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Stress and Cerebro-Hepatic Axis with Special Reference to Steroids Metabolism

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Introduction

We have studied many cases of acute or atypical psychoses from the endocrinological point of view (3). During the psychotic period, metabolic disturbances of androgen and estrogen were found frequently. These disturbances may be regarded as a result of hepatic dysfunctions. However, such abnormalities were also found in the psychotic state induced by *d*-lysergic acid diethylamide (LSD) and in the diencephalo-pituitary disorders, and were normalized upon remission without any protective treatment of the liver. Consequently, such hepatic dysfunctions during the psychotic period are not primary dysfunctions, but seem to indicate a breakdown of the cerebro-hepatic homeostasis. Although such a homeostatic breakdown is a non-specific bodily response, it may be an important factor in causing acute mental disorders. In order to further examine experimentally the cerebro-hepatic relationship, we have studied the effects of various stress conditions on the activities of several enzymes in rat liver (6). In this research, we examined the effects of electric stimulation of hypothalamus, administration of LSD, and forced running and immobilization, on the metabolism of androgen and estrogen in the liver of rabbits and rats.

Materials and Methods

Male and female Wistar rats and white male rabbits were used in all experiments. The ability of estrogen inactivation by the liver was measured by *Jailer*'s method (5). A 1 mg of estradiol pellet was transplanted into the spleen of the ovariectomized female rats. When the amount of estradiol in the spleen gradually decreased to the extent that the liver could completely inactivate the hormone, the cornified cells in the vaginal smear would disappear. The reappearance of cornified cells in the vaginal smear was regarded as a decrease in the ability of the liver to inactivate estrogen.

The metabolism of androgen was determined as follows. The liver of a male rat or a male rabbit was homogenized with a glass homogenizer in 5 volumes of cold 0.25 *M* sucrose. The homogenate was centrifuged at 1,000 *g* for 10 min. The resulting supernatant equivalent to 5 g of liver was incubated with Δ^4-androstane-3,17-dione 0.7 μM, reduced triphosphopyridine nucleotide (NADPH) 1.2 μM, $MgSO_4$ 100 μM and Tris-HCl buffer (pH 7.4) 200 μM for 90 min at 37 °C. The mixture was extracted by 150 ml of ethyl acetate. The ethyl acetate layer was washed with 15 ml of 10 % NaOH and water successively, and evaporated to dryness. The residue was dissolved by 15 ml of methanol and left overnight at −20 °C to remove excess lipids. The precipitate was filtered and the filtrate was evaporated. The residue was dissolved by 2 ml of benzene and loaded on an aluminum column (10 × 0.7 cm). The column was washed by 4 ml of benzene and eluted stepwise by the mixture of benzene 97:ethanol 3, benzene 84:ethanol 16 and benzene 65:ethanol 35. The fractions were combined and evaporated. Δ^4-androstane-3,17-dione, androsterone and etiocholanolone were analyzed quantitatively by gas chromatography (column XE 20, 210 °C, carrier gas N_2 1.2 kg, H_2 0.7 kg, air 1 kg).

In the experiment of the electric stimulation of hypothalamus, bipolar stainless electrodes were stereotaxically implanted unilaterally in the ventromedial nucleus of the hypothalamus (VMH) of male adult rabbits. The stimulating procedures were started 2 weeks after the electrode implantation. The animals had complete freedom of movement in their observation boxes and were stimulated for 20 sec, with 2-min rest intervals, for durations totaling 2 h. Monophasic square-wave pulses of 1 msec duration, 100 cycle/sec, with an amplitude of 3–5 V were delivered from an electronic stimulator. At the conclusion of the experiment, the disposition of the electrode tips was confirmed histologically using cresyl violet stains.

In the experiment of forced running, the rats were placed in a running drum 30 cm in diameter, revolving at a speed of 3 times per min. In the experiment of immobilization, the rats were placed face down upon a board with their legs fastened. Carbon tetrachloride and LSD were administered intramuscularly.

Results

A. Estrogen Metabolism

The results are summarized in table I. When the liver was injured by the injection of 0.2 ml of carbon tetrachloride, the cornified cells reappeared in 48 h. Forced running for 96 h and immobilization for 72 h also resulted in the reappearance of cornified cells. The result showed that stress conditions, such as forced running and immobilization, as well as the liver injury by carbon tetrachloride, decreased the ability of the liver to inactivate estrogen.

B. Androgen Metabolism

1. Electric Stimulation of VMH in Rabbits

The results are summarized in table II. In the control rabbits, 40 % of Δ^4-androstane-3,17-dione was converted to androsterone and etiocholanolone,

Table I. Effect of carbon tetrachloride, stress and LSD on estrogen metabolism in rat liver

	Rat No.	Appearance of cornified cells in vaginal smear				
		24 h	48 h	72 h	96 h	120 h
Carbon tetrachloride, 0.2 ml	1	–	+	+	+	
	2	–	+	+	+	
Forced running	1	–	–	–	+	
	2	–	–	–	±	+
Immobilization	1	–	–	+	+	
	2	–	±	+	+	
LSD, 25 μg	1	–	–	–	–	
	2	–	–	–	–	

Table II. Effect of electric stimulation of VMH on androgen metabolism in rabbit liver

	No. of rabbit	A/E	$(A + E/\Delta^4) \times 100$
Control	5	0.51 (± 0.13)	40 (± 7)
VMH stimulation, 2 h	11	0.07* (± 0.02)	27 (± 8)

A = androsterone; E = etiocholanolone; Δ^4 = Δ^4-androstane-3,17-dione; mean (± SE); * = $p < 0.01$.

and the ratio of androsterone and etiocholanolone was 0.51. When VMH was stimulated, the rabbits stopped moving, squatted and then began a running or circling movement. The motor reactions were stimulus-bound and it appeared that they were expressions of fear or rage. Following 2 h sessions of stimulation of VMH, the conversion ratio of Δ^4-androstane-3,17-dione to androsterone and etiocholanolone was 27 % and the ratio of androsterone and etiocholanolone was 0.07. The decrease of conversion ratio was not statistically significant, but the decrease of the ratio of androsterone and etiocholanolone was highly significant. The result indicates that the 5α-hydrogenation of Δ^4-androstane-3,17-dione to androsterone in rabbit liver is strongly inhibited by the stimulation of VMH.

Table III. Effect of LSD and forced running on androgen metabolism in rat liver

	No. of rat	A/E
Control	4	1.61 (± 0.30)
LSD administration, 25 μg, 6 h	4	0.75* (± 0.10)
Forced running, 6 h	3	0.79* (± 0.08)

A = androsterone; E = etiocholanolone; mean (± SE); * = $p < 0.05$.

2. Administration of LSD and Forced Running in Rats

The results are summarized in table III. In the rat, the activity of Δ^4-3-ketosteroid hydrogenase was much higher than that in the rabbits and only the ratio of androsterone and etiocholanolone was determined. In the control rats, the ratio of androsterone and etiocholanolone was 1.61. When the rats were killed 6 h after the administration of LSD (25 μg), the ratio decreased to 0.75. The addition of LSD *in vitro* (6×10^{-6} *M*) had no effect on the 5α-hydrogenation. When the rats were forcedly run for 6 h, the ratio decreased to 0.79. The results indicate that LSD and forced running inhibit the 5α-hydrogenation of Δ^4-androstane-3,17-dione to androsterone in rat liver probably through the cerebro-hepatic axis.

Discussion

We studied the fraction pattern of urinary estrogen and 17-ketosteroids in the cases of acute psychoses (3). As to the estrogen fraction pattern, the ratio between estradiol, estrone and estriol was approximately 1:2:3, respectively. During the psychotic phase, estrone was much higher than estriol. The result suggested that the inactivation of estrogen in the liver was disturbed in the psychotic period. We showed experimentally that the ability of the liver to inactivate estrogen was decreased by stress, such as forced running and immobilization.

Concerning the fraction pattern of 17-ketosteroids, fraction 4 contains the products of 5α-hydrogenation including androsterone and fraction 5 contains the products of 5β-hydrogenation including etiocholanolone. The ratio of fraction 4 and fraction 5 was over 1.5 in normal individuals or in the lucid phase of

psychotic patients. During the psychotic episodes, the ratio was below 1.5 or even below 1. The decrease of this ratio may reflect the decrease of 5α-hydrogenation of testosterone or Δ^4-androstane-3,17-dione in the liver. If the total activity of Δ^4-3-ketosteroid hydrogenase was determined by *Tomkins'* method (9) or by our method, neither stress nor LSD effected the enzyme activity. In this research, we determined 5α- and 5β-hydrogenation of Δ^4-3-ketosteroid separately, and demonstrated that electric stimulation of VMH, administration of LSD and forced running inhibited the 5α-hydrogenation, and decreased the ratio of androsterone and etiocholanolone. We did not obtain a definite result, when the other areas of brain, such as the lateral hypothalamus, the centre median, the hippocampus and the amygdala, were stimulated. However, the ratio of androsterone and etiocholanolone tended to decrease with the stimulation of these areas and the inhibition of 5α-hydrogenation may not be specific for VMH.

These results indicate that various stress conditions, which may affect the brain and hypothalamus, cause the metabolic disturbances of sex steroids in the liver probably through the cerebro (hypothalamo)-hepatic axis. Such a cerebro-hepatic relationship is supported by some other reports. *Higashimura et al.* (4) reported that the electric stimulation of VMH decreased the activities of arginase and arginine synthetase in the rabbit liver, and increased the level of blood ammonia. The hyperammonemia which is often found in the psychotic patients may be explained by the same mechanism (1). *Shimazu* (8) reported that the electric stimulation of hypothalamus increased tryptophan pyrrolase activity in the normal and the adrenalectomized rabbit liver and postulated some neural factors which control enzyme activity in the liver. *Nomura* (7) reported that the activity of tryptophan pyrrolase in rat liver was significantly increased by various stress conditions. Adrenalectomy did not prevent the increase in enzyme activity, but hypophysectomy eliminated the enzyme induction by stress. The result suggested that some unknown hypophyseal factor was involved in the regulation of rat liver tryptophan pyrrolase activity. *Colby* (2) recently reported that the effects of the gonadal hormones on hepatic Δ^4-steroid hydrogenase activity were manifested only in the presence of the pituitary gland. It is obvious that some endocrine as well as neural factors are involved in the regulation of some enzymatic activities in the liver. However, the exact nature of the cerebro-hepatic axis remains to be elucidated.

Summary

The ability to inactivate estrogen in rat liver was decreased by forced running and immobilization. The 5α-hydrogenation of Δ^4-androstane-3,17-dione to androsterone in rabbit and rat liver was inhibited by electric stimulation of the ventromedial nucleus of the hypothalamus, by administration of LSD and by forced running. These results indicate that

the metabolism of sex steroids in the liver was influenced by the various stress conditions probably through the cerebro-hepatic axis. This cerebro-hepatic axis may play an important role in the pathogenesis of acute psychoses.

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