WAYS OF INHIBITION OF CHOLESTEROL BIOSYNTHESIS BY CORTISOL IN THE RAT LIVER

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The mechanisms of interaction of glucocorticoids with the plasma membranes, reducing the permeability of the latter, have not yet been explained. A direct action of glucocorticoids on membranes is possible. In experiments with liposomes, for example, cortisol reduces their permeability [10]. Cortisol and its synthetic analogs stabilize membranes in experiments *in vivo* also [12]. The possibility cannot be ruled out that the action of glucocorticoids *in vivo* may be linked to a certain degree with changes in biosynthesis of cholesterol and phospholipids — the lipid components of membranes.

Data on the action of cortisol on cholesterol biosynthesis are few in number and contradictory in nature. Experiments *in vitro* have shown that dexamethasone reduces the incorporation of ^{14}C-acetate into cholesterol [8]. The authors cited consider that all steroids with glucocorticoid activity inhibit cholesterol biosynthesis. Meanwhile, an increase in the incorporation of labeled acetate into cholesterol of the liver 16 h after administration of cortisol has been observed *in vivo* [11]. According to Johnston et al. [5], incorporation of ^{3}H-acetate into cholesterol in HeLa tumor cells was increased after the addition of dexamethasone to the medium. It may be that these differences in the action of cortisol on cholesterol biosynthesis are connected with the duration of the hormonal effect. The ways in which glucocorticoids inhibit and activate cholesterol biosynthesis still remain unexplained.

The object of the present investigation was to study the ways in which cortisol affects cholesterol biosynthesis in the rat liver *in vivo*. For this purpose, the effect of cortisol had to be studied on cholesterol biosynthesis when acting for different periods, and also when labeled acetate and mevalonic acid were used as precursors.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male Wistar rats weighing 180-200 g. The animals were kept in a room with controlled alternation of light and darkness and were fed on dry pellets. In each series of experiments the rats were divided into two groups: control and experimental, with 15 animals in each group. In one series of experiments the rats were given hydrocortisone, from Gedeon Richter (Hungary), by intramuscular injection in a dose of 5 mg/kg body weight and cholesterol biosynthesis was investigated 5 h after injection of the hormone. In the other two series of experiments hydrocortisone was injected into the animals in the same dose for 5 days.

In all experiments the animals were deprived of food for 12-14 h and 2 h before sacrifice they were given in intraperitoneal injection of 2-^{14}C-acetate in a dose of 10 μCi/100 g body weight. The animals of the 3rd group were given 2-^{14}C-mevalonic acid lactone in the same dose intraperitoneally at the same times. Considering the biological rhythm of cholesterol synthesis, the experiments with labeled precursors were carried out in the late evening. After the end of the experiment the animals were decapitated and the liver perfused with cold NaCl solution. Weighed samples of liver were homogenized, lipids were extracted from the homogenates and blood serum, and the lipids extracts were purified by the method of Folch et al. [4]. Neutral lipids and phospholipids were separated by chromatography on a thin layer of silicagel [3]. The radioactivity of the samples was determined on a Nuclear Chicago liquid scintillation spectrometer (USA), using an external standard and PSD module. Considering that cortisol, by reducing the permeability of the tissues, may prevent absorption of the labeled precursor from the peritoneal cavity, specific radioactivity of cholesterol in the control and

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TABLE 1. Incorporation of ^{14}C-Acetate into Free Cholesterol of Rat Liver Tissue (in cpm/mg cholesterol), Depending on the Duration of Action of Cortisol ($\bar{X} \pm \sigma$)

Experimental conditions	Duration of administration	
	5 h	5 days
Control	2144±165	3165±80
Hydrocortisone	2796±189*	1601±61†

* $P<0,01$.
† $P<0,001$.

TABLE 2. Effect of Cortisol on Cholesterol Biosynthesis from ^{14}C-Mevalonic Acid in Rat Liver ($\bar{X} \pm \sigma$)

Experimental conditions	Tissue	Free cholesterol, mg/100 g tissue or/100 ml serum	Specific radioactivity, cpm/mg cholesterol
Control	Liver	71,0±3,4	1119±263
	Blood serum	17,1±1,8	360±45
Cortisol	Liver	78,2±6,0	2993±304*
	Blood serum	36,7±3,0*	676±73†

* $P<0,001$.
† $P<0,005$.

experimental groups was calculated with allowance for the pool of labeled precursor in the liver. The specific radioactivity of the free cholesterol fraction in the liver and blood serum of the rats was calculated. The labeled compounds used in the work were from the Radiochemical Centre, Amersham (England) and most reagents were from Sigma (USA).

EXPERIMENTAL RESULTS

There are no data in the literature on the action of cortisol on cholesterol biosynthesis depending on the duration of the hormonal effect. The times chosen for the present experiments (5 h and 5 days) were recommended in previous investigations [2], in which differences were found in the action of cortisol on biosynthesis of apoproteins of very low density lipoproteins. The action of cortisol *in vivo* for 5 days led to a decrease in specific radioactivity of cholesterol in the liver tissue, indicating inhibition of cholesterol biosynthesis by cortisol (Table 1). The action of cortisol when administered for longer periods (10 days) also was to inhibit cholesterol biosynthesis. The inhibitory effect was probably associated with prolonged administration of the steroid. For that reason, in the next experiment the period of action of the hormone was shortened to 5 h. It was found that cortisol, 5 h after a single injection, stimulated cholesterol synthesis. Depending on the duration of its action, cortisol thus differs in the direction of its effect on cholesterol biosynthesis in the rat liver.

To study the way in which cholesterol biosynthesis is inhibited by cortisol, in the next experiment 2-^{14}C-mevalonic acid was used as the precursor for biosynthesis. When cortisol was injected into rats for 5 days and mevalonic acid was used, cholesterol biosynthesis in the liver of the experimental animals took place at twice the control level (Table 2). The concentration of free cholesterol in the blood serum was increased at the same time. Consequently, the results showed that if acting for a long time cortisol depresses cholesterol biosynthesis from acetate but increases its formation from mevalonic acid.

It can be concluded from the results of these investigations and data in the literature that inhibition of cholesterol biosynthesis by cortisol is the result of a decrease in activity of the key enzyme of cholesterol biosynthesis, namely β-hydroxy-β-methylglutaryl-CoA reductase (HMG-CoA reductase), although the possibility cannot be ruled out that this is not the only key enzyme [1, 5, 7, 11]. The limiting stage in cholesterol biosynthesis is the conversion of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) into mevalonic acid. It has been suggested that glucocorticoids inhibit the activity of HMG-CoA reductase [8]. The results of the present experiments confirm this view. Cortisol inhibits the biosynthesis of cholesterol from acetate and increases its synthesis from mevalonic acid. Analysis of the scheme of cholesterol biosynthesis confirms that cortisol inhibits HMG-CoA reductase activity. Under physiological conditions inhibition of HMG-CoA reductase activity is not accompanied by an increase in the utilization of mevalonic acid for cholesterol biosynthesis, for no other sources of formation of mevalonic acid than from HMG-CoA have been found [1]. A more probable explanation is that during the action of cortisol biosynthesis of HMG-CoA from acetate is intensified in the rat liver, but the decrease in HMG-CoA reductase activity does not lead to the formation of mevalonic acid and it inhibits the later stages of cholesterol biosynthesis. Administration of exogenous mevalonic acid, against the background of inhibition of its endogenous synthesis, causes its more intensive utilization for cholesterol synthesis. The higher incorporation of

mevalonic acid into cholesterol under the influence of cortisol than in the control can be explained in this way.

HMG-CoA reductase is thus the stage of biosynthesis at which the inhibitory action of cortisol on cholesterol biosynthesis is exerted. In experiments both *in vitro* and *in vivo* a high degree of correlation was found between HMG-CoA reductase activity and the degree of stimulation of cholesterol biosynthesis [9]. However, when HeLa tumor cells were grown in medium with a low concentration of glucocorticoids, dexamethasone activated HMG-CoA reductase, but under these circumstances cholesterol biosynthesis was not increased [7]. Incorporation of ^{3}H-acetate into cholesterol of the cells was increased immediately after addition of dexamethasone to the medium for a maximum of 4 h, after which incorporation of the label fell to a low level, at which it remained for a long time [5].

The results reflect the dependence of the action of glucocorticoids on cholesterol biosynthesis on the duration of administration of the hormone. For short periods cortisol activates cholesterol biosynthesis, but if administered for a longer time the hormone inhibits it. There are no data in the literature on differences in the action of cortisol on cholesterol biosynthesis in the liver depending on the duration of administration of the hormone in experiments *in vivo*. However, it has been shown *in vitro* that dexamethasone activates HMG-CoA reductase and cholesterol biosynthesis during the first few hours after its addition to the incubation medium with a culture of HeLa cells. At these same times the hormone inhibits the activity of another enzyme — HMG-CoA synthetase, synthesizing the subtrate for HMG-CoA reductase. However, all the evidence of this effect of cortisol on cholesterol synthesis has been obtained entirely *in vitro* in experiments on cultures of HeLa cells. Whether cortisol has the identical action on HMG-CoA reductase activity *in vivo* to that which is observed *in vitro* will have to be decided on the basis of further experiments.

The writers' previous investigations showed that the action of cortisol on biosynthesis of apoproteins of very low density lipoproteins depends on the duration of its effect; 5 h after addition of the hormone protein synthesis was increased, but after a longer period of administration it was inhibited. The same relationship was found in the present investigation for the effect of cortisol on cholesterol biosynthesis. It can be tentatively suggested that cortisol has the same action on the formation of very low density lipoproteins, of which both apoproteins and cholesterol are important structural components.

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