RELATIONS BETWEEN THE GLUCOCORTICOID FUNCTION OF THE ADRENAL CORTEX AND BLOOD SERUM LIPOPROTEINS

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After a single injection of hydrocortisone into rats the serum concentration of very low density lipoproteins increased, but after repeated injections the serum level of very low density, low density, and high density lipoproteins all increased. Lipoproteins with d < 1.063 g/ml had an inhibitory action on steroid hormone production by rat adrenals in vitro.
KEY WORDS: glucocorticoids; lipoproteins; steroidogenesis.

Mechanisms linking "stress," the onset of hyperlipoproteinemia, and the development of ischemic heart disease are being actively investigated at the present time [5, 6, 8, 11, 12]. An excessive strain on the CNS [10] or prolonged administration of glucocorticoids to animals [3, 7, 12, 16] leads to an increase in the serum concentration of low density lipoproteins (LDLP) and very low density lipoproteins (VLDLP). However, in patients with atherosclerosis [2], and also in animals kept for a long time on an atherogenic diet [18], adrenal cortical function in some cases is depressed despite the existence of marked hyperlipoproteinemia.

EXPERIMENTAL METHOD

Experiments were carried out on female Wistar rats weighing 180-200 g. Cortisol was injected intraperitoneally in a dose of 2.5 mg/100 g body weight once only, or in a dose of 1.25 mg/100 g body weight daily for 5 days. The blood serum lipoprotein spectrum was determined 2.5 h after injection of the hormone by the method described earlier [9]. Preparative isolation of lipoproteins was carried out by disk electrophoresis in blocks of polyacrylamide gel prepared by the method of Davis [17], followed by electrophoretic elution of the lipoprotein fractions. The mold for the concentrating gel had two projections of different lengths, as a result of which after the end of polymerization, hollows of different capacity were obtained in the gel. Into the first of these hollows 0.05 ml of serum stained with Sudan black was poured as the standard, and 6 ml of a mixture of serum with glycerol in the ratio of 10 : 1 was poured in a layer into the larger hollow. Electrophoresis was carried out with a current of 10 mA/cm^2 for 2.5 h. The block of gel was then taken from the apparatus and areas of gel requiring extraction were determined from the position of the stained fractions. These areas were cut into small pieces measuring 2-3 cm^3 and transferred to special cuvettes for electrophoretic elution, filled with Tris–glycine buffer, pH 8.3. Special investigations showed that in the course of 3 h with an initial current of 20 mA/cm^2 practically all the dye bound with the lipoproteins passed from the gel into the cellophane bags suspended on the bottom of the cuvettes. After the end of elution dialysis was carried out for 24 h against 0.1 M phosphate buffer, pH 7.4, at a temperature of 3-5°C. The final concentration of lipoprotein in the incubated samples of adrenal slices was 0.5-1.5 mg/ml medium. Steroidogenesis was assessed from 11-hydroxycorticosteroid (11-HCS) production per 100 mg per hour. Incubation was carried out in a Warburg apparatus in Krebs–Ringer–phosphate buffer, pH 7.4, for 1.5 h. The ratio between 11-HCS production in the experimental (with the addition of lipoproteins) and in the control series (without lipoproteins) was calculated in per cent. The ACTH was added in a dose of 200 milliunits to 1.5 ml of incubation mixture.

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TABLE 1. Effect of Cortisol on Serum Lipoprotein Spectrum of Rats (M ± m)

Experimental conditions	VLDLP	LDLP	$HDLP_2$	$HDLP_3$
1. Before injection (15)	0,017±0,001	0,061±0,002	0,029±0,001	0,024±0,001
2. Single injection of cortisol (18)	0,020±0,001	0,059±0,002	0,029±0,002	0,026±0,002
3. Repeated injection of cortisol (10)	0,022±0,002	0,071±0,003	0,030±0,002	0,032±0,002
P_{2-1}	<0,05	>0,05	>0,05	>0,05
P_{3-1}	<0,05	<0,05	>0,05	<0,05
P_{3-2}	>0,05	<0,05	>0,05	<0,05

Legend. 1) Results given in optical density units. 2) Number of animals shown in parentheses.

TABLE 2. Effect of Blood Serum Lipoproteins on Steroidogenesis in Rat Adrenals in Vitro

Lipoprotein fraction	Adrenals of control rats + LPC	Adrenals of control rats + LPS	Adrenals of fasting rats + LPC	Adrenals of fasting rats + LPS	Adrenals of control rats + ACTH + + LPC
CM	74** (8)	81** (7)	73* (6)	71** (6)	96 (6)
VLDLP	80** (10)	81* (7)	72** (6)	78** (6)	81* (6)
LDLP	73*** (10)	81* (7)	83* (6)	98 (6)	89* (6)
$HDLP_2$	87 (9)	103 (7)	108 (6)	111 (6)	100 (6)
$HDLP_3$	91 (10)	101 (7)	113 (6)	105 (6)	106 (6)

Legend. 1) LPC) lipoproteins of control rats; LPS) lipoproteins of rats subjected to stress. 2) Number of experiments in parentheses. 3) One asterisk $P < 0.05$, two $P < 0.01$, three $P < 0.001$.

EXPERIMENTAL RESULTS AND DISCUSSION

A single injection of cortisol (TABLE 1) caused the serum VLDLP level of the rats to rise. During prolonged administration of the hormone, besides an increase in the VLDLP concentration, an increase also was observed in the levels of LDLP and high density lipoproteins ($HDLP_3$).

An increase in the VLDLP concentration under the influence of glucocorticoids was observed previously [3, 6, 7, 12, 16]. Corticosteroids are considered to induce hyperlipoproteinemia by stimulating VLDLP formation in the hepatocytes [19]. The absence of an increase in the HDLP concentration after a single injection of cortisol but an increase in their level during prolonged administration of the hormone may be the result of transformation of VLDLP into LDLP [3]. The discovery of an increase in the fastest lipoprotein fraction ($HDLP_3$) is of considerable interest.

In the next experiments the action of different serum lipoprotein fractions on steroidogenesis in the adrenals was studied in vitro (Table 2). The addition of "normal" lipoproteins to the adrenals of the control animals changed 11-HCS production. Chylomicrons (CM), VLDLP, and LDLP, i.e., lipoproteins with $d < 1.063$ g/ml, had an inhibitory action. HDLP had virtually no effect on 11-HCS production.

The inhibitory action of β-lipoproteins isolated from the blood serum of animals exposed to stress on liver and muscle hexokinase is known from the literature [1, 4, 13]. It is considered that under these circumstances the quantity of corticosteroids transported in the composition of β-lipoproteins is increased [1, 4]. No appreciable amounts of corticosteroids could be found by thin-layer chromatography in the total LDLP and VLDLP isolated from 50 ml of human and rat blood serum.

The addition of lipoproteins from rats exposed to stress (swimming for 3 h) to the incubation media of the adrenals gave an effect similar to the action of serum lipoproteins of the control animals. Considering the possibility of a difference in the "sensitivity" of the adrenals depending on the physiological state of the animal, investigations were carried out on the adrenals of animals exposed to stress (starvation for 48 h) and also with the addition of ACTH. In this series of experiments all the fractions of lipoproteins with $d < 1.063$ g/ml had an

inhibitory action which was independent of the original physiological state. It is interesting to note that in this series the LDLP had only a weak tendency toward activating 11-HCS production by the adrenals.

These results are evidence of a possible regulatory effect of the lipoproteins on adrenocortical function. The concrete mechanisms of this regulation have not been explained. It is likewise not clear whether this effect takes place in the whole organism. The depression of steroidogenesis by lipoproteins with $d < 1.063$ g/ml may perhaps be connected with their action on hexokinase, which leads to the inhibition not only of glycolysis, but also of the pentose cycle, the main source of NADPH. Inhibition by LDLP and VLDLP of cholesterol biosynthesis in human fibroblasts, in which the activity of β-hydroxy-β-methylglutaryl-CoA reductase is inhibited [14, 15], is a noteworthy fact. The presence of feedback between blood lipoproteins and glucocorticoids provides an explanation for the decrease in adrenocortical activity in some cases where marked hyperlipoproteinemia exists in patients with atherosclerosis and in animals kept on an atherogenic diet.

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