AGE AND REGIONAL CHANGES IN ANGIOTENSIN-CONVERTING AND KININ-DEGRADING ACTIVITY IN THE BRAIN OF AGGRESSIVE RATS

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Aggressive behavior is regarded as one form of hyperemotionalism, which is regulated with the participation of a complex system of various neurochemical mechanisms. Analysis of investigations conducted on experimental models of aggressiveness has demonstrated: 1) the participation of various chemical factors, including classical neurotransmitters, neuropeptides, and hormones, but without any demonstrable order in the relations between them, 2) absence of specificity of any group of these regulators for particular forms of aggressive behavior, 3) the small number of investigations of the age and regional characteristics of changes in activity of regulatory substances, or of the enzymes participating in their metabolism, which have been undertaken.

In the present investigation, a model of muricidal behavior ("mouse killing"), which allows precise quantitative testing, was chosen to study aggressive behavior. This model has been sufficiently widely used experimentally to study biochemical and physiological mechanisms of aggressiveness and to find drugs capable of modulating that behavior. Despite many publications establishing the importance of GABA, serotonin, monoamines, etc. [3-5, 10, 12], information on the participation of regulatory peptides in the formation and maintenance of different forms of aggressive behavior is still lacking. The results described below characterized changes in activity of the enzymes involved in angiotensin and bradykinin metabolism in certain parts of the brain of muricidal rats of different ages.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing initially 140-160 g (age 1-1.5 months), kept on a standard animal house diet without restriction of water and food, in individual cages measuring 15 × 15 × 32 cm. Electrolytic destruction of the paraseptal region of the brain was carried out on animals anesthetized with ether by means of a unipolar electrode, with a current of 1-2 mA at a voltage of 15 V; the duration of the procedure was 15 sec. The mortality of the animals after the operation was 5-7%.

Testing for aggressiveness (muricidal behavior) was done in accordance with the scheme [8], normally used in work with this model: an albino mouse weighing 15-20 g was placed in the same cage as a fed rat. If in the course of 2 min the rat, which had undergone the operation, attacked the mouse, biting it on the neck and head, it was considered to be muricidal. The whole group of rats undergoing the operation was retested every 5-7 days regardless of the results of previous testing. The following criteria of muricidal behavior of the animals were used: the number of muricidal rats as a percentage of the total group undergoing the operation, the latent period before the attack on the mouse, and the length of time the victim was held by the muricidal rats. To obtain a more reliable estimate of muricidal behavior, during each test another living mouse was placed in the cage with the rat and the latent period to the second attack was measured.

The following parts of the brain were removed from the rats in accordance with the scheme [7] after decapitation for biochemical testing: the pituitary, cerebellum, hypothalamus, and corpus striatum. The material was kept in plastic plates at −20°C until required for study of its enzymic activity. Activity of angiotensin-converting enzyme (ACE, dipeptidyl-carboxypeptidase) was determined by the method in [6] in our modification [11], using

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TABLE 1. ACE (in nmoles His-Leu/min/mg protein) in Brain Zones of Muricidal Rats (M ± m)

Brain zone	Rats aged 2 months			Rats aged 7 months	
		undergoing operation		undergoing operation	
	intact (n = 7)	nonmuricidal (n = 7)	muricidal (n = 9)	nonmuricidal (n = 4)	muricidal (n = 7)
Pituitary	1,78±0,09	2,38±0,11**	2,16±0,29	6,65±1,13***	4,63±0,26***
Cerebellum	1,42±0,11	1,49±0,18	2,24±0,25*	2,34±0,22***	1,75±0,33
Hypothalamus	0,77±0,08	0,60±0,12	0,05±0,05*	2,60±0,13***	2,04±0,51***
Corpus striatum	3,33±0,30	3,15±0,37	1,94±0,09*	4,98±0,93	2,65±0,52

Legend. *p < 0.05 compared with nonmuricidal rats (here and in Table 2); **p < 0.05 compared with intact animals; ***p < 0.05 compared with animals of the same group aged 2 months.

TABLE 2. Total KDA (in nmoles bradykinin/min/mg protein) in Brain Zones of Muricidal Rats (M ± m)

Brain zone	Rats aged 2 months			Rats aged 7 months	
		undergoing operation		undergoing operation	
	intact (n = 7)	nonmuricidal (n = 7)	muricidal (n = 9)	nonmuricidal (n = 4)	muricidal (n = 7)
Pituitary	4,93±0,48	5,02±0,50	9,96±0,52*	4,29±0,12	4,37±0,20
Cerebellum	6,20±0,39	7,48±0,88	7,18±0,44	2,25±0,46	1,84±0,23
Hypothalamus	5,49±0,36	4,33±0,15	9,79±0,49*	2,35±0,22	1,86±0,11*
Corpus striatum	5,49±0,38	5,97±0,36	8,95±0,41*	2,52±0,23	2,11±0,13

Legend. Activity in rats aged 2 months was determined in supernatant fraction after centrifugation at 10,000g for 15 min, in rats aged 7 months — in homogenate.

hippuryl-histidyl leucine (from "Serva," West Germany) as the substrate. Since several enzymes capable of hydrolyzing the bradykinin molecule have been identified in brain tissue, the method in [2, 9], based on determination of the final quantity of unreacted bradykinin after fractionation on a cellulose phosphate column, was used to estimate total kinin-degrading activity (KDA). Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

Local electrolytic destruction of the paraseptal zone of the telencephalon led to the appearance of muricidal behavior in the rats. The time course of development of muricidal behavior is illustrated in Fig. 1. Starting from the 3rd postoperative day the indicators of muricidal activity gradually changed: the percentage of muricidal rats increased from 10 to 42 by the end of the 3rd week, the latent periods before attacks on the mouse decreased, and the time of holding the victim increased. Maximal values of all the above parameters, reached at the time indicated in Fig. 1, were maintained at this level for a long time. Regular testing showed conclusively that the high degree of aggressiveness was still maintained by the majority of muricidal rats 6 months after the operation.

With this fact in mind, biochemical tests were carried out in two groups of rats aged 2 and 7 months undergoing the operation. Each group contained rats which had or had not developed muricidal behavior. The data in Table 1 show that ACE activity in rats aged 2 months, which had not developed muricidal behavior after the operation, was the same as that in intact animals. Only in the pituitary was there found a small increase in ACE activity due to the operation. In muricidal rats of this age, ACE activity showed a significant change: it was increased by 50% in the cerebellum, and reduced by 91.6 and 38.4% in the hypothalamus and corpus striatum respectively. These changes are significant when compared both with animals developing or not developing muricidal behavior after the operation and with intact rats.

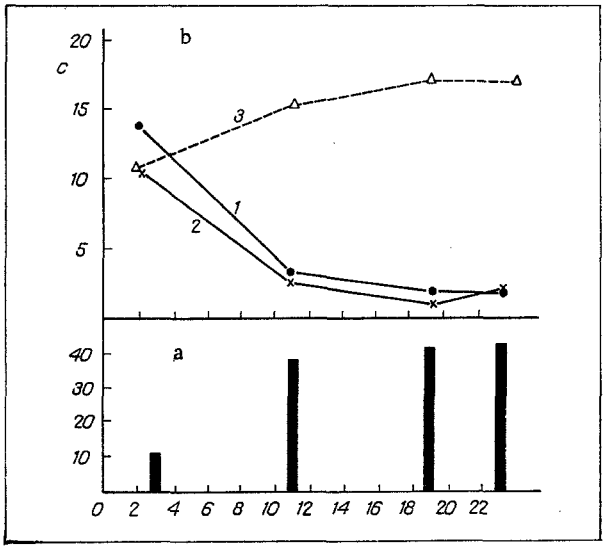

Fig. 1. Time course of muricidal response of rats after electrolytic destruction of the paraseptal zone of the brain. Abscissa, time after operation (in days); ordinate, percentage of muricidal animals (a) and change in temporal characteristics (in sec) of muricidal response (b). 1) Latent period to 1st attack, 2) latent period to 2nd attack, 3) time of holding victim. Mean results for 127 rats are shown.

Another series of tests was carried out on muricidal and nonmuricidal rats 6 months after the operation of destruction of the paraseptal zone. In these animals the ACE level was increased in all brain zones and in the pituitary, to correspond to the trend of age-related changes in activity of this enzyme [1]. However, the difference between muricidal and nonmuricidal rats aged 7 months was now virtually not significant. There was only a tendency for the ACE level in the pituitary and corpus striatum of the muricidal animals to fall.

The results of determination of KDA in the brain zones of the muricidal animals are given in Table 2. In rats aged 2 months the development of muricidal behavior was seen to be associated with a considerable rise of enzyme activity: by 98% in the pituitary, by 149% in the hypothalamus, and by 50% in the corpus striatum. Determination of these same parameters in rats aged 7 months showed that, just as with ACE, preservation of aggressive behavior in the mature animals was not associated at that stage with changes in KDA in the brain regions studied.

Two main conclusions can be drawn from these results. First, the formation of aggressive behavior in young rats undergoing this brain operation is accompanied by changes in ACE and KDA activity in the majority of brain regions studied. Second, in rats aged 7 months, although their muricidal behavior still persisted, activity of the above enzyme systems did not differ from that in the corresponding control. Moreover, the ACE level in adult muricidal rats in the pituitary and hypothalamus was significantly higher than in muricidal rats aged 2 months. If ACE activity is projected on changes in the angiotensin II concentration in the corresponding brain regions, it must be assumed that aggressive behavior itself cannot be directly connected with a rise or fall of the peptide level. More complex temporal and regional relationships are evidently involved.

There is no doubt that the formation of muricidal behavior and persistence of this form of aggressive behavior for certain lengths of time are brought about by a complex system of biochemical and neurophysiological mechanisms. However, whatever the case, an initial moment in the development of muricidal behavior in the rats must be distinguished, which is accompanied by a change in neuropeptidase activity that indicates a fall in levels of both angiotensin and bradykinin, exhibited primarily in the hypothalamo-hypophyseal region of the brain. Subsequent persistence of this form of aggressive behavior (in this case for 6 months) now involves the participation of other neurochemical mechanisms, in which the role of angiotensin and bradykinin is not so evident.

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EFFECT OF METRAZOL-INDUCED EPILEPTIC ACTIVITY ON TRANSPORT Ca-ATP-ASE ACTIVITY IN RAT BRAIN SYNAPTIC MEMBRANES

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According to the membrane hypothesis of the pathogenesis of epileptic activity (EA) of the brain, the creation of a generator of pathologically enhanced excitation (GPEE) of neurons may be attributed to specific disturbances of the structure of neuronal membranes, leading to the opening of additional ionic channels or to the longer existence of these channels in the open state [3, 5, 7]. Under these conditions the accumulation of intracellular Ca^{++} (probably up to 10 μM) facilitates prolonged secretion of excitatory mediators by hyperactive neurons, i.e., the conditions are created for long-term, persistent depolarization of the neurons. It was shown previously [2, 6] in the writer's laboratory that inactivation of electrogenic Na,K-ATPase of synaptic membranes is an important (evidently the trigger) factor in epileptization of neurons. This phenomenon corresponds in principle to the fact that inactivation of the electrogenic Na,K-pump may be the trigger factor coupling depolarization with transmitter secretion by nerve endings [4].

It can be tentatively suggested that pathological hyperactivity of neurons may also be maintained by the fact that the systems for the outflow of Ca^{++} which accumulates in the synaptoplasm are "switched off" because of structural changes in the membrane of nerve endings. In other words, inactivation of the Ca-pump and (or) the direct Na^{+}/Ca^{++} exchange system leads to increased efficiency of the secretory process of hyperactive neurons.

The aim of the present investigation was accordingly to study the state of Ca-ATPase activity of rat brain synaptic membranes at different stages of development of generalized EA induced by injection of metrazol.

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