BLOOD ACTH AND CORTISOL LEVELS IN EXPERIMENTAL FOCAL EPILEPSY

T. V. Goryacheva, M. N. Karpova,
R. N. Glebov, I. O. Mikhal'skaya,
M. E. Kislova, and S. Yu. Titov

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It was shown previously [1] that the blood plasma level of ACTH and also of other hormones and peptides is considerably elevated at the height of development of generalized epileptic activity (EA) induced by injection of metrazol into rats. Changes in the hormonal balance in EA are still largely unexplained, and the available facts are contradictory [6]. When the role of hormones and neuropeptides in the development and suppression of EA is studied it is important to consider the influence of secondary nonspecific factors (stress, hypoxia, etc.), characteristic of the seizure process in EA. From this aspect it is important to investigate plasma hormone levels in the course of development of EA, including on different experimental models of epilepsy.

The aim of the present investigation was to assess the role of stress hormones (ACTH and cortisol) in the development and disappearance of seizure disorders on experimental models of focal EA with a minimal stresss component.

EXPERIMENTAL METHOD

There were two series of experiments. The experiments of series I were conducted on 87 male Wistar rats weighing 180-200 g. Using a technique described previously [2], 24 h before the experiment a burr-hole (2 × 4 mm) was drilled in the animal's skull above the sensomotor area of the cortex and monopolar cortical silver electrodes were applied to record electrical activity from the region of the cortex indicated.

Experiments of series II were carried out on 7 cats of both sexes, weighing 2.5-3.5 kg, anesthetized with pentobarbital (25-35 mg/kg, intraperitoneally). After trephining of the skull and catheterization of the external jugular and femoral veins the animals were immobilized with D-tubocurarine (0.1-0.2 mg/kg, intravenously) and artificially ventilated.

An epileptic focus was created by application of filter paper soaked in a solution of the Na salt of benzylpenicillin (12,000 IU/ml) to the sensomotor cortex of rats, and 20,000 IU/ml to the orbital or coronal gyrus of cats. The electrocorticogram (ECoG) was recorded on an EE 68 electroencephalograph (Hungary), the rats with a focus of EA being unrestrained during the recording. Amplitude versus frequency characteristics were determined. The power of the epileptic focus was calculated by multiplying the discharge frequency by their amplitude and expressed in relative units (rel. u.). Application of penicillin (12,000 or 20,000 IU/ml) led to the appearance of EA: after 2-3 min, against the background of the spontaneous ECoG, separate epileptic discharges appeared and their amplitude gradually increased. After 15-20 min EA of stable frequency and amplitude was generated in the newly appearing epileptic focus, and continued for 25-45 min, after which the amplitude and frequency of the discharges decreased. The mean life span of the epileptic foci from application until complete disappearance of the epileptic discharges was 100 min for rats and 140 min for cats. The clinical picture of activity of the foci of EA showed that for every spike discharge there were corresponding myoclonic spasms of the fore and(or) hind limbs and of the neck muscles and muscles of mastication in rats.

The rats were killed by decapitation at the height of development of EA in the cortex (20-30 min after application of penicillin) and 2-3 h after cessation of EA in the cortex.

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TABLE 1. Changes in Plasma ACTH and Cortisol Levels of Rats at Height of Development and after Cessation of EA Induced by Penicillin Application (12,000 IU/ml) to Cerebral Cortex Compared with Animals Undergoing Mock Operations and Intact Animals (M ± m)

Control intact animals (n = 6)		Mock operation (20-30 min after beginning of recording ECoG; n = 10)		At height of development of EA (20-30 min after application of penicillin; n = 12)					Mock operation (4-5 h after beginning of recording ECoG; n = 14)		After cessation of EA (after 2-3 h; n = 14)	
ACTH, pg/ml	cortisol, ng/ml	ACTH, pg/ml	cortisol, ng/ml	no. of dischg./min	ampl. of dischg., mV	power of focus, rel.u.	ACTH, pg/ml	cortisol, ng/ml	ACTH, pg/ml	cortisol, ng/ml	ACTH, pg/ml	cortisol, ng/ml
37,2 ±5,8	10,8 ±0,6	59,0 ±1,1	11,0 ±1,2	14,0 ±1,1	0,84 ±0,03	12,0 ±1,1	132,0 ±3,5*	17,0 ±2,5*	46,0 ±3,9	15,0 ±2,5	60,0 ±4,5	12,0 ±3,0

Legend. Asterisk indicates data for which $p < 0.05$ compared with initial experimental values; n) number of animals in series.

TABLE 2. Changes in Plasma ACTH and Cortisol Concentration in Cats during Development of EPA (M ± m)

Background			At height of development of EA					During decline of EA					After cessation of EA	
No. of cat	ACTH, pg/ml	cortisol, ng/ml	number of discharges/min	amplitude of discharges, mV	power of focus, rel.u.	ACTH, pg/ml	cortisol, ng/ml	number of discharges/min	amplitude of discharges, mV	power of focus, rel.u.	ACTH, pg/ml	cortisol, ng/ml	ACTH, pg/ml	cortisol, ng/ml
1	133	197	14	1,9	26,8	10	46	17	0,9	14,6	309	222	347	250
2	389	344	11	1,4	15,6	195	377	10	0,4	4,2	69	334	93	327
3	838	499	19	2,8	54,1	366	318	8	0,3	2,1	306	279	681	289
4	150	79	12	1,4	16,6	29	65	14	0,3	4,3	17	57	458	125
5	366	139	12	1,8	20,6	627	199	8	0,5	3,9	787	250	390	251
6	106	88	17	2,8	47,3	286	214	11	0,8	8,4	204	150	213	105
7	158	97	9	1,8	16,5	206	119	8	0,3	2,2	524	95	843	130

Animals undergoing mock operations and also intact rats served as the control. Blood was collected in cold plastic test tubes with a 6% solution of EDTA-Na_2 (0.1 ml of 5 ml of blood). Blood samples were centrifuged without delay at 1000g for 15 min at 4°C. Plasma samples were kept at −20°C until the time of determination of the hormones (not more than 30 days). Blood was taken from the jugular vein of the cast through a catheter. In each of 7 cats four blood samples taken before application of pencillin (background), at the height of development of EA in the cortex, during the period of quenching of the focus, i.e., the decline of EA, and 5-10 min after cessation of EA in the cortex, were investigated. Taking of the blood, separation of the plasma, and its preservation followed the same rules as for rats. ACTH was determined by radioimmunoassay using kits of reagents manufactured by CEA-Sorin (France) and cortisol was determined with the aid of Soviet "Steron $K^{125}I$" kits.

EXPERIMENTAL RESULTS

The results showed (Table 1) that the plasma ACTH level in rats at the height of development of focal EA was twice as high as the level of this hormone in the blood of animals undergoing mock operation, and three times higher than its concentration in the blood of intact rats. The blood level of cortisol at the height of development of EA was rather higher than the control ($p < 0.05$). The ACTH and cortisol concentrations in the blood plasma 2-3 h after cessation of EA were indistinguishable from the control values. Incidentally, the mock operations led to a small but significant rise (by 50%) of the plasma ACTH (but not cortisol) level compared with that of intact rats.

Thus in unrestrained animals the appearance of a focus of EA in the cerebral cortex induces the release of ACTH into the blood stream, and this may result in a rise of the blood cortisol level.

Our previous investigations [1] showed massive release of ACTH into the blood stream of rats with generalized metrazol-induced EA, five or six times more marked than in the control

as early as 90-150 sec after exposure to the epileptogen, during clonicotonic convulsions, followed 30 min later by a significant rise of the plasmas cortisol level. The fact that not only generalized EA with a powerful stressor component, but also focal epilepsy in unrestrained animals, is accompanied by elevation of the blood ACTH concentration, followed by that of cortisol, evidently indicates that this response of the pituitary gland during epileptic fits arises not only under the influence of the stress factor, but also at the minimum of the stress reaction, i.e., it exhibits features of specificity of epileptization of the neurons. The effect of a focus of EA created in the cerebral cortex on release of ACTH by pituitary cells may be mediated through the spread of excitation from the cortex to structures of the hypothalamus, and thereafter through the intervention of releasing factors acting on the pituitary, and resulting in ACTH release. The degree of ACTH release by the adenohypophysis evidently correlates positively with the degree of excitatory influences from the cerebral cortex. For instance, generalized EA leads to a five- to sixfold rise of the blood hormone level, whereas focal EA only doubles the blood ACTH level.

The time course of changes in the blood levels of these hormones in immobilized and anesthetized cats was rather different from that of rats (Table 2). During peak EA in the cerebral cortex the plasma ACTH level was elevated in 3 of 7 animals but the cortisol level was high in only one. In the period of decline of EA the plasma ACTH concentration was raised in 4 of 7 animals but the cortisol level was high in only 3 cats. In 2 cats EA in the cerebral cortex was not accompanied by elevation of the ACTH and cortisol concentrations above the background level. In one of them (No. 2) the focus of EA had the lowest power, whereas in the other (No. 3) the background ACTH level was exceptionally high (more than four times the average value).

After cessation of EA in the cerebral cortex the plasma ACTH level was on average 2.5 times higher than initially in 5 of the 7 animals. The plasma cortisol concentration in these five cats was on average 1.4 times higher than initially.

In 6 of the 7 animals the plasma ACTH concentration was higher after cessation of cortical EA than during the period of quenching of the focus, i.e., during the decline of EA.

Focal EA in anesthetized and immobilized cats, just as in unrestrained rats, is thus accompanied by elevation of the ACTH and cortisol levels in the blood, evidence that this phenomenon is connected with EA and not with the stress component in the case of generalized convulsions. These data alone are insufficient to answer the question whether the hormones mentioned are pro- or anti-epileptogens. There is no general agreement likewise in the literature on this matter. It has been suggested [7] that the three- to fourfold rise of the plasma ACTH level observed in patients in the course of 1 h after a seizure may be a hormonal marker of an epileptic fit. There is also evidence of a connection between convulsive disorders in man and elevation of the plasma cortisol (and prolactin) level after a seizure [3, 4]. Some workers consider that only seizures with a marked motor component are a factor in the rise of the blood cortisol level [9]. According to some workers ACTH can potentiate EA evoked by electric shock [8], and it can facilitate epileptic kindling in animals [11]; fragments ACTH 1-10, 4-10, and 4-9, injected intraventricularly into rabbits, can induce EA [13]. According to other data, however, ACTH exhibited some degree of anticonvulsant activity in rats during kindling [12] and had weak anticonvulsant and sedative action in gerbils when given by intraventricular injection [5]. We also know that in some forms of epilepsy in children treatment with ACTH and corticosteroids gives a positive effect [6, 10], although the mechanism whereby this effect is achieved has not been studied.

A fact that deserves attention in the results of the present study in that elevation of the plasma ACTH level at the height of development of EA in the cortex was observed in only some of the anesthetized and immobilized cats, and the peak of the rise of the plasma ACTH concentration was observed after cessation of EA. The absence of elevation of the plasma ACTH level in some of the anesthetized and immobilized animals is probably connected with disturbance of the transmission of excitation to the pituitary, due to the action of general anesthetics, as a result of which the plasma ACTH level actually was observed to fall during a period of marked EA in the cortex, evidence that even those influences on the pituitary gland that are essential for maintaining the background level of ACTH release into the blood stream had ceased.

The "delayed" rise of the plasma ACTH level after cessation of EA in the cerebral cortex may possibly be due to the accumulation of substances in the brain tissue during functioning of the focus of EA which can induce the release of ACTH into the blood stream after disappearance of the effect of the anesthetic.

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ROLE OF ADRENERGIC MECHANISMS IN REGULATION OF VENULAR PERMEABILITY DURING SHORT- AND LONG-TERM IMMOBILIZATION

M. P. Gorizontova

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Immobilization leads to phasic disturbances of vascular permeability in various organs [1, 2, 5]. The writer's previous investigations [3] showed that catecholamines can play different (depending on the duration of immobilization) roles in the regulation of venular permeability. At the same time, there is evidence that β-agonists are able to reduce venular permeability when disturbed by histamine, bradykinin, and prostaglandins [10, 12, 14], i.e., by biologically active subtances which accumulate in excess in the tissues during stress.

The aim of this investigation was to study the role of α- and β-adrenergic mechanisms in the regulation of venular permeability during short- and long-term immobilization.

EXPERIMENTAL METHOD

Experiments were carried out on 169 male Wistar rats weighing 200-250 g. The animals were immobilized in the supine position for 30 min (short-term immobilization) or 24 h (long-term immobilization).

Quantitative evaluation of venular permeability in the rate mesentery was carried out by contact luminescence biomicroscopy (CLB) based on the LYUMAM KF-1 microscope (Leningrad Optical-Mechanical Combine). Rabbit globulin labeled with fluorescein isothiocyanate (FITC) was used as indicator of disturbances of venular permeability.

Biomicroscopic evaluation of the state of the microcirculation conducted on an apparatus based on the "Docuval" microscope (Carl Zeiss, East Germany).

The morphological and functional state of the mesenteric mast cells (MC) was determined after fixation of the tissue with 96° alcohol and staining with 0.5% toluidine blue.

Laboratory of General Pathology of the Microcirculation, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.
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