CORTISOL, ACTH, AND BIOSYNTHESIS OF APOLIPOPROTEINS IN RAT HEPATOCYTES

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The effect of cortisol (5 mg/kg, 5 and 10 days) on biosynthesis of apoproteins of very low density lipoproteins in the liver and on synthesis of apoproteins of very low, low, and high density lipoproteins in blood serum of intact animals was investigated *in vivo*. Cortisol, within the periods specified, inhibits biosynthesis of apoproteins of very low density lipoproteins (apo-VLDL) in liver. After adrenalectomy apo-VLDL synthesis is intensified and this effect is abolished during replacement administration of cortisol. Apoprotein synthesis is activated 5 h after a single injection of cortisol and ACTH; a single dose and prolonged administration of cortisol give opposite results. Investigation of the specific radioactivity of apolipoproteins in the blood serum indicates a change in lipoprotein metabolism: disturbance of conversion of very low density into low density lipoproteins. An important role in the pathogenesis of the hyperlipidemia induced by cortisol within the specified period is played not by increased lipoprotein synthesis in the liver, but by a disturbance of their metabolism in the blood.
KEY WORDS: *cortisol; ACTH; apolipoproteins; rat liver and blood.*

Lipoproteins synthesized in the liver circulate in the blood plasma in the form of very low density lipoproteins (VLDL) and high density lipoproteins (HDL). Under the influence of triacylglycerohydrolase, hydrolysis of triglycerides of VLDL takes place in the blood and low density lipoproteins (LDL) are formed [6]. Besides neutral lipids and phospholipids, the lipoproteins also contain specific transport proteins, or apoproteins (apolipoproteins, apo-LP) [13]. According to Alaupovic's classification [2], apo-LP can be divided into three classes: apo-A, apo-B, and apo-C. Later investigations showed the heterogeneity of apo-LP isolated from individual classes of lipoproteins. Apoproteins were separated into subclasses, represented by polypeptide chains: apo-A_I and II, apo-$C_{I,II}$, and III [13]. This classification likewise does not reflect the heterogeneity of apo-LP: More than 20 components have been discovered by the method of isoelectric focusing in polyacrylamide gel in apo-VLDL alone.

Apo-B is the basic protein of all LDL, but its content varies: in chylomicrons it accounts for 20%, in apo-VLDL for 40% and in apo-LDL for 95% of the protein. Apo-A is the basic protein of HDL (90% of the protein in HDL). Apo-C is a component of VLDL and LDL: In apo-VLDL it accounts for 50%, and in apo-HDL for 10% of the protein. Activation of post-heparin triacylglycerohydrolase and intensification of hydrolysis of the triglycerides of VLDL lead to displacement of apo-C from VLDL and HDL [4]. In the process of translocation of apo-C from VLDL into HDL, apo-B remains the basic protein of the newly formed HDL. The most closely studied function of apo-LP is their interaction with lipids, by means of which lipids are able to "dissolve" in the extracellular fluid and to be transported with the lowest expenditure of energy. During sonication of a mixture of apo-HDL and phospholipids complexes similar in structure and lipid composition to native HDL are formed [11].

There are no data in the literature on the effect of glucocorticoids on the synthesis of lipoproteins and apoproteins. The object of the present investigation was to study the synthesis of apoproteins of individual classes of lipoproteins in the rat liver under the influence of cortisol and ACTH.

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TABLE 1. Biosynthesis of Apo-VLDL in Rat Hepatocytes and Specific Radioactivity of Apoproteins of Rat Blood Serum under Different Experimental Conditions (in counts/mg protein/min; M ± m, n = 15)

Index	Control	Cortisol	Adrenalectomy	Adrenalectomy + cortisol
Apo-VLDL:				
liver tissue	7 367±302	4 619±194 $P<0,01$	11 482±292	6 121±201 $P<0.01$
blood serum	17 500±371	13 843±251	19 008±241	18 250±301
Apo-LDL:				
blood serum	14 090±284	4 301±108	17 188±139	16 533±229
Apo-HDL:				
blood serum	14 964±327	10 533±134	20 088±292 $P<0,05$	12 783±254 $P<0.01$

EXPERIMENTAL METHOD

Experiments were carried out on 90 male Wistar albino rats weighing 180-200 g. The animals were kept on a fat-free diet. In the experiments of series I hydrocortisone (from Gedeon Richter, Hungary) was injected into the animals of group 1 in a dose of 5 mg/kg daily for 5 days. The animals of the control group received injections of the same volume of 0.15 M NaCl. Animals of group 2 received the identical dose of cortisol for 10 days. The rats of group 3 were given a single injection of hydrocortisone and decapitated 5 h later. The animals of group 4 received a single injection of 50 µg ACTH (Synacten, from Ciba, Switzerland) and also were decapitated after 5 h. In the experiments of series II, total adrenalectomy was performed on 30 animals under ether anesthesia. The animals took part in the experiment 2 weeks after the operation. From the 11th day after the operation 15 rats received an injection of hydrocortisone (5 mg/kg). Instead of water, the remaining animals received 0.15 M NaCl.

In all the experiments, the animals were deprived of food for 12-14 h and then 2 h before sacrifice received an intraperitoneal injection of 3,4-leucine-^{3}H in a dose of 10 µCi/100 g body weight. The animals were decapitated, blood was collected, and pieces of liver were washed with cold 0.15 M NaCl, and dried. To obtain VLDL from rat liver the tissue was homogenized in 10 volumes of medium with a density of 1.006 g/ml (0.196 M NaCl + 100 mg EDTA), followed by centrifugation on a Spinco L-5-65 ultracentrifuge (40 rotor, 39,000 rpm, 15°C, 18 h). VLDL, LDL, and HDL were isolated from blood serum by successive ultracentrifugation in media of densities of 1.006, 1.063, and 1.210 g/ml on the same centrifuge, but using the Ti-65 rotor [9]. The density of the buffer was verified against readings of the IRF-22 refractometer. The isolated VLDL were recentrifuged in medium of the same density. To obtain apoproteins the lipoproteins were delipidized with a cold mixture of ethanol and ethyl ether (3:1) [12]. The precipitated apoproteins were isolated by ultracentrifugation and washed with 5% TCA containing unlabeled leucine in a concentration of 1 mg/ml. Protein was dissolved in 0.1 N KOH and the protein concentration was determined by Lowry's method [10]. Completeness of delipidization was verified by testing for lipids in a solution of alkali by the sulfovanillin test and investigation of triglycerides by an enzymic method. Samples of protein were counted on a liquid scintillation spectrometer after the addition of Tritizol scintillator [3]. The results were subjected to statistical analysis by means of Student's t-test.

EXPERIMENTAL RESULTS

In experiments *in vivo* cortisol inhibited the synthesis of apo-VLDL (Table 1). After adrenalectomy synthesis of apoprotein was considerably intensified. The activation effect was abolished during replacement therapy with hydrocortisone. Consequently, the inhibitory action of cortisol on apo-VLDL biosynthesis is manifested both in intact animals and after adrenalectomy. The data given in Table 1 reflect the effect of cortisol when administred for 5 days. Similar results were obtained also when cortisol was given in a longer period (10 days). The inhibitory effect of cortisol on the specific radioactivity of apo-VLDL also was observed in blood serum. The effect of replacement therapy after adrenalectomy on the specific radioactivity of apo-VLDL was weaker. Similar results also were obtained for the second apoprotein, namely apo-HDL, the specific radioactivity of which was lowered. Replacement therapy after adrenalectomy had the same action. Cortisol *in vivo* thus inhibits

TABLE 2. Incorporation of Leucine-^{3}H into Apo-VLDL of Rat Hepatocytes and into Blood Serum Apoproteins under Different Experimental Conditions (in counts/mg protein/min; M ± m; n = 10)

Index	Control	Adrenalectomy	Adrenalectomy + cortisol
Apo-VLDL:			
liver tissue	1948±65	3364±138 $P<0,05$	3465±162 $P<0,05$
blood serum	750±59	1625±99 $P<0,05$	2155±101 $P<0,05$
Apo-LDL:			
blood serum	3210±109	2516±95	3223±122
Apo-HDL:			
blood serum	541±32	474±24	509±19

the synthesis of apoproteins of lipoproteins. Injection of cortisol into intact animals led to a marked decrease in specific radioactivity of apo-LDL in the blood serum. This decrease was greater than the decrease in the synthesis of apo-VLDL, which also include apo-LDL, in the liver. This difference can be explained by the inhibitory effect of glucocorticoids on the conversion of VLDL into LDL, when apo-B remains as a component of VLDL and LDL formation is reduced. This may be connected with the inhibitory effect of cortisol on post-heparin triacylglycerohydrolase activity. During longer administration of cortisol the same results also were obtained.

In experiments *in vivo* 5 h after a single injection of cortisol the synthesis of apo-VLDL was activated (Table 2). A single injection of ACTH had the same effect. Cortisol and ACTH increased the specific radioactivity of apo-VLDL in the blood serum; no increase in apo-HDL biosynthesis was observed at this time. In such a short period cortisol and ACTH did not disturb the conversion of the VLDL into LDL and the specific radioactivity of apo-LDL was the same in the experimental and control groups of animals.

In experiments *in vivo* cortisol thus significantly inhibits the synthesis of apo-VLDL in the liver. Cortisol does not disturb the secretion of synthesized VLDL into the blood stream but it inhibits the conversion of VLDL into LDL. Synthesis on apo-HDL is also inhibited by cortisol. Considering the role of apo-LP in the formation of lipoproteins, it can be tentatively suggested that cortisol has an inhibitory effect on lipoprotein synthesis

Information on the effect of glucocorticoids on protein synthesis in the liver is contradictory. A single injection of cortisol into experimental animals increases the synthesis of plasma proteins, fibrinogen, and transferrin [10]. In pharmacological doses, however, glucocorticoids depress the accumulation of protein in the liver. Inhibition of protein biosynthesis in experiments *in vivo* is preceded by a short period of stimulation of protein synthesis. Repeated injections of cortisol inhibit protein synthesis more strongly [7]. After adrenalectomy protein synthesis in the liver is more than doubled in intensity, whereas a single dose of cortisol inhibits it [5]. Kyner et al. [8] observed a hypolipidemic effect of glucocorticoids and attributed it to inhibition of apo-LP biosynthesis. The main glucocorticoid of rats is corticosterone, but the plasma membranes of the rat liver can bind both corticosterone and cortisol specifically [14].

The mechanism of the inhibitory effect of cortisol on protein synthesis is connected with changes in the properties of the membranes of the subcellular formations. Cortisol inhibits protein synthesis of polysomes, but the effect is exhibited only on polysomes fixed on membranes [5]. The inhibitory action of glucocorticoids on the incorporation of labeled precursors into polypeptide chains is not exhibited in the soluble fraction of hepatocytes [7].

The writers previously demonstrated the effect of glucocorticoids on lipoprotein metabolism, namely the development of hyperlipoproteinemia [1]. A study of the lipid composition of VLDL and LDL showed a disturbance of metabolism and accumulation of VLDL in the blood. In the present investigation this fact was confirmed by a study of the degree of metabolism of apoproteins. The pathogenesis of the hyperlipoproteinemia in these experiments, it can tentatively be suggested, was due not to increased synthesis of VLDL in the liver but to a disturbance of lipoprotein metabolism.

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EFFECT OF ADRENALIN, HYDROCORTISONE, INSULIN, AND DIBUTYRYL-CYCLIC AMP ON GLYCOLYSIS AND GLYCOGENOLYSIS IN SURVIVING LIVER SPLICES FROM ALBINO RATS

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Adrenalin, hydrocortisone, and dibutyryl-cyclic AMP inhibited glycolysis and glycogenolysis in surviving liver slices from albino rats. An inhibitory effect also was found when glucose-6-phosphate (G6P), but not fructose-1,6-diphosphate, was used as the substrate for glycolysis. This indicates that activity of hexokinase and also, probably, of phosphorylase and phosphofructokinase, is inhibited under the influence of these hormones and dibutyryl-cyclic AMP. In a reconstituted cell-free system the hormone had no effect and dibutyryl-cyclic AMP inhibited hexokinase only. For the hormones to exert their effect interaction between them and the cell membrane was essential. Inhibition of glycogen and G6P breakdown to lactic acid in liver slices is not connected with the action of the hormones on the corresponding enzymes (phosphorylase and phosphofructokinase) directly through cyclic AMP and protein kinase. The results are evidence of an additional mechanism modifying the action of cyclic AMP on the activity of the above-mentioned enzymes. Insulin had no effect in all cases.
KEY WORDS: *adrenalin; hydrocortisone; insulin; cyclic AMP; glycolysis; glycogenolysis.*

Considerable progress has recently been made in the study of the hormonal regulation of metabolism. The mechanism of regulation of enzyme activity by the phosphorylation–dephosphorylation principle has been discovered [8]. The molecular mechanisms of action of adrenalin and glucagon on tissue phosphorylase activity have been elucidated [11, 13]. The important role of cyclic nucleotides as intermediate mediators of hormone action has been demonstrated. However, several fundamental problems still remain unexplained. This applies in particular to the mechanism of action of glucocorticoids and insulin on the state of glycolysis and glycogenolysis. It has been shown, for example, that hormones participate in the suppression and induction of hexokinase synthesis respectively in the liver [1, 14], whereas their role in the allosteric regulation of the key enzymes of anaerobic carbohydrate breakdown is not clear. It is not known whether penetration of glucocorticoids into the cell

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