ACTH, CORTICOSTERONE, AND β-ENDORPHIN IN RAT BLOOD PLASMA AFTER PROLONGED IMMOBILIZATION STRESS

E. A. Kiyatkin, Yu. V. Polyntsev,
N. E. Kushlinskii, and M. G. Amiragova

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Data on involvement not only of the hypothalamo-hypophyseo-adrenal system [12], but also of endogenous opioid peptides, in the development of acute and, in particular, of chronic stress have been obtained in recent years; an important role in the changes observed in behavior and autonomic and hormonal parameters under these circumstances is ascribed to the latter [4, 9]. Strongest correlation has been found between changes in β-endorphin (BE) and corticosterone (CS) levels [7] in stress induced by electrodermal stimulation.

Data on synthesis of ACTH and BE from their common precursor [15], their coexistence, and their combined secretion during stress from the same secretory granules of pituitary cells [6], and the presence of opiate receptors on secretory cells of the hypothalamus and pituitary and adrenal glands [4] suggest that close functional relations exist between these substances.

In the present investigation, to assess functional relations between changes in ACTH, BE, and CS levels, plasma concentrations of these hormones were studied in rats during the development of prolonged immobilization stress.

EXPERIMENTAL METHOD

Experiments were carried out on 37 noninbred male rats weighing 180-200 g. Immobilization of the rats for 30 h in Plexiglas constricting cages was used as the model of prolonged stress. Hormone concentrations were determined after immobilization for 10-20 min, and 1, 5-6, and 25-26 h. The first two periods corresponded to the initial stage of development of the state of stress, when the most dramatic changes are observed in the animals' behavior and in the levels of autonomic, hormonal, and neurochemical parameters [2, 3, 11]. The last two times were chosen on the basis of a previous analysis of changes in firing pattern and chemical sensitivity of central neurons [1] and corresponded: the first – to the most marked changes in functional properties of the neurons, the second – to restoration of deviations of these parameters, evidently due to adaptation of the animals to conditions of prolonged immobilization.

Unrestrained rats, kept in single cases with free access to food and water, were used as the control.

The time of decapitation and beginning of blood sampling in all the animals was separated from the time of the first contact of the rats with the experimenter by an interval of 8-20 sec. The time of blood sampling from immobilized rats (10-15 h) was chosen allowing for data showing no significant changes in the blood levels of these hormones during this time of the 24-h period [5, 8].

Because of great differences in the values obtained for the test hormone, and CS in particular, in "intact" rats [7, 8, 10, 13], concentrations of ACTH, CS, and BE also were studied during relatively short-term exposure of the animals to ecologically adequate adverse stimuli. The animals used in this series were kept in single cages in the room in which the rats were decapitated and blood was taken, and for 5-15 min they heard acoustic stimulation (vocalization) associated with decapitation of the other rats.

Blood was collected after decapitation of the animals in cooled test tubes with the addition of a solution of EDTA in phosphate buffer, pH 7.4, at 20°C (7.5 mg EDTA to 5 ml of whole blood). After thorough mixing the samples were centrifuged at 1500 g in the cold (2-4°C) for 15 min. The plasma was kept until required for hormone assay for 4-6 weeks at −70°C.

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TABLE 1. Plasma Levels of CS, Immunoreactive ACTH, and BE in Rats at Different Periods of Immobilization Stress

Experimental Conditions	Parameter	ACTH, pg/ml	CS, μg/100 ml	BE, pg/ml
Control (1)	$X \pm m$	62,00±15,43	2,05±0,50	193,67±23,96
	CV, %	49,78	54,43	21,43
Immobilization stress:				
10-20 min (2)	$X \pm m$	836,00±141,34	19,58±1,80	326,67±21,04
	CV, %	37,80	22,52	15,78
1 h (3)	$X \pm m$	572,00±89,13	29,50±3,61	440,67±43,67
	CV, %	34,84	30,00	25,27
5—6 h (4)	$X \pm m$	206,67±45,82	14,08±3,62	325,33±17,26
	CV, %	54,31	63,05	12,99
25—26 h (5)	$X \pm m$	143,40±25,23	12,77±4,36	30,417±16,62
	CV, %	39,34	83,65	13,38
	P_{1-2}	<0,001	<0,001	<0,01
	P_{1-3}	<0,001	<0,01	<0,01
	P_{1-4}	<0,05	<0,05	<0,01
	P_{1-5}	<0,05	<0,05	<0,01
	P_{2-3}	n.s.	<0,05	<0,05
	P_{2-4}	<0,01	n.s.	n.s.
	P_{2-5}	<0,001	n.s.	n.s.
	P_{3-4}	<0,01	<0,01	<0,05
	P_{3-5}	<0,001	<0'05	<0,05
	P_{4-5}	n.s.	n.s.	n.s.

Legend. Control group contained four animals, all other groups contained six animals. Here and in Table 2: X) arithmetic mean, m) standard error, CV) coefficient of variation, n.s.

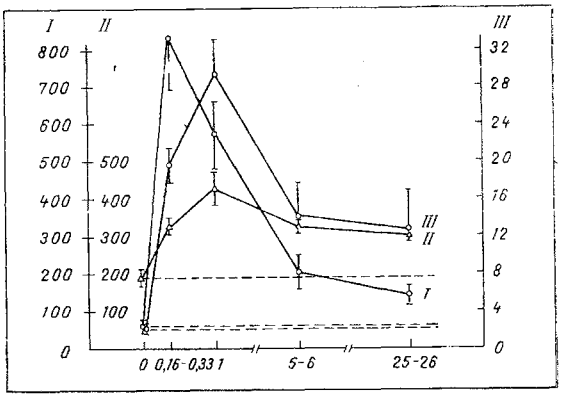

Fig. 1. Changes in concentrations of ACTH (I), BE (II), and CS (III) in blood plasma of rats after different periods of immobilization stress. Broken lines – initial hormone levels. Abscissa, duration of immobilization (in h); ordinate: I) pg/ml, II) pg/ml, III) μg/100 ml.

Plasma hormone concentrations were determined by radioimmunoassay: ACTH with the kit from CIS-IRE-Sorin (France–Italy), BE with the kit from Immuno Nuclear Corp. (USA). A particular feature about the kit used to determine BE was the presence of 50% cross-reactivity of the antiserum against β-lipotrophin. To determine CS a highly specific antiserum produced by the Laboratory of Endocrinology (Head, G. V. Katsiya), Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, was used.

The results were analyzed by standard statistical methods on the Élektronika D3-28 microcomputer.

EXPERIMENTAL RESULTS

The development of immobilization stress in the rats was accompanied by significant rise in the plasma ACTH, CS, and BE levels at all stages of exposure (Table 1; Fig. 1).

The fastest and most marked changes were observed in the case of ACTH, whose concentration at the 10th-20th min of immobilization was 13.5 times higher than in the control animals. Later ACTH activity fell, but still remained higher than initially: by 9 times after 1 h, by 3.3 times after 5-6 h, and by 2.3 times after

TABLE 2. Plasma CS, Immunoreactive ACTH, and β-Endorphin Levels in Rats during Short Exposure to Ecologically Meaningful Adverse Factors

Experimental conditions	Paremeter	ACTH, pg/ml	CS, μg/100 ml	BE, pg/ml
Experimental (6)	$X \pm m$	227,25±40,32**	11,01±1,53***	226,17±17,96*
	CV, %	35,49	41,74	15,63
Control (4)	$X \pm m$	62,00±15,43	2,05±0,50	193,67±23,96
	CV, %	49,78	54,43	21,43

Legend. *P < 0.05, **P < 0.01, ***P < 0.001. Number of animals given in parentheses.

25-26 h. The CS and BE levels rose during the first 10-20 min of immobilization and reached a peak after 1 h, when they were 14.4 and 2.2 times higher than the control respectively. During subsequent periods of immobilization of the rats the CS and BE concentrations fell parallel to one another, but remained 6.9 and 1.7 times higher respectively than initially after 5-6 h and 6.2 and 1.6 times higher after 25-26 h.

Analysis of the coefficients of variation of individual values of plasma hormone levels (Table 1) showed that in all states the highest variability within the group was observed for CS and ACTH, and much lower for BE.

To determine correlations between changes in the blood CS, ACTH, and BE concentrations the coefficient of linear correlation of paired values of these substances in the experimental animals was used. The closest correlation was found between CS and BE levels ($r = 0.711$; $P < 0.001$), weaker between CS and ACTH ($r = 0.656$; $P < 0.001$), and weakest of all between BE and ACTH ($r = 0.510$; $P < 0.01$). Differences in the strength of correlation between CS–BE and BE–ACTH were significant ($P < 0.05$).

Similar correlations between blood CS and BE levels were found previously at different times of the 24-h period [14], and also during stress of different intensities, caused by electric shocks of increasing strength [7]. These data, together with differences in the dynamics of the ACTH and BE concentrations discovered in the present experiments in the initial periods of immobilization stress, and the weakest correlation between the levels of these hormones in individual animals may indicate differences not only in the regulation of secretion of ACTH and BE, but also in the degree of their utilization and inactivation during the development of stress.

Investigation of hormonal responses arising during the development of a negative emotional response due to relatively brief exposure of the animals to ecologically meaningful stimuli arising from other rats (Table 2), revealed a significant and marked rise in the blood levels of all three hormones. The most significant changes were found with CS (by 5.5 times), less marked for ACTH (by 3.3 times), and minimal for BE (by 1.4 times).

Much evidence has been obtained recently of the high variability of glucocorticoid levels in waking animals during various experimental procedures, and their close correlation with the level of behavioral activity and the degree of emotional excitation during changes in the external environment (its novelty). For instance, simply moving rats from the familiar cage into a new one led to a three-fourfold rise in the blood CS level up to 18-22% [7, 10]. The highest sensitivity of the adrenals to ACTH also is known to be observed in the presence of physiological blood levels, and sensitivity falls at ACTH levels characteristic of stress [10].

Relatively short exposure of animals to weak but ecologically meaningful factors can thus induce significant changes not only in CS and ACTH concentrations, but also in the BE level. Significant differences in the concentrations of these hormones in rats after immobilization for 10-20 min and after exposure to ecologically meaningful stimuli for 5-15 min (Tables 1 and 2) are evidence of differences in the character and intensity of the hormonal responses depending on the type of stress-inducing factor. These differences may also be connected with the character of changes in the animals' behavior in these two different models of exposure to stress. For the initial periods of immobilization, for instance, a sharp increase in motor activity of the rats was characteristic, whereas ecologically adverse stimulation was not accompanied by any significant change in motor activity or caused it to be inhibited.

The results also explain to some extent the considerable fluctuations of "background" plasma CS levels in intact animals cited in the literature – from 2 to 30 mg% [7, 8, 10, 13]. These fluctuations are probably largely due to technical differences during blood sampling and with disregarding of experimental factors that are extremely informative for animals.

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EFFECT OF PROSTAGLANDIN E_2 ON ACTIVITY OF SOME ENZYMES DURING PARENTERAL FEEDING

R. M. Glantsov and L. A. Lyubinets

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The writers demonstrated previously the anabolic effect of prostaglandin E_2 (PGE_2) during parenteral feeding (PF) [3]. Considering that the principal processes during PF takes place at organ-tissue, subcellular, and molecular levels [1, 2] and do so in accordance with the mediator–enzyme–tissue or hormone–enzyme–tissue principle, and also considering relations between prostaglandins and enzymes [4], it was decided to study the effect of PGE_2 on activity of aspartate aminotransferase (AaAT), alanine aminotransferase (AlAT), and aldolase, which play important roles in the mechanisms of anabolic effects at the organ-tissue level.

EXPERIMENTAL METHOD

Experiments were carried out on 65 male albino rats weighing 180-250 g. AsAT and AlAT were determined by the method in [6], and aldolase by the method in [5] (activity was expressed in micromoles/g wet weight of tissue). The tests were undertaken on objects important for PF such as the liver and striated muscles.

The experimental animals were kept for 6 days on a nonprotein diet, consisting of starch, sugar, yeast, salt mixture, and vitamins. Against the background of this diet on the 7th day of the experiment rats of group 1 received an intramuscular injection of physiological saline, rats of group 2 received casein hydrolysate in a dose of 0.3 g conventional protein per 100 g body weight, and rats of group 3 received casein hydrolysate and PGE_2 (prostenon, USSR), synthesized in the Sector of Pure Substances, Institute of Chemistry, Academy of Sciences of the Estonian SSR, under the direction of Professor Yu. E. Lille, PGE_2 was injected intramuscularly in a dose of 40 μg/100 g body weight. The experimental results were compared with data obtained on healthy rats, kept on the usual animal house diet, and on animals kept on a nonprotein diet and receiving injections of physiological saline.

Experimental Division, L'vov Institute of Nematology and Blood Transfusion. (Presented by Academician of the Academy of Medical Sciences of the USSR O. K. Gavrilov.)

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