MECHANISM OF REGULATION OF STEROID FORMATION IN THE ADRENALS BY BLOOD SERUM LIPOPROTEINS

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UDC 612.451.015.32-06:612.123

High-density lipoproteins obtained by ultracentrifugation and loaded with cholesterol and pregnenolone, were shown to stimulate glucocorticoid production by rat adrenals in vitro. Very high density native lipoproteins have an inhibitory effect. The action of very low density lipoproteins is linked with the protein component. The mechanism of action of different classes of blood serum lipoproteins on steroid production in the rat adrenals differs substantially.

KEY WORDS: blood serum lipoproteins; apoproteins; adrenals; steroid production.

Studies of the regulatory effects of blood serum lipoproteins on steroid production in the adrenals have recently been published. Intravenous injection of low-density lipoproteins (LDLP) into rats has been shown to reduce activity of β-hydroxy-β-methylglutaryl-CoA reductase and β-hydroxy-β-methylglutaryl-CoA synthetase, key enzymes of biosynthesis of cholesterol, the main precursor of the steroid hormones, in the adrenals [8]. Inhibition of lipoprotein formation in the liver, accompanied by a sharp fall in the blood lipoprotein concentration, leads to intensification of steroid synthesis in the adrenals [7]. The writers showed previously that chylomicrons, very low-density lipoproteins (VLDLP) and LDLP obtained by preparative disc electrophoresis, lower glucocorticoid production by rat adrenals in vitro [3, 5]. The main suppliers of cholesterol for steroid synthesis are considered to be high-density lipoproteins (HDLP) of the blood serum [10].

This paper describes a continuation of the study of mechanisms of regulation of steroid production by blood serum lipoproteins.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. The animals were killed by decapitation, the adrenals removed in the cold and divided into two parts, from which sections were cut containing about equal quantities of tissue from the left and right adrenals of one animal in parallel samples. The sample to which lipoproteins were added was the experimental, and that without addition of lipoproteins the control. The contents of tissue in each sample was 18-22 mg. Sections were placed in vessels containing 1.5 ml Krebs–Ringer-phosphate buffer, pH 7.4, and incubated at 37°C for 2 h. Steroid production was assessed from the rate of production of 11-hydroxycorticosteroids (11-HCS), expressed in μg/100 mg tissue/h. The 11-HCS concentration in the incubation medium was determined fluorometrically [4]. Preparative isolation of the blood serum lipoproteins was carried out by ultracentrifugation in salt solutions [11] in the presence of 0.005M EDTA on a Beckman L-75 centrifuge, using the 75 Ti rotor. HDLP was saturated with precursors of steroid hormone (cholesterol and pregnenolone) by the method in [13]. The lipoproteins were delipidized with a mixture of chloroform and methanol [2]. The lipoproteins and apoproteins (APO) thus obtained were dialyzed for 48 h against 0.15M NaCl, containing 0.005M EDTA, pH 7.4, at 4°C. Lipoproteins were added to the samples in a dose of 0.5-1.5 mg, APO in a dose of 0.1-0.2 mg, and ACTH* in a dose of 0.7 unit/ml medium without preincubation of the tissue. The experimental results were subjected to statistical analysis.

*ACTH was produced by the Moscow Research Institute of Technology of Blood Substitutes and Hormone Preparations and was generously provided by F. Yu. Ryzhka, to whom the authors are grateful.

Laboratory of Biochemistry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 9, pp. 267-269, September, 1979. Original article submitted August 6, 1978.

0007-4888/79/8809-0965$07.50

TABLE 1. Effect of HDLP Saturated with Cholesterol and Pregnenolone on 11-HCS Production by Rat Adrenals under Normal Conditions and during Stimulation of Steroid Production by ACTH (M ± m)

Experimental conditions	Normal HDLP (experiment)	Without HDLP (control)	HDLP + cholesterol (experiment)	Without HDLP (control)	HDLP + pregnenolone (experiment)	Without HDLP (control)
Without ACTH	1,82±0,26 (9)	1,83±0,23 (9)	1,97±0,05 (5)	1,61±0,10 (5)	2,64±0,19 (5)	2,28±0,13 (5)
ACTH	3,26±0,07 (5)	3,14±0,06 (5)	4,68±0,13* (5)	3,33±0,10 (5)	5,76±0,17† (5)	3,26±0,11 (5)

*P < 0.001.

†P < 0.0001.

Legend. Number of experiments shown in parentheses.

TABLE 2. Effect of Different Lipoproteins Obtained by Preparative Ultracentrifugation on Steroid Synthesis in Rats

Lipoprotein fraction	Experiment	Control	No. of expts.	P
VLDLP:				
Rat	1,64±0,06	2,10±0,08	9	<0,01
Human	1,21±0,20	1.84±0,17	6	<0,001
LDLP:				
Rat	1,82±0,07	2,10±0,18	9	>0,05
Human	1,68±0,12	1,69±0,07	6	>0,05
HDLP:				
Rat	1,83±0,23	1,82±0,26	9	>0,05
Human	1,84±0,25	2,20±0,31	6	>0,05

Legend. 11-HCS production without addition of lipoproteins served as the control, its production with addition of lipoproteins as the experiment.

TABLE 3. Effect of Various Apoproteins on 11-HCS Production by Rat Adrenals (M ± m)

Index	APO-VLDLP (experiment)	Without APO (control)	APO-HDLP (experiment)	Without APO (control)	Albumin (experiment)	Without albumin (control)
11-HCS	1,22±0,12* (16)	1,87±0,19 (16)	1,95±0,19 (15)	2,06±0,20 (15)	2,50±0,38 (3)	2,49±0,44 (3)

*P < 0.01.

Legend. Number of experiments indicated in parentheses.

EXPERIMENTAL RESULTS

Addition of HDLP to the incubation medium had no effect on steroid production (Table 1). HDLP loaded with cholesterol increased the output of steroid hormones by 21%, and HDLP loaded with pregnenolone increased it by 27% (T = 2.42). ACTH in the presence of HDLP caused virtually no change in 11-HCS production compared with the action of the hormone alone. The situation was sharply altered if ACTH was added to the slices together with HDLP loaded with cholesterol. In that case steroid production was increased by 41%.

Cholesterol is known to be present in HDLP chiefly as esters and the content of metabolically active free cholesterol does not exceed 4%. Incubation of HDLP with free cholesterol leads to an increase in its content in HDLP up to 17-22% [12]. The results now obtained can be regarded as evidence that ACTH increases the supply of cholesterol from HDLP to the adrenal cortical cells. This is confirmed by other authors' observations [10].

Conversion of cholesterol with the participation of the desmolase complex into pregnenolone in the adrenal mitochondria is the limiting process of steroid synthesis [9]. In fact, it was found that the presence of HDLP saturated with pregnenolone in the incubation medium together with ACTH led to even greater intensification of steroid synthesis. The rate of secretion of 11-HCS was 171% compared with the action of ACTH alone.

A decrease in the rate of steroid synthesis was found previously to be induced by VLDLP and LDLP isolated from rat serum by preparative disc electrophoresis [3, 5]. VLDLP and LDLP obtained by preparative ultracentrifugation also inhibited glucocorticoid production by rat adrenals (Table 2). The most marked effect was given by human VLDLP (inhibition by 34%); rat VLDLP were rather less effected (22%). LDLP had weak inhibitory properties. It is interesting to note that human HDLP lowered 11-HCS production to some extent, probably due to the nonhomogeneity of the lipid and protein composition of rat and human HDLP.

The degree of saturation of lipoproteins with lipids (cholesterol) and also their concentration in the blood are known to have a significant effect on processes of steroid synthesis [6]. It is also known that many regulatory properties of lipoproteins are due to their protein components. The results relating to the effect of apoproteins on the rate of secretion of 11-HCS by adrenal slices are given in Table 3.

APO-VLDLP were found to have an inhibitory action and they thus reproduced the effect of intact VLDLP. APO-HDLP, like intact HDLP of rat serum, had no action on steroid synthesis. Serum albumin, which also did not affect glucocorticoid production by rat adrenals, was used as the control.

The results are evidence that HDLP, as a transport form of cholesterol, can under certain conditions stimulate steroid synthesis in the adrenals. Their effect on cell metabolism likewise cannot be ruled out. VLDLP give the opposite effect. The inhibitory action of VLDLP is linked with the protein component. The mechanism of action of different classes of blood serum lipoproteins on processes of steroid synthesis in the rat adrenals differ significantly.

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