N. Xu. ACTH decreases serum cholesterol and apolipoprotein B in a hyperlipidemic rabbit model.
Submitted.

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Abbreviations

ACAT	Acyl CoA:cholesterol transferase
ACTH	Adrenocorticotrophic hormone
apo	Apolipoprotein, apoprotein
CETP	Cholesterol ester-transfer protein
CRH	Corticotropin-releasing hormone
CVD	Cardiovascular disease
ELISA	Enzyme linked immunosorbant assay
ER	Endoplasmic reticulum
FH	Familial hypercholesterolemia
GH	Growth hormone
HDL	High-density lipoprotein
HL	Hepatic lipase
HMG-CoA	β -hydroxyl- β -methylglutaryl-CoA reductase
HPA	Hypothalamic-pituitary-adrenal
HSL	Hormone sensitive lipase
IDL	Intermediate-density lipoprotein
kDa	Kilo Dalton
LDL	Low-density lipoprotein
Lp(a)	Lipoprotein(a)
LpL	Lipoprotein lipase
LRP	LDL-receptor related protein
MC	Melanocortin
mRNA	Messenger ribonucleic acid
MSH	Melanocyte-stimulating hormone
MTP	Microsomal triglyceride transfer protein
NZW	New Zealand White rabbit
PC	Prohormone convertase
POMC	Pro-opiomelanocortin
TH	Thyroid hormone
VLDL	Very low-density lipoprotein

General introduction

Lipoprotein metabolism

Lipoproteins

Our body consists of water, lipids, proteins, carbohydrates and minerals. A continuous intake of nutrients is essential to provide a source of energy, sustaining organ structures and metabolic processes, and the energy needed is provided by oxidation of carbohydrates and lipids.

Lipids are, due to their hydrophobicity, insoluble in plasma and need to be transported as lipoproteins, macromolecular complexes of lipids and proteins in the circulation (Figure 1a). These spherical lipoproteins have a core of non-polar (neutral) lipids such as cholesteryl esters and triglycerides, while more polar lipids, such as phospholipids and unesterified (free) cholesterol, are arranged in a monolayer surrounding the lipoprotein. In the amphipathic surface specific proteins called apolipoproteins (apo/apoprotein) are interspersed; they stabilise the lipoprotein and are involved in the metabolism of the lipoprotein mediating activation of metabolic enzymes and recognition by cell surface receptors for uptake. Most apoproteins occur in numerous copies and transfer easily between lipoprotein particles, and thus apoprotein composition continually changes during the lifespan of the particle. ApoB is the exception (Scott 1989; Young 1990): it is present in a single copy and remains in its original particle during its residence in plasma and is essential for assembly, secretion and receptor-mediated uptake of the lipoprotein.

Lipoproteins are classified by their difference in density, a property reflecting their composition. A larger core, consisting of triglycerides and cholesteryl esters, result in a lower density of the lipoprotein. The major classes are chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), lipoprotein (a) (Lp(a)) and high-density lipoprotein (HDL) (Table 1 and 2). Lipoproteins are dynamic populations of particles and there is an obvious heterogeneity in size and composition within each lipoprotein class.

Table 1. Lipoprotein characteristics

Class	Density (g/mL)	Diameter (nm)	Major apolipoprotein
Chylomicron	<0.94	>70	B48, E, C, A
VLDL	0.94-1.006	30-80	B100,E, C
IDL	1.006-1.019	25-35	B100,E
LDL	1.006-1.063	18-25	B100
Lp(a)	1.040-1.130	25-30	B100, apo(a)
HDL	1.063-1.210	4-12	A-I, A-II, C, E

Abbreviations: VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Lp(a), lipoprotein (a).

Reference: Rifai 1999, Kane 1996, Nilsson-Ehle 2003.

Table 2. Lipoprotein composition (% of mass)

	Surface components			Core components	
	C	PL	Apo	TG	CE
Chylomicrons	2-4	3-10	1-2	85-95	2-3
VLDL	7-10	15-20	8-10	55-80	5-12
IDL	9	19-25	19-22	20-50	29
LDL	8	20-22	20-25	5-15	37-42
HDL ₂	5	33	40	5	17
HDL ₃	4	25	55	3	13

Abbreviations: VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Lp(a), lipoprotein (a); C, cholesterol; PL, phospholipids; Apo, apolipoprotein; TG, triglyceride; CE, cholesteryl ester.

Reference: Rifai 1999, Kane 1996, Nilsson-Ehle 2003, Ginsberg 2005.

Lipid transport: Exogenous pathway

In the intestine dietary fats, mainly triglycerides and some phospholipids, cholesterol and fat-soluble vitamins, are absorbed by the enterocytes. Absorption occurs in the small intestine and requires hydrolysis to fatty acids, monoglycerides, lysophospholipids and cholesterol. After absorption these are re-esterified and packaged into chylomicrons along with the specific intestinal protein, apoB48, and apoA-I (Figure 2). After secretion into the lymphatic system the lipoproteins enter the circulation via the thoracic duct (van Greevenbroek and de Bruin 1998). Other apoproteins (apoE and apoCs) are incorporated into the surface through interaction with HDL (see reverse cholesterol transport).

Chylomicrons are seen in plasma within half an hour after feeding and are very fat-rich, containing about 90% triglycerides (Ginsberg et al. 2005). As the chylomicrons pass through the capillaries, the core triglycerides are rapidly hydrolysed by lipoprotein lipase (LpL) that resides anchored to the endothelial cells lining the capillaries throughout the body (Nilsson-Ehle et al. 1980). The metabolites free fatty acids and monoglycerides are taken up by extrahepatic cells, and are either metabolised by active cells such as muscle or stored in adipose tissues for future needs. As the lipoprotein shrinks, due to the hydrolysis of core triglyceride, phospholipids, unesterified cholesterol and apoproteins on the surface become redundant. These are transferred to HDL generating a denser chylomicron remnant containing less than 30% of the original triglycerides. ApoB48 and apoE remain in the shrinking particle, and the remnants are taken up by the remnant receptor in liver within a day, a process facilitated by apoE (Mahley 1988).

Thus a considerable amount of cholesterol from the diet is transported to the liver. It can then be secreted into bile as such, or it can be used for bile acid synthesis whereby it is eliminated from the body. However, part of it is esterified and stored in the liver or redirected into the circulation by VLDL secretion (see endogenous transport).

Transport of dietary lipids is efficient and rapid. After a meal triglyceride levels in plasma rise 2-3 fold and reach a peak at after 3-4 hours. This transient lipemia is mirrored by apoB48 levels, i.e. amount of chylomicrons, and can be exploited as a tool to study the dynamic phase of lipoprotein metabolism after fat intake (see paper I).

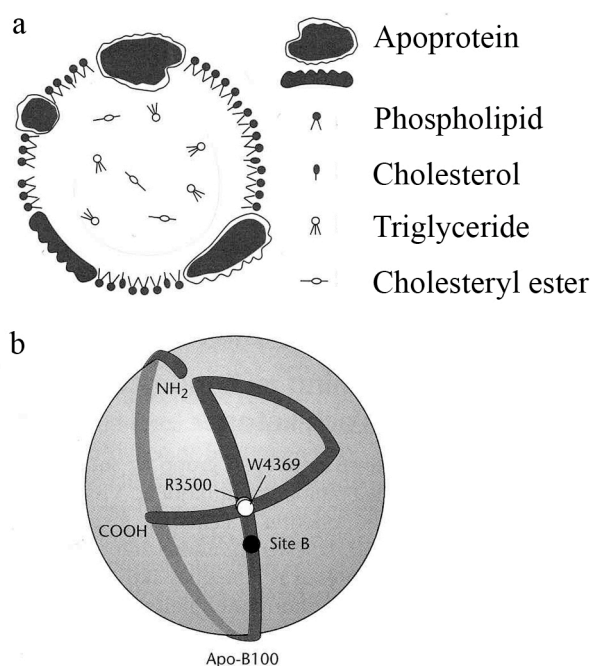


Figure 1

a. Schematic structure of plasma lipoproteins. A core of neutral lipids is surrounded by an amphipathic layer containing apoproteins.

b. A model of LDL with an apoB-100 molecule (showing mutagenic and receptor-binding sites).

Adapted from Nilsson-Ehle 2003.

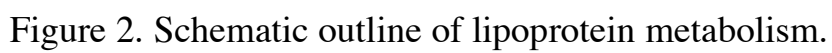
Lipid transport: Endogenous pathway

All cells throughout the body have a constant need for lipids, serving as energy, structural components and for synthesis of biologically active compounds. To meet this demand the liver continuously synthesises and secretes lipoproteins (Figure 2). Hepatic triglycerides, derived by fatty acid mobilisation from adipose tissue or by lipogenesis from carbohydrates, are packaged along with cholesterol, phospholipids, apoA and the liver specific apoB100 into large lipid-rich VLDL particles which contain 60-80% triglyceride (Ginsberg et al. 2005). These are released into the circulation via the space of Disse. In analogy with chylomicron triglycerides, the core triglycerides of VLDL is hydrolysed by LpL in the capillaries, and free fatty acids are locally absorbed and metabolised or stored.

As VLDL circulates, transfer from HDL results in an enrichment of apoCs and apoE. Cholesterol ester-transfer protein (CETP) mediates an exchange of triglycerides (and some phospholipids) for cholesteryl esters (Kane 1996). Presumably, there is also a role for hepatic lipase (HL) in the later phase of VLDL degradation (Santamarina-Fojo et al. 2004). The depletion of triglycerides, and a concomitant re-transfer of apoCs back to HDL, generate smaller VLDL remnants, the intermediate-density lipoproteins (IDL). These are very short-lived lipoproteins that undergo further delipidation, generating even denser remnants, the low-density lipoprotein (LDL). A small amount of IDL is taken up in the liver by the LDL receptor, due to recognition of apoE.

LDL is the dominant lipoprotein class and carries 70% of the cholesterol in plasma. LDL also accumulates cholesteryl esters in exchange for triglycerides from HDL, a process which is facilitated by CETP (Brewer 1996). LDL consists largely of cholesterol (50% as compared to 15% in the initial VLDLs (Ginsberg et al. 2005)). During the lipolytic process, the apoB molecule undergoes conformational changes which expose receptor-binding sites that are not exposed on the lipoprotein surface in the original VLDL (Figure 1b)(Scott 1989).

LDL is removed from the circulation by receptor-mediated endocytosis. About 70% is eliminated by the LDL receptor pathway, a process tightly related to cellular cholesterol homeostasis (Brown and Goldstein 1986). Most LDL receptors reside in the liver (Dietschy et al. 1993), but other cells with requirement for cholesterol, like the adrenals, ovaries and testes also express LDL receptors. Thus cholesterol is mainly excreted in bile (as cholesterol and bile acids) or utilised for steroid hormone synthesis.



A minor portion of LDL is removed from the circulation by less specific receptors, generally referred to as scavenger receptors or SR-BI receptors. In contrast to the LDL receptor, they also bind biologically modified LDL, and uptake via these receptors exerts no feed-back inhibition on intracellular cholesterol synthesis. Thus, they are considered to have a key role in the development of atherosclerotic lesions (see Lipoproteins and cardiovascular disease).

A special class of LDL is lipoprotein (a) (Lp(a)), characterised by the presence of apo(a). This particular apolipoprotein, which demonstrates considerable analogies with plasminogen, is synthesised by the liver. It is covalently associated to the apoB100 moiety of LDL, rendering the lipoprotein unable to be processed by apoB100 pathways (Scott 1989). Lp(a) concentrations are dependent on genetic traits and is an independent cardiovascular risk factor (Dahlen and Stenlund 1997).

Reverse cholesterol transport

The apoB-containing lipoproteins carry lipids from the liver and the intestine to peripheral organs throughout the body. HDL transfers lipids, mainly free and esterified cholesterol, back to the liver for excretion via the bile (Glomset 1968), a process essential in the human body as most mammalian cells readily synthesise, but do not catabolise cholesterol. HDL carries about 25 % of plasma cholesterol and serves as a circulating pool for apoAs, apoCs and apoE. A major function of HDL is to mediate the turnover and metabolism of surface components from VLDL, IDL and chylomicrons (Figure 2) (Brewer 1996).

Nascent HDL, discoid lipid-poor particles containing mainly the surface components phospholipids, cholesterol and apoA-I, are synthesised in liver and intestine (Fielding and Fielding 1995). On secretion into the circulation, the HDL particles turn spherical by the action of lecithin:cholesterol acyltransferase (LCAT), an enzyme that circulates bound to HDL. Cholesterol is esterified and migrates to the core of the HDL particle, generating a small and dense subclass of HDL, HDL₃, (Tall and Breslow 1996). HDL₃ can assimilate more phospholipids and unesterified cholesterol from peripheral cells (and from the chylomicrons and VLDL as their lipolytic process proceeds, see above). By continuous action of LCAT on these surface components, core components are generated resulting in the transition into a larger HDL₂.

HDL now contains cholesteryl esters, which can be exchanged for triglycerides from VLDL and chylomicrons by the action of CETP. The triglycerides in HDL₂ are then hydrolysed by HL, resulting in re-transformation to a lipid-poor, denser HDL₃ particles, which enter the cycle again.

Cholesterol esters in HDL can be selectively taken up by SR-BI receptors in liver and steroidogenic tissues (Moore and Freeman 2006), providing a complementary pathway for cholesterol supply to these organs. HDL circulates for 4-5 days and is eventually removed by receptor-mediated pathways recognising apoA in the liver and kidney (Ajees et al. 2006).

Apolipoprotein B

ApoB is a large glycoprotein with a fundamental role in transport and distribution of dietary and endogenously synthesized lipids. It is critical for the assembly and secretion of lipoproteins, for their intravascular transport and for the receptor-mediated uptake of these lipoproteins into various tissues.

In mammals two forms exist. ApoB100 is the full-length protein of 512 kDa, in humans exclusively synthesised in the liver and present in VLDL. ApoB48 is a shorter 240 kDa protein, a truncated form of B100 synthesised in the human intestine during chylomicron synthesis (Teng et al. 1990; Anant and Davidson 2002). Both apoBs contain several short hydrophobic sequences, capable of interacting with the neutral lipid core, and amphipathic structures that contribute to the lipid binding capacity of apoB (Shelness and Sellers 2001).

The human genome possesses a single *APOB* gene exclusively expressed in liver and intestine (Knott et al. 1985) and there are considerable similarities in the assembly and secretion of all apoB-containing lipoproteins (Hussain 2000; Shelness and Sellers 2001). It has, however, been proposed that the regulatory elements controlling *APOB* gene-expression are different in liver and intestine (Wang et al. 2003).

ApoB100

In the liver apoB is continuously synthesised in excess (Pullinger, et al. 1989). It is shunted to a pathway of lipoprotein assembly when lipids are available and to presecretory degradation when lipid supply is insufficient (Dixon and Ginsberg 1993). This ensures efficient VLDL synthesis when larger amounts of lipids reach the liver, for instance during extensive mobilisation of fatty acids from

adipose tissue. Lipidation occurs in two steps in the cellular endoplasmic reticulum (ER) and is facilitated by microsomal triglyceride transfer protein (MTP); a modest lipidation occurs while the translation of the protein is proceeding to *ApoB48* assure stabilisation (Boren et al. 1992; Spring et al. 1992), followed by maturation as the primordial apoB particle fuses with larger lipid droplets (van Greevenbroek and de Bruin 1998; Shelness and Sellers 2001). The amount of apoB-containing lipoproteins secreted from the liver is mainly regulated by the access to lipids.

ApoB48

Intestinal apoB48 results from RNA-editing, a mechanism that converts a site-specific Cytidine to Uridine, introducing an early stop in the message sequence (Chen et al. 1987; Powell et al. 1987). This C-to-U conversion requires apobec-1, a multicomponent enzyme confined to the small intestine in humans. Editing results in the loss of domains responsible for LDL-receptor binding and apo(a) association (Chen et al. 1987; Young 1990; Anant and Davidson 2001). Synthesis and secretion of apoB in enterocytes proceeds in two steps similar to lipoprotein assembly in hepatocytes; a primordial apoB48 acquires a small amount of lipids and then rapidly fuses with a large triglyceride-rich droplet (Cartwright and Higgins 2001). The requirement for MTP in this process seems to be lower than for apoB100, probably due to the shorter length of apoB48 (Yao et al. 1997). ApoB in the intestine also seems to be synthesised in excess, with little intracellular degradation, providing a pool of apoB48 that is readily shunted towards secretion from the ER upon cellular lipid load (Morel et al. 2004).

Lipoprotein receptors

Lipoproteins circulating in plasma serve to transport lipids to different tissues. The uptake of triglycerides occurs mainly through lipolytic processes on endothelial cells while receptor-mediated processes mediate the uptake of cholesterol. Intracellular cholesterol, especially in its unesterified form, is toxic (Tabas 2002), and its levels are controlled by several mechanisms. These include regulation of synthesis (HMG-CoA reductase), storage (ACAT activity) and influx, the latter regulated mainly by the LDL receptor. The LDL receptor is the only receptor responsive to intracellular cholesterol status.

LDL receptor

Most cells in the body express LDL receptors. The majority is found in the liver, which removes more than half of LDL from the circulation. Its efficiency is due to the clustering of LDL receptors in coated pits on the cell surface and their ability to recycle, thus allowing large amounts of cholesterol to be internalised (Brown and Goldstein 1986). The number of LDL receptors on the cell surface is regulated by a strong feed-back inhibition by the intracellular cholesterol level (Brown and Goldstein 1997). The LDL receptor preferably binds apoB-100, the main protein of VLDL and its remnants IDL and LDL. The receptor-binding region of apoB does not seem to be exposed on the surface of nascent VLDL, but lipoprotein hydrolysis mediates a rearrangement of apoB leading to recognition by the LDL receptor (Knott et al. 1986; Scott 1989). The LDL receptor also recognises apoE when present in several copies in chylomicrons, VLDL, IDL and HDL (Brown and Goldstein 1986; Mahley 1988; Lestavel and Fruchart 1994).

LRP

A larger receptor, structurally similar to the LDL receptor, is the LDL receptor related protein (LRP) (Lestavel and Fruchart 1994). Its main function is to bind lipoprotein remnants, thereby directing cholesterol to the liver and adrenals (Strickland et al. 2002). The LRP is, like the LDL receptor, able to recycle through endosomal compartments, but it is not under control by the cellular cholesterol level (Hussain et al. 1999). The ligands recognised by LRP are numerous, including lipoproteins, bacterial toxins and viruses (Herz and Strickland 2001).

SR-BI

Scavenger receptors are integral membrane proteins with ubiquitous expression. Highest activities are found in cells with requirement for cholesterol, like liver and steroidogenic tissue (adrenal, ovaries and testes) (Moore and Freeman 2006). SR-BI has a specific role in lipoprotein metabolism due to its ability to bind chemically modified forms of LDL (oxidized, glycosylated, acetylated and aggregated) in addition to the native lipoproteins (Acton et al. 1994; Acton et al. 1996; Krieger 1999). Macrophages express SR-BI and thus avidly bind these modified lipoproteins. Since SR-BI is not regulated by intracellular cholesterol levels excessive cholesterol load may result in the transformation of

macrophages into foam cells. This is considered to be an early event in the development of atherosclerotic plaques.

A recent focus of interest is the ability of SR-BI to mediate the exchange of cholesterol between diverse cells and HDL. This process facilitates selective delivery of cholesteryl esters from HDL to liver and steroid hormone producing tissues, thus giving it a role in reverse cholesterol transport (Acton et al. 1996; Moore and Freeman 2006). This selective transfer without lipoprotein uptake appears to be bi-directional (Connelly et al. 2003).

Regulation of lipoprotein metabolism

Lipoproteins transport energy, mainly as triglycerides, and also supply tissues with vital cell elements. Cholesterol is required for the formation and maintenance of cell membranes, but is also a precursor for bile acids, vitamin D and steroid hormones. Most of the cholesterol is synthesized in the body, while dietary cholesterol is a minor component in cholesterol balance. The cholesterol skeleton cannot be degraded in the body, but is processed by liver and steroidogenic tissues (i.e. secretion of unprocessed cholesterol to bile, oxidation to water-soluble oxysterols and bile acids, and steroid hormone production) (Ikonen 2006).

Regulation of lipid metabolism is efficient and integrated with carbohydrate metabolism; through well defined hormone signals energy flux in both fasting and fed state is regulated to ensure efficient channelling of energy to all organs (Nilsson-Ehle 2003).

Short-term regulation of energy fluxes is mediated mainly by insulin, glucagon and catecholamines. Insulin is induced after feeding, thus increasing glucose utilisation and promoting energy storage in adipose tissue by stimulating LpL activity and reducing lipid mobilisation by inhibition of hormone sensitive lipase (HSL) activity. In contrast, glucagons and catecholamines increase glucose mobilisation, and the latter also promote energy mobilisation from adipose tissue by activating HSL.

In a longer perspective, glucocorticoids, growth hormone (GH) and thyroid hormone (TH) also contribute to the regulation of energy metabolism. They all increase glucose availability. Deficiency in GH and TH are well known to induce hyperlipemia, mediated in part by lower activities of LpL and LDL receptors. The glucocorticoids stimulate energy mobilisation from adipose tissue.

ACTH, the hormone studied in this thesis, has only recently been implicated in the regulation of lipoprotein metabolism.

Lipoproteins and cardiovascular disease

In Sweden, cardiovascular diseases (CVD) accounted for 43 % of the deaths in 2004 (Socialstyrelsen 2007).

Hypercholesterolemia, hypertension and smoking are major risk factors for the development of cardiovascular disease (The pooling project 1978). The risk for CVD is largely mediated by LDL and reduction of LDL cholesterol levels is correlated to the regression of atherosclerotic lesions (Brown et al. 1990). Variants of LDL, like Lp(a), small dense LDL and modified LDL (like oxidized and aggregated) seem to be especially atherogenic (Steinberg et al. 1989; Lundstam et al. 2002). HDL, on the other hand, is an inverse risk factor (Gordon et al. 1977), which is attributed to its role in reverse cholesterol transport (see above). Recent data suggest that apoprotein concentrations (A-I and B100), which rather reflect the number of particles, can be better predictors of CVD than concentrations of lipoprotein components such as LDL cholesterol and HDL cholesterol (Maciejko et al. 1983; Kukita et al. 1984).

LDL, especially biologically modified LDL, seem to be involved in the atherosclerotic process by the formation of foam cells, occurring when macrophages in the vascular wall accumulate cholesterol-rich lipoproteins (Stary et al. 1994). Large amounts of lipids can be taken up through the macrophages scavenger receptors, since this pathway is not responsive to the intracellular cholesterol level. This, in turn, stimulates the complex inflammatory reactions which are typical of progressive atherosclerotic lesions.

Statins, LDL-lowering drugs which retard and even reverse the atherosclerotic process, act by upregulating LDL receptors preferentially in the liver, thereby promoting cholesterol excretion from the body and decreasing LDL uptake by the (atherogenic) scavenger pathway.

Disorders of lipoprotein metabolism

Disturbances in lipoprotein metabolism resulting in hyperlipemia and increased risk for CVD are common. They are either hereditary or secondary to clinical diseases or, if less pronounced, to life-style factors.

The most well-known genetic disorder is familial hypercholesterolemia (FH) which is manifested by increased plasma cholesterol levels, most notably in the LDL class. FH is due to mutations in the LDL receptor or in some cases its ligand apoB (Brewer 1996).

Overweight and abdominal obesity frequently result in insulin resistance and diabetes (type II), and are associated with a strong risk of developing CVD (Smith 2007). Like diabetes type I, these conditions display disturbances in both carbohydrate and lipid metabolism. The typical lipoprotein pattern is low HDL levels and elevated triglyceride concentrations, probably due to both higher production and reduced catabolism of VLDL.

Other common disorders inducing secondary hyperlipemia are liver and kidney diseases, which are also frequently associated with low levels of HDL. VLDL and LDL levels are increased, in liver diseases due to LDL receptor malfunction and in the nephrotic syndrome due to increased production.

Hypothyroidism results in increased LDL cholesterol, due to down-regulation of the LDL receptor and elevated VLDL and IDL levels as a consequence of decreased HL activity.

Cushing's disease and Cushing's syndrome are comparatively rare conditions characterised by elevated glucocorticoid concentrations. It is caused by adrenal tumors or by ACTH-producing pituitary adenomas (in which case ACTH is also elevated). The clinical features include hypercholesterolemia, truncal obesity and sometimes also insulin resistance (Yanovski and Cutler 1994; Shibli-Rahhal et al. 2006). As described below observations of the lipoprotein pattern in such patients before and after adrenalectomy provided the first indications that ACTH as such might influence lipoprotein metabolism independently of glucocorticoids.

Successful treatment of secondary hyperlipemia is entirely dependent on treatment of the underlying disease. If treatment does not restore organ functions (e.g. in liver or renal diseases), additional drug treatment is necessary. Needless to say, this is always the case with the genetic disturbances. Today the major hypolipidemic drugs (statins, cholesterol synthesis inhibitors) act on cholesterol synthesis primarily in hepatic cells; the decreased cholesterol synthesis leads to upregulation of LDL receptors and accelerated lipoprotein removal through the LDL receptor pathway. Fibrates, in contrast, influence intracellular lipid metabolism in the liver by multiple mechanisms which lowers VLDL output from the liver. In addition, fibrates have been reported to enhance the intravascular metabolism of VLDL.

ACTH - Adrenocorticotrophic hormone

Origin and function

Synthesised as a cleavage product

ACTH belongs to the melanocortins, peptide hormones derived through a series of proteolytic cleavages from the precursor pro-opiomelanocortin (POMC), see Figure 3 (Yeo et al. 2000; Pritchard et al. 2002). The melanocortins have a broad spectrum of physiological actions including regulation of pigmentation, immunomodulatory effects, and control of the cardiovascular system, as well as regulation of body weight (Smith et al. 1992; Li et al. 1996; Zemel and Shi 2000; Voisey and van Daal 2002).

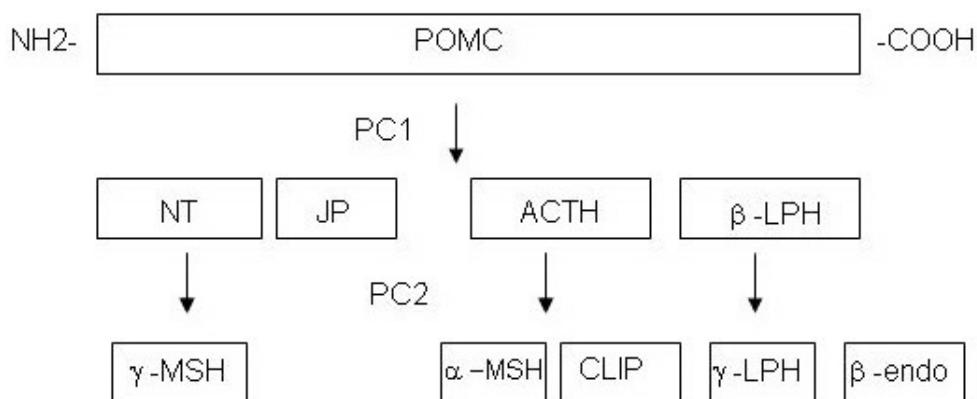


Figure 3. ACTH and various melanocortins derived from POMC by cleavage and post-translational modification by PC1 and PC2 in the pituitary gland.

Abbreviations: POMC, pro-opiomelanocortin precursor; PC, prohormone convertase; NT, N-terminal peptide; JP, joining peptide; LPH, lipotrophin; MSH, melanocyte-stimulating hormone; CLIP, corticotropin-like intermediate peptide; β-endo, β-endorphin.

Reference: Seidah 1999, (Metherell et al. 2006)

The POMC gene is expressed in several tissues, most notably in the pituitary gland, and the tissue-specific peptide release is due to strict control of the prohormone convertase (PC) enzymes (Autelitano et al. 1989; Seidah et al. 1999). Human pituitary POMC-processing by PC1 results in the bioactive peptide ACTH (White and Gibson 1998). However, both PC1 and PC2 are expressed in other tissues and circulating “ACTH-precursors” are cleaved by PC2 in other tissues, yielding ACTH₁₋₁₃, known as α -melanocyte-stimulating hormone (MSH).

The synthesis of POMC, its post-translational modifications, and the secretion of ACTH are under the control of corticotropin-releasing hormone (CRH) synthesized in hypothalamus. Both CRH and ACTH are under the negative feed-back control by circulating glucocorticoids, often referred to as the hypothalamic-pituitary-adrenal (HPA) axis.

Effects on the adrenals

Normal plasma levels of ACTH are 2-10pmol/L. Release from the pituitary is episodic, with highest plasma concentrations at the onset of wakening and the lowest around midnight.

ACTH has its major endocrine function in regulating steroid hormone synthesis in the adrenal cortex, i.e. glucocorticoids, androgens and the short-term secretion of mineralcorticoids. Glucocorticoids are the dominating plasma steroid hormones; cortisol is the most important human glucocorticoid with effects on cardiovascular, metabolic and immunologic functions. It is an important mediator of the stress response of the organism.

Since there is no secretory pool of glucocorticoids a rapid regulation of synthesis is required. Cholesterol for synthesis of glucocorticoids is derived from de novo synthesis, from mobilisation of stored cholesteryl esters in adrenals or from receptor-mediated uptake (Kraemer 2007). Almost 80% of the cholesterol used in steroid synthesis derives from receptor-mediated uptake, mostly representing LDL cholesterol (Borkowski et al. 1967).

Sequence preservation and relevance

ACTH consists of 39 amino acids and is highly conserved with only a few substitutions among most species (Costa et al. 2004). The core peptide, amino

acids 6-9, is shared by ACTH and MSH and is evolutionary preserved, suggesting that it holds the biological activity.

The first 24 amino acids are needed for maximal stimulation of corticosteroid activity (Konig 1993, see Synacthen). Amino acids 15-18 are involved in ACTH receptor binding and amino acids 25-39 confer stability, thus increasing the half-life of ACTH (Costa et al. 2004).

Fragments of ACTH eliciting a cellular response (ACTH₄₋₁₀, ACTH₆₋₉ and ACTH₄₋₁₂) are all part of the α -MSH region of ACTH (Hruby et al. 1987; Sawyer et al. 1990; Hirobe and Abe 2000).

Receptors

The melanocortin (MC) receptor family consists of the ACTH-receptor (MC2) and four MSH-receptors (MC1, MC3 to MC5) (Mountjoy et al. 1992). Each receptor has a characteristic tissue distribution and biological significance. ACTH is recognised by all five melanocortin receptors (Clark and Cammas 1996), but is the only ligand binding to the ACTH receptor (Schioth et al. 1996) due to requirements for a highly basic motif present solely in the ACTH molecule (Elias and Clark 2000), besides the conserved core sequence (Hruby et al. 1987).

ACTH receptor

The ACTH receptor is most abundant in the adrenals, where activation induces steroid synthesis. It is upregulated by its own ligand (i.e. ACTH) resulting in increased transcriptional rate of the receptor and prolonging its mRNA half-life (Beuschlein et al. 2001). There is little evidence of receptor expression in other tissues besides skin and lymphocytes in humans (Yamaoka et al. 1992; Yamamoto et al. 1995; Slominski et al. 1996).

MSH receptors

The MC5 receptor (α -MSH receptor) is the only receptor present in a variety of tissues, such as exocrine glands, skeletal muscle, lung, heart, adipose and kidney, among others (Labbe et al. 1994; Takeuchi and Takahashi 1998; Jacobs et al. 2002), thus identifying this receptor as a candidate mediator of many peripheral melanocortin actions (Labbe et al. 1994). The mRNA sequence of

MC5 receptor is shorter but similar to the sequence of the ACTH receptor (Gantz et al. 1994).

The classical α -MSH receptors, MC1, is mainly expressed in human skin where it affects hair and skin pigmentation (Abdel-Malek et al. 1999; Schaffer and Bologna 2001). It is also suggested to have an immunomodulatory role due to its expression in lymphocytes and dendritic cells (Becher et al. 1999; Neumann Andersen et al. 2001). Recently, the MC1 receptor was identified in the human liver (Gatti et al. 2006), providing a hypothetical link to explain the effects of ACTH on hepatic lipid metabolism

MC3 (γ -MSH) and MC4 receptors (α -MSH) are found mainly in the brain (Gantz et al. 1993; Roselli-Rehfuss et al. 1993) and are thought to play a role in the regulation of body weight and food intake, and also in the autonomic control of the cardiovascular system (Adan and Gispen 1997).

Synacthen

Synacthen, also known as tetracosactid, is a synthetic analogue of the naturally occurring ACTH. It consists of the first 24 amino acids, which are known to harbour all adrenocorticotrophic effects (Konig 1993). Synacthen is most commonly used in diagnostics of adrenal function. It has been used in the treatment of conditions of autoimmune or inflammatory nature. Synacthen also retards the development of neurological diseases such as infantile spasms and multiple sclerosis (Filippini et al. 2000; Brunson et al. 2002)

Introduction to ACTHs lipid-lowering effects

In the 90's an observation in patients with Cushing's disease suggested that not only the peripheral hormones, corticosteroids, but also ACTH may influence lipid metabolism (Berg et al. 1990). It was noted that alterations in lipid metabolism seen in these patients were not altogether reversed by adrenalectomy. This promoted a series of studies, both in healthy subjects and patients with renal disease, on the actions of ACTH. A direct effect on lipid metabolism, independent of glucocorticoid action, was confirmed.

Lipids and Lipoproteins

Plasma cholesterol levels are markedly and consistently decreased by 20-40% by ACTH administration. Often, plasma triglycerides are decreased, especially in persons with high initial levels (Berg and Nilsson-Ehle 1994; Berg and Nilsson-Ehle 1996; Arnadottir et al. 1997; Berg et al. 1999; Arnadottir et al. 2001; Hardarson et al. 2001). The reduction in plasma cholesterol is accounted for by a reduction in LDL and VLDL levels, and is seen after only a few days' of ACTH administration. These effects cannot be reproduced by equipotent doses of glucocorticoids.

HDL cholesterol is moderately increased (Berg and Nilsson-Ehle 1994; Berg and Nilsson-Ehle 1996; Berg et al. 1999) or sometimes unaffected by ACTH administration (Arnadottir et al. 1997; Arnadottir et al. 2001; Hardarson et al. 2001). The changes in HDL cholesterol are generally mirrored by plasma apoA-I levels. ACTH primarily affects HDL₂, which increases by 10-20%. Part of these changes (in HDL cholesterol) seem to be mediated by glucocorticoids (Berg and Nilsson-Ehle 1994).

Postulated mechanisms behind the effects of ACTH

The hypolipidemic effect of ACTH includes both VLDL and LDL, i.e. the apoB-containing lipoproteins, and is mirrored by reductions in plasma apoB by 20-45% (Berg and Nilsson-Ehle 1994; Berg and Nilsson-Ehle 1996; Arnadottir et al. 1997; Berg et al. 1999; Arnadottir et al. 2001). In vitro, in hepatic cell cultures, ACTH reduced the levels of apoB mRNA as well as apoB secretion by 40% (Xu et al. 2001). These data strongly suggests that ACTH interferes with the production and/or secretion of apoB, thereby lowering lipoprotein levels.

This interpretation is supported by the fact that Lp(a), a modification of LDL, is also reduced in response to ACTH administration, possibly secondary to the decreased VLDL and LDL formation (Berg and Nilsson-Ehle 1994; Berg and Nilsson-Ehle 1996; Arnadottir et al. 1997; Arnadottir et al. 1999; Hardarson et al. 2001). However glucocorticoids had a similar effect on Lp(a) demonstrating that other mechanisms are also involved.

The possibility that the prominent reduction in LDL cholesterol would be explained by increased clearance of LDL has been explored. ACTH is known to up-regulate the LDL receptor in adrenals in humans (Liu et al. 2000), and in an early study, support was found for an increased receptor-specific uptake and degradation of LDL particles in hepatic cell cultures (Berg and Nilsson-Ehle 1994; Bartens et al. 1997). However, glucocorticoids were also capable of eliciting LDL receptor response. A more recent study, with a different approach, displayed no change in LDL receptor activity nor LDL receptor mRNA (as well as SR-BI) levels in hepatic cell cultures in response to ACTH treatment (Xu et al. 2001). Thus, clearance of LDL by the receptors is unlikely to be a major mechanism behind the lipid-lowering actions of ACTH.

We have also reported that ACTH administration leads to an enrichment of VLDL with apoE (Arnadottir et al. 2001), which might conceivably increase the rate of removal of apoB-containing lipoproteins due to the high affinity of apoE for lipoprotein receptors, i.e. without upregulation of the receptors. This possible mechanism of action has not been fully explored.

Aims of the Studies

The focus of my work has been to gain further insights into the mechanisms behind the well established hypolipidemic effect of ACTH seen in man. More specifically, the following questions were addressed:

- The prominent reduction in plasma lipoproteins during ACTH administration has tentatively been ascribed to reduced synthesis and secretion of lipoproteins, as seen in hepatic cell cultures. Is reduced synthesis and secretion of apoB a mechanism of action of ACTH in man?
- Plasma lipids, in particular those associated with apoB-containing lipoproteins, are also reduced after ACTH administration. Is this secondary to alterations in apoB processing, or does ACTH also affect synthesis and/or secretion of lipoprotein lipids, i.e. cholesterol and triglycerides?
- To investigate molecular mechanisms in detail, animal models need to be explored. Are the rat and/or rabbit suitable experimental models to study the effects of ACTH?

Present Investigation

Effects of ACTH in man

Effects of ACTH on postprandial output of chylomicrons (Paper I)

Intestinal apoB production as a tool to study effects of ACTH in man

The mild lipemia occurring after fat intake is largely accounted for by intestinal chylomicron production, seen as a transient elevation in plasma apoB48 concentrations. The production, assembly and secretion of intestinally derived apoB-containing lipoproteins can be used as a representative model of the hepatic lipoprotein production, since the synthesis and secretory pathways share several characteristics as outlined above. However, the postprandial phase is complex. While the early phase mainly reflect lipoprotein production and secretion, the later phase is also influenced by intravascular metabolism and elimination of lipoprotein particles.

Methods

The postprandial rise in chylomicrons was studied in 10 healthy men and women after four days administration of ACTH and glucocorticoids. Intestinal secretion of lipoproteins was monitored as triglyceride, retinyl palmitate and apoB48 concentrations in plasma. Retinyl palmitate, a marker for chylomicron and chylomicron remnant turnover (Berr and Kern 1984; Bitzen et al. 1994), was analysed by high performance liquid chromatography and apoB48 by a competitive ELISA (Lovegrove et al. 1996).

ApoB and lipids in the fasted state

In analogy with earlier studies, plasma apoB, exclusively representing apoB100, was reduced by ACTH but was unaffected by glucocorticoids. However, plasma apoB48 levels were unexpectedly elevated after ACTH administration (from 0,13 to 0,19mg/L); this was also the case after glucocorticoid administration (to 0,20mg/L). This finding may reflect a retention of remnants in the circulation, but needs further investigation.

This study also demonstrated that phospholipids, as expected, were reduced by ACTH which reflects the reduced VLDL/LDL levels.

ApoB after fat intake

After fat intake the enterocytes rapidly secrete lipid-rich chylomicrons, as illustrated by a 3-fold elevation in plasma apoB48 concentrations. The rise of apoB48 during the early postprandial phase (0-4 hours) was reduced by almost 85% after ACTH administration and to a smaller extent after glucocorticoids (65%). In the later postprandial phase, after 4-6 hours, a second peak in plasma apoB48 was seen c.f. (Silva et al. 2005). It may be a consequence of the “saturable mode” of remnant catabolism by the liver seen already after the intake of 70g fat (Berr 1992).

Plasma lipids after fat intake

Despite the reduction in apoB plasma concentrations during the postprandial phase, the lipemic response (i.e. triglyceride and retinyl palmitate levels) increased slightly after ACTH administration as well as after glucocorticoid administration. Also, plasma lipid concentrations tended to peak earlier after both hormone administrations. This indicates that the absorption, and also secretion, was accelerated, which may be a consequence of the known effects of stress hormones on the gastrointestinal (GI) motor complex (Fukudo et al. 1998; Monnikes et al. 2001).

Retinyl palmitate is associated with the core of the chylomicron until clearance of the lipoprotein (Berr and Kern 1984). The comparatively slow turnover of retinyl palmitate in the present study (cf. Bitzen et al. 1994) was probably due to the composition of the meal (fat, carbohydrates and proteins).

Conclusions

The number of chylomicrons (i.e. apoB48 levels) in plasma after fat intake was essentially constant after ACTH administration, strongly suggesting that ACTH administration leads to a reduced apoB synthesis and/or secretion in man. Unexpectedly, glucocorticoids were able to partly mimic the effects, which might indicate that the effects of ACTH differ between hepatocytes and enterocytes. This requires further investigation.

Lipid transport, however, was unimpaired after ACTH administration. Taken together these data strongly suggest that the effects of ACTH on enterocytes result in the formation of fewer, but larger, more lipid-rich chylomicrons.

Thus ACTH seems to selectively decrease apoB production without influencing lipid processing in the enterocytes. These results are consistent with the hypothesis from in vitro studies (HepG2 cells) that ACTH acts primarily by down-regulation of apoB synthesis.

Effects of ACTH on lipid processing *in vitro* (Paper II)

HepG2 cells

To further document the lack of effects of ACTH on synthesis and processing of lipids, HepG2 cell cultures were employed. These cells are of human origin and are well established experimental models for studies of lipoprotein metabolism, since they retain many liver-specific metabolic functions including synthesis and secretion of lipoproteins (Wang et al. 1988; Javitt 1990). Lipid synthesis and secretion were assessed in subconfluent cultures using labelled precursors, [¹⁴C]-acetate and [³H]-glycerol. Cellular and secreted lipids were extracted with chloroform/methanol, separated by thin layer chromatography and quantitated by radioactivity measurements of the respective fractions.

Lipid synthesis and secretion

Hepatic cells prelabelled with precursors for lipid synthesis did not demonstrate any change in secretion of cholesterol, cholesteryl ester, triglycerides and phospholipids when incubated with ACTH for 24 hours. Insulin, used as a positive control, lowered the secretion of lipids by about one third as expected.

Similar results were obtained using a continuous labelling during exposure to ACTH, indicating that decreased synthesis of lipids was also unaffected by ACTH.

Lipids were assessed at time points and under conditions where apoB synthesis and secretion is inhibited by ACTH (Xu et al. 2001). Effects of ACTH on apoB were more pronounced in the presence of oleic acid which stimulates both apoB secretion and lipid synthesis (Dashti and Wolfbauer 1987; Pullinger et al. 1989; Moberly et al. 1990; Dixon et al. 1991; Xu et al. 2001). However, synthesis and secretion of lipids were unaffected by ACTH in the presence as well as in the absence of oleic acid.

Conclusions

ACTH did not affect lipid synthesis or secretion in cultured hepatic cells, although it has been demonstrated that it reduces apoB output. This supports the view that ACTH inhibits apoB output specifically, and not as a consequence of altered lipid availability.

That ACTH does not influence lipid metabolism in the hepatocyte is in line with the results obtained from the postprandial study in man. A logical inference from our data in HepG2 cell cultures is that ACTH has similar effects in hepatic cells as in enterocytes, i.e. promotes the production of fewer but larger lipoprotein particles. Furthermore, preliminary result in undifferentiated CaCo2cells (enterocytes), which mainly produce apoB100, indicate that ACTH does not affect lipid synthesis, but reduces apoB expression also in these cells.

ACTH in animal models

A better understanding of the mechanisms behind the hypolipidemic effects of ACTH requires additional experimental models. Therefore two such models, the rat and the rabbit, were explored.

Effects of ACTH in the rat (Paper III)

Design

Plasma lipid concentrations and tissue distribution of ^{125}I Human-LDL was assessed in Sprague-Dawley rats after three days' treatment with ACTH.

Effects of ACTH in the rat

In contrast to our data from patients and healthy volunteers, plasma LDL cholesterol levels increased after ACTH administration in the rat. The increase in LDL cholesterol could be explained by a slightly retarded LDL clearance after ACTH administration i.e. the ^{125}I -LDL retained in the circulation was significantly increased. ACTH did not change the distribution of LDL uptake in different tissues.

Conclusions

The observed differences in LDL metabolism between rat and man makes the rat unsuitable as a model for studies of the mechanisms of ACTH on lipid transport. The discrepancy may be explained by basic species differences. For example, the predominant lipoprotein in rat is HDL, while LDL concentrations are considerable lower than in man.

Effects of ACTH in the rabbit (Paper IV)

The rabbit as an experimental model

The New Zealand White (NZW) rabbit is a well characterised animal and frequently used for studies on lipid metabolism. The predominant lipoprotein class is LDL. In contrast to the rat, the rabbit does not display editing of apoB mRNA in the liver. However plasma lipid levels are low, as are HL and CETP

activities; they all increase considerably upon cholesterol feeding (Warren et al. 1991).

Design

NZW rabbits fed a high fat/cholesterol diet displayed hyperlipidemia with elevated serum levels of total cholesterol, LDL cholesterol, HDL cholesterol, apoB, apoA-I, Lp(a) and slightly elevated triglycerides. ACTH was administered on 6 consecutive days resulting in a rise in plasma cortisol (396 $\pm$ 221 nmol/L to 463 $\pm$ 101).

Hypolipidemic effect of ACTH

ACTH administration significantly decreased serum levels of total cholesterol, LDL cholesterol, HDL cholesterol, apoB and Lp(a) in the hyperlipidemic rabbits. Serum levels of apoA-I and triglycerides, in contrast, increased slightly. Thus the effects are similar to those recorded in man.

Preliminary data strongly indicate that ACTH reduces the hepatic expression of apoB in such rabbits, corroborating the mechanism suggested in our earlier experimental models. Interestingly, hepatic expression of the remnant receptor LRP was also reduced. Possibly, such a mechanism could explain the elevated chylomicron levels seen after ACTH administration in man (Paper I).

Conclusions

Taken together, these data indicate that diet-induced hyperlipemia in the rabbit is a representative model of human lipoprotein metabolism and its regulation by ACTH, especially with regard to the metabolism of apoB-containing lipoproteins. The effects of ACTH recorded in this model lend support to our hypothesis that ACTH acts by reducing apoB synthesis in vivo.

Concluding remarks and Perspectives

Contribution of present thesis

The ability of ACTH to lower plasma LDL cholesterol is an interesting concept for future clinical applications. Since ACTH affects synthesis rather than elimination of lipoproteins, it may provide an interesting complement to lipid-lowering by statins. However, this requires that the mechanisms of ACTH are defined.

This thesis demonstrated that reduced apoB synthesis, as seen in hepatic cell cultures, is also operative in a representative *in vivo* model and also in man, using intestinal lipoprotein production as a substitute for hepatic metabolism.

The present studies also indicate that the effects of ACTH on the intestine, and also probably the liver, result in the production of fewer but larger lipoproteins. This is interesting since evidence is accumulating that lipoprotein atherogenicity is related to the number of particles (i.e. apoB levels) rather than to the molar concentrations of lipoprotein components (i.e. LDL cholesterol).

For future more detailed studies on mechanisms we have identified a representative *in vivo* model, the diet-induced hyperlipidemia in the rabbit.

Future areas of interest

The implication that ACTH induces the secretion of fewer but larger lipoproteins, probably from both hepatic and intestinal cells, is interesting since it would imply that the pattern of lipoprotein alterations induced by ACTH are indeed anti-atherogenic. A more detailed study on lipoprotein size and composition after ACTH treatment has been initiated. The possibility to study the effects of ACTH in apoE deficient mice, which develop atherosclerotic lesions rapidly, would be of interest in this respect. Such a model would also be interesting since it would give clues as to the possible involvement of apoE concentrations in the effects of ACTH (Arnadottir et al. 2001).

Preliminary results suggest that shorter fragments of ACTH, notably ACTH₄₋₁₀ harbour the lipid-lowering effects. Interestingly, this peptide does not elicit adrenocorticotrophic effects in man (Fehm et al. 2001). This opens the possibility not only to separate the lipid-lowering effects of ACTH from its steroidogenic actions, but also to provide a simpler means of administration.

Synacthen requires intramuscular administration, while shorter fragments are absorbed through mucosal membranes.

The rabbit provides an *in vivo* model to study in detail hepatic expression of genes involved in lipid metabolism, such as receptors, apoproteins and enzymes involved in lipid synthesis. Another line of future investigations is to identify and characterize a so far hypothetical receptor for ACTH actions in the liver and intestine.

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Populärvetenskaplig sammanfattning

Introduktion

Adrenokortikotropiskt hormon (ACTH) stimulerar frisättningen av steroidhormoner, främst kortisol, från binjurarna. Kortisol är livsnödvändigt och finns alltid i blodet och förhöjs kraftigt vid stress. I början av 90-talet fann vår forskningsgrupp en koppling mellan ACTH och fettomsättningen som inte involverade steroidhormonerna. Detta blev startskottet för ett flertal kliniska studier som visade att ACTH som ges till friska personer och patienter med njursjukdomar kraftigt sänker blodfetthalterna, fr a kolesterol och triglycerider.

Då fetter är svårlösliga i vatten transporteras de som partiklar (lipoproteiner) tillsammans med olika proteiner (äggviteämnen) i blodet. Kolesterolssänkningen motsvarades av en sänkning av lipoproteiner av typen LDL ("det onda kolesterolet"), som är förknippat med snabb utveckling av åderförkalkning och hjärt-kärlsjukdomar. Däremot ökar lipoproteinerna av HDL-typ ("det goda kolesterolet") som förebygger hjärt-kärlsjukdom.

Det viktigaste proteinet i LDL är apoB, och det visade sig att alla lipoproteiner som innehåller apoB sjönk i blodet som en effekt av ACTH. Från cellodlingsförsök drog man slutsatsen att den viktigaste mekanismen bakom ACTHs blodfettsänkande effekt var att bromsa syntesen av just apoB.

Avhandlingens mål

Avhandlingen fokuserar på två delfrågeställningar. Den första är att vidare utröna de mekanismer som ligger bakom ACTHs blodfettsänkande effekter, framför allt att verifiera att minskad syntes av apoB (och därmed en minskad produktion av lipoproteiner) är en viktig mekanism också hos människan. Det andra målet är att hitta en representativ djurmodell som kan förenkla det framtida arbetet kring ACTHs mekanismer.

Mekanismer bakom ACTH's blodfettsänkande effekter

Vi följde sekretionen av apoB-innehållande lipoproteiner från tarmen, som kan ses i blodet som en övergående ökning av apoB-48 och triglycerider. Tarmens lipoproteinsyntes användes här som en modell för lipoproteinsyntesen i levern, eftersom de aktuella processerna i dessa organ har stora likheter. Vi fann att behandling med ACTH minskade utsöndringen av antalet lipoproteinpartiklar

från tarmen, vilket stämmer väl överens med hypotesen att ACTH primärt påverkar syntesen av apoB.

ApoB-utsöndringen skulle teoretiskt kunna bero på att ACTH minskar fettsyntesen i tarm- och leverceller, d v s att tillgången på fett i dessa celler är avgörande för lipoproteinbildningen. Våra resultat stöder inte denna möjlighet, eftersom triglyceriderna insöndrades till blodet i lika stor mängd före och efter ACTH behandling. Inte heller i odlade leverceller påverkade ACTH syntesen eller utsöndring av fetter. Det tycks alltså som att ACTH-behandling leder till att lever och tarm utsöndrar färre, men större (och mera fettrika) lipoproteinpartiklar.

Ur klinisk synpunkt är fynden intressanta eftersom blodfetternas kärlskadande effekt tycks vara mera knuten till antalet lipoproteinpartiklar (LDL) än till koncentrationerna av blodfetter (kolesterolvärdet, mätning av det ”onda” och ”goda kolesterolet”).

ACTHs effekter i två djurmodeller

Råttor och möss är de vanligaste försöksdjuren, även om det är väl känt att deras fettomsättning i vissa avseenden skiljer sig från människans. Hos råttan visade det sig att ACTH snarast höjde blodfetthalterna och att de effekter vi noterat hos människa inte kunde reproduceras. Vi drar slutsatsen att råttan inte är en användbar försöksmodell för att studera mekanismerna bakom ACTHs blodfettsänkande effekter.

Normalt har kaniner låga halter av blodfetter, men lipoproteinkoncentrationerna i blodet ökar kraftigt om de tillförs en fettrik diet. Hos sådana kaniner med kost-inducerad hyperlipemi minskade blodfetthalterna, fr a LDL kolesterol och apoB under ACTH behandling. Detta tyder på en minskad produktion av lipoproteiner och motsvarar den effekt vi ser av ACTH hos människan. Denna försöksmodell verkar alltså lämplig för vidare detaljerade studier av ACTHs blodfettsänkande verkan.

Slutsatser och utvecklingslinjer

Att ACTH minskar syntesen av apoB och därmed sänker koncentrationen av lipoproteiner som innehåller apoB är av kliniskt intresse. Dagens mediciner, som statiner och fibrater verkar främst genom att eliminera kolesterol från blodet, och/eller genom att påverka synteshastigheten av fett fr a i levern. ACTH

eller derivat av ACTH, som har en annan verkningsmekanism, kan vara attraktiva komplement eller alternativ till nuvarande behandlingar. I pågående studier försöker vi identifiera det aktiva segmentet i ACTH-molekylen för att ev kunna utnyttja fragment eller sekvenser i ACTH, som kan administreras på enklare sätt är nu tillgängliga preparationer.

Ytterligare frågeställningar av intresse är t ex vilken/vilka receptorer i lever och tarm som medierar ACTHs effekter. Detta kan undersökas i djurexperimentella modeller, t ex kaninen.

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