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ZONAL CORTICOSTEROID HORMONE BIOSYNTHESIS IN THE ADRENAL CORTEX IN RATS EXPOSED TO EMOTIONAL STRESS COMBINED WITH SALT LOADING

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The adrenal cortex is a complex endocrine organ which specifically changes the secretion of corticosteroid hormones under the influence of stress and water-salt environmental factors [1]. Nevertheless, the combined action of these factors on corticosteroid biosynthesis in the adrenals remains virtually unstudied. Investigation of salt loading which, like emotional stress, has a definite pathogenic influence on the state of the cardiovascular system [2, 4], is particularly interesting.

The aim of this investigation was to study the pattern of biosynthesis of corticosteroid hormones in the zona glomerulosa (ZG) and the combined zona fasciculata + zona reticularis (ZF-ZR) of the adrenals, which are responsible for the mineralocorticoid and glucocorticoid function of the glands, during simultaneous exposure of animals to salt loading and emotional stress.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 230-260 g. For 8 days the animals took a normal or excessive amount of salt with their food: 2.5 or 50 meq sodium per rat per day. In the latter case the rats received a semisolid diet with a 2% concentration of salt and (to drink) 1.8% NaCl solution. A state of emotional stress was induced in the animals 30 min before decapitation (at 10-11 a.m.) on account of group conflict arising after 15-18 rats were put together in a cage measuring 46 × 30 × 15 cm, each rat having a rubber ring fitted on its hind limb, to compress it and cause irritation. The adrenals were divided into capsular and decapsulated parts and parallel samples were incubated in vitro with the addition of ^{3}H-progesterone (53 Ci/mmole) to each sample in a dose of $3 \cdot 10^5$ cpm. The technique of incubation, of chromatographic isolation of the ^{3}H-corticosteroids, and their quantitative assay were described previously [5]. The numerical data were subjected to statistical analysis by Student's t test.

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TABLE 1. Production (I) and Specific Activity (II) of Corticosteroid Hormones Formed by Decapsulated Part of Adrenals during Emotional Stress in Rats Differing in Their Salt Intake (M ± m)

Experimental conditions	DOC		18-OH-DOC		Corticosterone	
	I	II	I	II	I	II
Normal salt intake						
control (n = 4)	0,86±0,06	15 112±2 104	1,08±0,06	6647±504	5,6±0,28	4057±227
control + ACTH in vitro, relative units/g tissue (n = 4)	1,76±0,28*	5 945±664*	2,0±0,18*	4380±161*	7,7±0,81*	2824±187*
stress (n = 4)	1,46±0,2*	12 275±1 360	2,6±0,34*	4552±558*	9,1±0,54*	1871±75
Salt loading:						
control (n = 5)	0,78±0,08	19 050±2 720	1,04±0,12	3245±252**	7,44±0,8**	4301±345
stress (n = 5)	1,54±0,18*	9 840±783*	5,1±0,24*	1515±117*	19,6±0,8*	1135±61*

Legend. I) μg/100 mg tissue/h, II) cpm/μg. *) Difference significant compared with the corresponding control within the group; **) compared with control of normal salt intake, $P < 0.05$-0.01. n) Number of separate incubations in the given group. Capsular and decapsulated parts of adrenals from five or six rats were combined in each incubation.

TABLE 2. Production (I) and Specific Activity (II) of Corticosteroid Hormones Formed by Capsular Part of Adrenal Cortex during Emotional Stress in Rats Differing in Their Salt Intake (M ± m)

Experimental conditions	DOC		18-OH-DOC		Corticosterone		Aldosterone	
	I	II	I	II	I	II	I	II
Normal salt intake								
control (n = 4)	1,56 ±0,08	17 831 ±1 285	1,28 ±0,12	2987 ±347	4,14 ±0,14	5251 ±600	1,38 ±0,02	5521 ±408
control + ACTH (n = 4)	2,14 ±0,12*	11 248 ±607*	2,0 ±0,2*	1727 ±265*	5,44 ±0,24*	3530 ±209*	2,1 ±0,12*	3400 ±339*
stress (n = 4)	2,5 ±0,12*	9 677 ±1 213*	2,54 ±0,38*	2610 ±117	6,24 ±0,9	1610 ±0,280*	2,24 ±0,28*	1605 ±273*
Salt loading:								
control (n = 5)	1,92 ±0,16**	23 271 ±1 822**	0,6 ±0,05**	2993 ±702	4,63 ±0,3	5531 ±378	0,58 ±0,05**	5405 ±492
stress (n = 5)	4,6 ±0,38*	6 058 ±361*	4,84 ±0,44*	980 ±110*	14,7 ±0,78*	1176 ±96*	1,0 ±0,08*	1322 ±45*

EXPERIMENTAL RESULTS

When rats with a normal salt intake were exposed to emotional stress their production of corticosterone, 18-hydroxy-11-deoxycorticosterone (18-OH-DOC), and of their common precursor — deoxycorticosterone (DOC) — in both parts of the adrenals and of aldosterone in the capsular part increased (Tables 1 and 2). The specific activity of the ^{3}H-labeled corticosteroids, meanwhile, decreased significantly, and in the modern view this indicates selective stimulation of the pregnenolone pathway of corticosteroid hormone biosynthesis in the cells of ZF and ZG of the adrenal cortex [6, 7]. This general increase in the rate of corticosteroid hormone biosynthesis was evidently due to the activating effect of endogenous ACTH, mobilized under conditions of stress, on the adrenal cortex. There is a similarity in principle between data in the literature [7] and comparison of the effect of stress with the action of ACTH when added to the incubation medium of adrenal tissues of control animals: production of corticosteroids rises under the influence of ACTH but their specific activity declines (Tables 1 and 2). Keeping rats for 8 days on a diet containing an excess of salt led to a small but significant increase in corticosterone production by the decapsulated part of the adrenal cortex and to reduced formation of aldosterone and 18-OH-DOC by the capsular part. Emotional stress, induced in rats against the background of salt loading, was characterized by a sharp rise of corticosterone and 18-OH-DOC production (Fig. 1) and a sharp fall in the specific activity of the hormones in both parts of the adrenal cortex, which was much more marked than during stress in rats receiving a normal salt intake. Aldosterone production by the capsular part increased, and this was accompanied by a significant decrease in its specific activity. Excessive salt intake thus increased the functional sensitivity of the adrenal cortex of the rats to emotional stress. It can be suggested that this phenomenon is connected to some degree with general activation of the hypothalamo-hypophyseo-neurosecretory system during salt loading [3] and, in particular, with increased formation of vasopressin, which stimulates ACTH

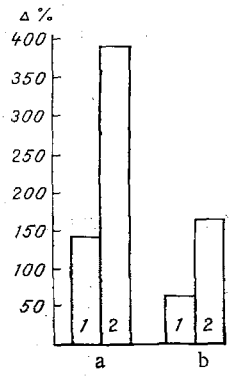

Fig. 1

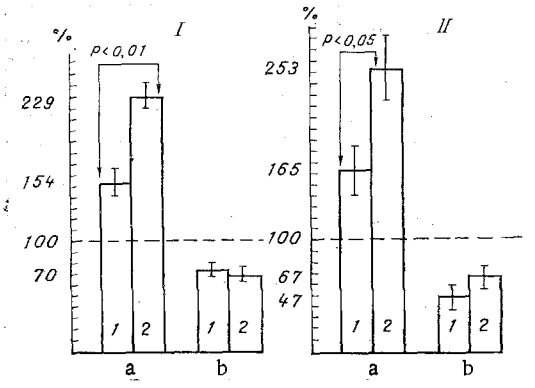

Fig. 2

Fig. 1. Comparison of increases in productionof 18-OH-DOC (a) and corticosterone (b), expressed as percentages of the control level (Δ%), in decapsulated part of rat adrenals during emotional stress against the background of normal (1) and excessive (2) salt intake.

Fig. 2. Effect of emotional stress against the background of normal (1) and excessive (2) salt intake on incorporation of tritium label into 18-OH-DOC and corticosterone, formed by decapsulated (I) and capsular (II) parts of adrenals (in % of control, M ± m). Incorporation of label: cpm/μg hormone/100 mg tissue/h; change in per cent incorporation relative to control level significant in every case: $P < 0.05$ or <0.01; differences in per cent incorporation into 18-OH-DOC in states 1 and 2 are significant.

secretion by the pituitary [13]. The observed increase in the rate of hormone synthesis from unlabeled precursors of the pregnenolone pathway is in agreement with this suggestion. The functional importance of the pregnenolone pathway of biosynthesis probably lies in the urgent mobilization of gluco- and mineralocorticoids in response to acute situations.

Attention is drawn to the considerable increase in 18-OH-DOC production during stress (Fig. 1). Although the physiological importance of 18-OH-DOC is not yet sufficiently clear, the definition of this hormone as an "ACTH-dependent mineralocorticoid" indicates some of its characteristic properties: an increase in its concentration in the incubated samples and blood under the influence of ACTH [10, 14, 15], ability to retain sodium and to raise the blood pressure in rats [8, 11], an increase in secretion in essential hypertension [9]. The marked decrease in specific activity of 18-OH-DOC is evidence of increased formation of the hormone via the pregnenolone pathway, but at the same time the results reveal definite participation of the progesterone pathway of biosynthesis in its increased production. It will be clear from Fig. 2 that emotional stress systematically increased incorporation of the tritium label into 18-OH-DOC and reduced its incorporation into corticosterone, evidence of stimulation of hydroxylation of endogenous ^{3}H-DOC in position 18 and its weakening in position 11. It is important to note that similar reciprocity was found in the adrenals of rats with hereditarily determined salt hypertension [12]. The authors cited conclude that 18- and 11-hydroxylation of DOC are under the joint control of one gene, through a change in the properties of the mitochondrial membrane. Their data suggest that factors of emotional stress selectively stimulate hydroxylation of ^{3}H-DOC in position 18, by acting on the state of the mitochondrial membranes of adrenal cortical cells; moreover, the process takes place to a more marked degree in stress superposed on salt loading. It can be concluded on the whole that excessive salt intake leads to a sharp increase in the formation of corticosterone and 18-OH-DOC by the adrenal cortex during emotional stress. Under these circumstances, aldosterone production is also at a sufficiently high level. In this situation, therefore, the general mineralocorticoid potential, aimed at retaining sodium in the body, is inappropriately increased.

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HYPOXIA-INDEPENDENT MECHANISM OF ORGAN INJURY IN HYPERTHROMBOPLASTINEMIA

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KEY WORDS: hemocoagulation; glycocalyx; lysosomes; kinins; thromboplastin.

Injury to organs during intravascular hemolysis, trauma, and certain obstetric complications is linked with disturbances of regulation of the aggregate state of the blood (RASB), caused by the entry of thromboplastin into the bloodstream [5]. It is considered that hyperthromboplastinemia causes activation of thrombin and the formation of hypercoagulation, followed by response activation of plasmin and the development of hypocoagulation. Disturbances of the microcirculation under these circumstances lead to hypoxic damage of organs [2, 13, 14].

EXPERIMENTAL METHOD

The consequences of hyperthromboplastinemia were studied in three series of experiments on 20 dogs and 85 albino rats after infusion of a suspension or extract of allogeneic (AGB) or xenogeneic (XGB) brain in a dose of 25 ml/kg body weight. In the experiments of series I (four dogs, 25 rats) a suspension of fragments of XGB cell membranes was injected intravenously. An extract of XGB was injected into the animals in the experiments of series II (25 rats, eight dogs — three males and five females). In the experiments of series III (25 rats, eight dogs — three males and five females) extract of AGB was used. In the control, 10 rats received an injection of isotonic NaCl solution, pH 7.4, in a dose of 25 ml/kg body weight.

To obtain 25 ml of a suspension of XGB, 1 g of thromboplastin was mixed with 55 ml of physiological saline, homogenized, and the sample was centrifuged for 3 min at 17 sec^{-1}. To obtain 25 ml of extract 10 g of native material of AGB was mixed with 45 ml of physiological saline and homogenized; the homogenate or suspension of XGB was then centrifuged twice at 58 sec^{-1} for 20 min each time. The supernatant with activity of 16-24 sec^{-1} [1] was used for infusion. The level of free kinins in arterial and venous blood was determined in the male dogs before and at the 2nd minute of infusion, and 5 min and 1 h after infusion, by a biological method [4] after stabilization [9], and the blood pressure was measured. The ureters of the female dogs were exteriorized by the Pavlov—Tsitovich method and partial renal function was studied [7] before and 2, 5, and 10 days after infusion. Parameters of the coagulogram of the dogs [1] were determined before and at the 1st-2nd minute of infusion, and again 5, 60, and 120 min and 24 h after infusion. Under ether anesthesia the rats were decapitated 3-4 and 10 min and 1 and 10 days after infusion. Under these circumstances the liver, kidneys, and heart were removed and treated for histological investigations by staining with hematoxylin and eosin [11] and by Selye's method [12]. The ultrastructure of these organs 3-4 min after infusion was studied by the acid phosphatase (AP) reaction and with ordinary contrast staining [6].

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