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CHANGES IN PLASMA HORMONE AND PEPTIDE CONCENTRATIONS IN RATS WITH EXPERIMENTAL EPILEPSY

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UDC 616.853-092.9-07:[616.154:577.175]-074

KEY WORDS: epileptic activity, metrazol, rat blood plasma, ACTH, renin-angiotensin system.

The mechanisms and pathogenetic importance of changes in blood hormone levels during epileptic activity (EpA) remain largely unexplained [2, 3, 6]. In the study of this problem, attention must be paid to the effect of secondary nonspecific factors (stress, anoxia, etc., for example) during the seizure process on changes in blood hormone levels. From this aspect it is important to determine hormone levels at different stages of development of the epileptic process, starting with the latent period and until the onset of convulsions.

In this investigation plasma levels of seven hormones were studied in rats during the development of generalized convulsive EpA induced by metrazol.

EXPERIMENTAL METHODS

Experiments were carried out on noninbred male rats weighing 200-220 g, kept under standard animal house conditions (temperature 22 ± 2°C, alternation of 12 h daylight and 12 h darkness, standard diet). The experiments were carried out in the spring and summer. An experimental epileptic syndrome was induced by intraperitoneal injection of a 10% solution of metrazol in a dose of 75 mg/kg; control animals received the same volume of physiological saline [4].

Blood was taken during the latent period, namely 30 sec after injection of metrazol in the absence of any clinical signs of seizure activity or electrocorticographic manifestations of EPA, 90-150 sec after injection of metrazol during the development of clonico-tonic convulsions with the animals falling on their side and with a marked tonic extension phase, and 5-10 and 30 min after injection of metrazol, during continuing EpA. The duration of EpA in live animals lasted 9-11 hours. Blood was taken at the same time of day in order to exclude

Laboratory of Biochemistry, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 6, pp. 670-672, June, 1988. Original article submitted December 14, 1987.

0007-4888/88/0006-0793$12.50

TABLE 1. Changes in Blood Plasma Hormone Levels of Rats during Development of Generalized Metrazol-Induced EpA (M ± m)

Hormone	Experimental conditions	Group of animals			
		1 (latent period, 30 sec after injection of metrazol)	2 (90-150 sec after injection of metrazol)	3 (5-10 min after injection of metrazol)	4 (30 min after injection of metrazol)
ACTH, pmoles/liter	Control	37,2±5,8 (6)	40,6±5,7 (21)	34,6±5,4 (7)	35,7±4,4 (10)
	Experiment	43,9±7,0 (7)	236,9±32,1* (14)	180,9±7,1 * (5)	91,5±10,6* (10)
Aldosterone, nmoles/liter	Control	2,3±0,1 (7)	2,6±0,1 (9)	3,1±0,3 (7)	3,1±0,3 (8)
	Experiment	3,0±0,3 (7)	3,3±0,4 (7)	3,8±0,7 (7)	9,5±0,5* (7)
Vasopressin, pg/ml	Control	15,6±0,3 (6)	101,3±21,6 (7)	—	34,5±13,1 (7)
	Experiment	20,3±4,0 (6)	132,3±18,1 (7)	—	69,3±10,8* (6)
Renin, ng/ml/h	Control	7,8 (14)	3,1 (14)	—	2,9 (7)
	Experiment	11,9 (14)	11,6** (14)	—	3,2 (7)
Angiotensin I, ng/ml	Control	3,0 (14)	3,3 (14)	—	6,1 (7)
	Experiment	6,6 (17)	6,1** (15)	—	4,9 (10)
Insulin, μU/ml	Control	47,2±5,9 (6)	81,3±6,9 (6)	—	108,3±7,6 (6)
	Experiment	70,1±7,1 (6)	62,8±6,1 (8)	—	87,8±11,3 (8)
Glucagon, pg/ml	Control	80,5±4,2 (6)	82,0±3,0 (7)	—	84,8±16,5 (6)
	Experiment	67,4±5,8 (6)	150,7±10,3* (11)	—	59,0±5,5 (8)

Legend. *$p < 0.05$ by Student's test compared with control, **$p < 0.05$ for Wilcoxon–Mann–Whitney nonparametric U test. Number of experiments given in parentheses. Plasma vasopressin level in intact animals not receiving 0.14 M NaCl was 15.6 ± 1.4 pg/ml (5), renin level 2.1 ± 0.3 ng/ml (6) per hour, and angiotensin I 2.1 ± 0.5 ng/ml (7).

any influence of circadian rhythms on the parameters determined. The rats were killed by decapitation and blood was collected in cold plastic test tubes containing 6% EDTA-Na_2 solution (0.1 ml to 5 ml of blood). The blood samples were quickly centrifuged for 15 min at 3000g and 4°C. Plasma samples were kept in plastic tubes until the time of determination, for not more than 30 days at −20°C. Hormone concentrations and renin activity in the plasma were studied by standard methods of radioimmunoassay; kits from Oris (France) were used to determine ACTH, from Bühlmann Laboratories AG (Switzerland) for vasopressin assay, from CEA-Sorin (Fracne-Italy) to determine aldosterone and activity of the renin-angiotensin system, and from Biodatda (Itly) to determine glucagon; insulin and cortisol concentrations were determined by kits of USSR origin, in the latter case with the Sterone-K^{125} I kit.

EXPERIMENTAL RESULTS

Data on hormone concentrations and renin activity in the blood plasma during development of the epileptic syndrome are given in Table 1. The ACTH concentration remained unchanged during the latent period of EpA but rose sharply during EpA development (by 5.2-5.8 times; $p < 0.05$). There was virtually no difference in the ACTH level in the animals of groups 2 and 3. In the rats of group 4, 30 min after injection of metrazol the ACTH level remained higher than in the control (2.6 times higher; $p < 0.05$), although it was a little slower than in the rats of groups 2 and 3. The plasma aldosterone level was unchanged during metrazol convulsions in the animals of the first three groups, but was increased threefold in the rats of group 4. The plasma cortisol level in rats is known to be rather on the low side, but the method of radioimmunoassay which we used was sensitive and was able to reveal definite amounts of this adrenocortical hormone. The plasma cortisol level was unchanged during EpA in the rats of the first three groups, and not until 30 min after injection of the epileptogen was it found to be raised (by 1.7 times; $p < 0.05$).

Activity of the renin-angiotensin system in the blood plasma of the control rats remained virtually unchanged during 30 min after injection of 0.14 M NaCl and was comparable with the renin activity and angiotensin I concentration in intact animals not receiving physiological saline. However, the vasopressin level in the control animals was considerably increased, especially 90-150 sec after intraperitoneal injection of 0.14 M NaCl (Table 1). Later the values of this parameter during EpA in the experiments was compared with the corresponding control values for each group of animals.

The plasma vasopressin level was raised during EpA only when 30 min had elapsed after injection of metrazol. The renin activity and angiotensin I level were raised during EpA only in the rats of group 2 (i.e., 2 min after injection of the epileptogen). Normal values of these parameters were restored 30 min after injection of metrazol.

The plasma insulin level in the rats of groups 1, 2 and 4 remained unchanged during EpA, and elevation of the glucagon level was observed only in the animals of group 2 (by 1.8 times, $p < 0.05$).

Analysis of the results draws attention to the state of the pituitary-adrenal system during metrazol convulsions. The significant rise of the plasma ACTH concentration during metrazol convulsions can be explained by hyperactivity of the pituitary cells (and also of ACTH-containing neurons) and by secretion of this hormone into the blood. ACTH is known to accumulate in the CSF of patients with temporal epilepsy [1]. It is also well known that there are cases where ACTH has been used in treating epilepsy [1]. In connection with this fact, which remains difficult to explain, it is worth noting a recently expressed view that ACTH may be a factor in adaptation of the brain to stress [8]. Our own observations are further evidence of the complexity of the interpretation of data on the role of ACTH in the development and suppression of the epileptic syndrome: in three rats weighing 300-350 g, which had greater sensitivity to metrazol, the convulsions led quicky (after 5-7 min) to the animals' death. The ACTH concentration in the blood plasma of these rats, decapitated immediately before death, was the same as in the control.

If accumulation of ACTH in the blood during metrazol EpA can be explained by the action of stress, elevation of the blood level of corticosteroids may also be expected due to their increased secretion and resynthesis in cells of the adrenal cortex. Plasma levels of two hormones of these glands, namely cortisol and aldosterone, were unchanged in the early stages of EpA. Not until 30 min after injection of metrazol was the cortisol level observed to rise. This delayed effect can be explained by the short time interval for manifestation of the action of ACTH on the adrenal cortex. In human patients [5, 7] and also in monkeys with generalized EpA, caused by injection of bicuculline, cortisol accumulates in the blood plasma [9], and in the latter case it appears 35 min after intravenous injection of the epileptogen.

Activation of the renin-angiotensin system and elevation of the plasma vasopressin level during EpA may be connected with disturbance of osmotic homeostasis of the animal and, in particular, with sodium deficiency and hyponatriemia, which in turn leads to lowering of the seizure threshold, i.e., it potentiates epileptization of the neurons [11]. In monkeys with generalized EpA, 14 min after intravenous injection of bicuculline, these experiments, like our own, showed a considerable increase in the plasma glucagon concentration, which then quickly returned to normal. A delayed, slow rise of the plasma insulin level was observed in these same experiments [9, 10].

The facts require verification with the use of other ways of producing experimental epilepsy, such as focal EpA in the brain, for metrazol, the epileptogen which we used, may give rise to various effects and, in particular, it may modify the behavioral responses of the animal. Accumulation of certain hormones in the blood up to a critical level, it will be recalled, may intensify or weaken EpA in the brain through a feedback system (penetration of hormones into the brain when the permeability of the blood-brain barrier is increased) [2, 3].

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