CHANGES IN ANGIOTENSIN CONVERTING ENZYME ACTIVITY IN RAT BRAIN STRUCTURES AFTER FRONTAL LOBECTOMY

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It has been shown [3, 4] that damage to the frontal region of the cerebral cortex in rats is accompanied by disturbances of conditioned reflex behavior and also by corresponding changes in function of the monoaminergic system of the brain. The most marked changes in monoamine metabolism have been observed on the 9th day after damage to the frontal cortex. The action of drugs on monoamine metabolism (administration of 5-hydroxytryptophan, the serotonin precursor in the brain, and of p-chlorophenylalanine, an inhibitor of tryptophan hydroxylase) had a significant effect on restoration of conditioned-reflex connections when disturbed by frontal lobectomy. Data on functional interaction of the monoaminergic and renin-angiotensin systems of the brain, which is reflected in the regulation of catecholamine secretion by angiotensin, and dependence of the action of the peptide on the sensitivity of dopamine receptors [7-9, 11], have been published in recent years. Isolated studies have demonstrated the involvement of angiotensin II in learning and memory processes [6]. These observations are in agreement with the concept of the polyfunctionality of action of the regulatory peptides and, in particular, of angiotensin II, i.e., the ability of the same peptides to exert their influence on different physiological functions [2].

The aim of the present investigation was to determine activity of angiotensin-converting enzyme (ACE) in different structures of the rat brain when integrative activity of the CNS was disturbed after frontal lobectomy.

EXPERIMENTAL METHOD

The investigation was conducted on 27 noninbred male albino rats weighing 180-200 g. The animals were divided into three groups: 1) intact rats, 2) rats undergoing a mock operation, consisting of trephining of the skull without injury to the cerebral cortex; 3) animals with extirpation of the frontal zones of the cortex. Bilateral removal of the frontal cortex was carried out on rats anesthetized with pentobarbital in a dose of 40 mg/kg (intraperitoneally). Not more than 40-50% of the total area of the frontal cortex was extirpated. On the 9th day after the operation, i.e., at a time of most marked changes in neurotransmitter metabolism [3, 4] the rats were decapitated and the brain removed for biochemical investigation. ACE activity was determined in the parietal cortex, hippocampus, corpus striatum, thalamus, hypothalamus, midbrain including the substantia nigra and the ventral part of the tegmentum mesencephali, the medulla, and pituitary gland. Structures were isolated from sections obtained on a special block [10]. Homogenates of brain structures were prepared in 0.05 M potassium-phosphate buffer, pH 7.5, containing 0.01% Triton X-100. ACE activity was determined by the method in [14], using carbobenzoxyphenylalanine-histidyl-leucine (CPHL) as the substrate and histidyl-leucine (HL) as the standard. The homogenate (10 μl) was incubated with 10 μl of 10 mM CPHL in 70 mM potassium-phosphate buffer, pH 8.0, containing 300 mM NaCl (final volume of the sample 100 μl) for 120 min at 37°C. The reaction was stopped by addition

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TABLE 1. Protein Concentration (in mg/ml homogenate) in Structures of Rat Brain and Pituitary Gland on 9th Day after Frontal Lobectomy

Brain structure	Group of animals			Difference, percent	
	1 (n=10)	2 (n=8)	3 (n=9)	between 1 and 2	between 2 and 3
Parietal cortex	9,1±0,3	9,7±0,3	8,8±0,2	7	—10
Hippocampus	8,8±0,4	8,9±0,5	8,1±0,4	1	—9
Corpus striatum	8,9±0,3	7,6±0,2	7,4±0,4	—14	—3
Thalamus	3,5±0,2	3,8±0,3	4,4±0,2	10	15
Hypothalamus	3,9±0,1	3,9±0,1	5,2±0,3	0	32
Midbrain	1,9±0,2	2,1±0,09	2,1±0,1	8	0
Medulla	9,6±0,3	9,7±0,3	10,2±0,3	1	6
Pituitary gland	0,5±0,01	0,9±0,09**	1,4±0,1*	72	56

Legend. Here and in Table 2, * indicates significance of differences between parameters in lobectomized rats and rats undergoing mock operation, **) between rats undergoing mock operation and intact rats ($p < 0.05$); n) number of animals in group.

TABLE 2. ACE Activity in Brain Structures and Pituitary Gland of Rats on 9th Day after Frontal Lobectomy

Brain structure	ACE, nmoles His-Leu/min/mg protein					ACE, nmoles His-Leu/min/mg tissue				
	group of animals			difference, percent		group of animals			difference, percent	
	1 (n=10)	2 (n=8)	3 (n=9)	between 1 and 2	between 2 and 3	1	2	3	between 1 and 2	between 1 and 2
Parietal cortex	2,3±0,2	2,2±0,2	9,2±0,7*	—1	309	0,2±0,02	0,22±0,02	0,81±0,06*	10	268
Hippocampus	5,8±1,0	7,1±1,4	28,8±2,9*	23	305	0,56±0,08	0,63±0,13	2,25±0,16*	13	257
Corpus striatum	62,9±3,1	68,4±5,3	119,9±9,5*	9	75	5,7±0,3	5,3±0,4	8,6±0,6*	—7	66
Thalamus	27,0±5,3	26,3±2,3	39,5±4,0*	—3	51	2,1±0,2	2,4±0,2	3,4±0,3*	14	41
Hypothalamus	10,3±1,2	13,3±1,8	15,5±1,1	29	16	0,89±0,12	1,14±0,1	1,63±0,09*	28	43
Midbrain	11,8±1,2	12,0±1,2	12,2±1,3	2	2	0,93±0,09	1,02±0,1	1,03±0,13	10	0
Medulla	15,8±1,7	18,8±1,8	19,0±1,5	19	1	1,5±0,11	1,8±0,09	1,9±0,08	20	5
Pituitary gland	44,3±2,4	56,0±3,3**	59,9±4,5	26	7	12,3±1,2	25,7±2,0**	42,8±2,8*	108	67

of 1 ml 0.1 N NaOH, after which 25 μl of 2% o-phthaleic dialdehyde was added to the samples, and after incubation for 30 min (in darkness at room temperature) the reaction was completed by the addition of 1 ml of 0.8 N HCl. Fluorescence of the samples was determined at a wavelength of excitation of 360 nm and of emission of 500 nm. The protein concentration in the samples was determined by Lowry's method. The results were subjected to statistical analysis by Student's test [5].

EXPERIMENTAL RESULTS

Extirpation of the frontal cortex of the rats caused considerable changes in ACE activity in the different brain structures.

Data on the protein content in structures of the animal brain are given in Table 1. Comparison of the protein concentration in the brain structures of the animals of groups 1 and 2 revealed an increase in the pituitary gland of the latter by 72% ($p < 0.05$). The protein content in the pituitary gland and hypothalamus of animals of group 3 exceeded the corresponding values in the rats of group 2 by 56% ($p < 0.05$) and 32% respectively. Active protein biosynthesis in the pituitary gland was evidently connected with stimulation of its excretory endocrine function as a result of the surgical operation.

Table 2 gives data characterizing ACE activity in brain structures of animals of the same experimental groups. ACE activity was expressed in the form of specific activity per milligram protein and also as activity per milligram weight of tissue, due to differences in the protein content in the brain structures studied.

Comparison of ACE activity in the brain zones of the animals of groups 1 and 2 revealed no statistically significant differences. Consequently, the surgical operation without injury to the cerebral cortex did not affect ACE activity in the brain structures. After frontal lobectomy ACE activity increased in the brain zones of the rat compared with the corresponding value in animals undergoing the mock operation. Changes in ACE activity differed in this case in different regions

of the brain. For instance, the most marked increase in ACE activity was found in the parietal cortex and hippocampus (by 309 and 305%; $p < 0.05$). ACE activity by weight was increased in these zones within the same limits. Considerable changes in specific activity of the enzyme and its activity expressed per weight of tissue are characteristic of the corpus striatum, in which there were increased of 75 and 66% ($p < 0.05$) respectively. In the thalamus of the animals of group 3, specific and relative ACE activity also exceeded the corresponding values in animals of group 2, although the differences were smaller. In the hypothalamus no significant change in ACE activity was observed. Meanwhile, relative activity of the enzyme was increased by 43% ($p < 0.05$). This difference in the degree of increase of ACE activity can be explained on the grounds that the protein concentration in the hypothalamus of the animals of group 3 exceeded the corresponding value in the animals of group 2.

The pituitary gland occupies a special place in the general picture of the biochemical changes. Relative ACE activity was increased by 108% ($p < 0.05$) in the pituitary of the animals of group 2 compared with those of group 1, and by 67% ($p < 0.05$) in the animals of group 3 compared with group 2. The figures relating to the increase in relative ACE activity were much lower, due to the increased protein concentration in the pituitary gland of the lobectomized rats.

No significant differences in ACE activity could be found in the medulla and midbrain of the animals of groups 3 and 2.

It can thus be concluded from the result that on the 9th day after injury to the frontal cortex of rats ACE activity was increased in the cortex and deep brain structures, evidence of a probable increase in angiotensin II formation.

We know that ACE in the rat brain is located in nerve terminals or postsynaptic cell membranes of the corpus striatum, the thalamo-hypothalamic region, hippocampus, and neocortex. ACE activity is highest in the corpus striatum [15]. In our investigations the greatest increase (fourfold) in ACE activity was found in the parietal cortex and hippocampus, evidently in connection with the important role of these brain zones in the organization of integrative activity of the CNS [1]. The marked changes in ACE activity in the corpus striatum are evidently connected with possible involvement of ACE in the hydrolysis of other regulatory peptides (substances P and K, enkephalins), the concentration of which is higher in the corpus striatum than that of angiotensin II [13]. An increase (by 1.5-2 times) in ACE activity also was found in the thalamus and hypothalamus. Characteristically, brain regions in which significant changes in ACE activity were found have extensive morphological and functional connections with the frontal cortex [12].

It can be tentatively suggested that extirpation of the frontal cortex causes compensatory activation of ACE in zones of the cortex and deep brain structures adjacent to the region of injury. This process is regional in character and is expressed most strongly in brain regions playing an active role in the reorganization of activity of the CNS after cortical damage.

Activation of ACE and the probable increase in angiotensin II formation probably have an adaptive role. Angiotensin, which interacts with other physiologically active substances (secretion of catecholamines, sensitivity of dopamine receptors), may exert its influence on the formation of new morphological and functional connections in the damaged brain.

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TYROSINE HYDROXYLASE ACTIVITY IN TWO-DIMENSIONAL MONOMOLECULAR FILMS

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Tyrosine hydroxylase (TH) is a neurospecific regulatory enzyme of catecholamine biosynthesis, catalyzing oxidative conversion of L-tyrosine into L-dihydroxyphenylalanine (L-dopa). TH is an important component of the catecholaminergic systems of the brain. Agonists and antagonists [4] of presynaptic dopamine receptors are its specific allosteric modulators. It was shown previously that determination of the kinetic parameters of TH in the presence of various pharmacologically active substances in vitro can be effectively used to detect a catecholaminergic component in the molecular mechanism of action of these substances [4, 5]. It is accordingly interesting to use TH as a sensory element for the primary detection of compounds with a dopaminergic mechanism of action. With this aim we have therefore investigated the kinetic characteristics of TH in two-dimensional monomolecular films, formed on solid surfaces.

EXPERIMENTAL METHOD

Highly purified TH was isolated from the rat hypothalamus with the aid of biospecific chromatography, using as the adsorbent di-iodothyroninesepharose 4B [3]. A 10% homogenate of hypothalamic tissue in 0.32 M sucrose was centrifuged at 1000*g* (10 min) and the supernatant was centrifuged at 100,000*g* (60 min). The residue was suspended in 50 mM Tris-maleate buffer, pH 6.0, containing 0.1% Triton X-100. After centrifugation at 15,000*g* (30 min) the supernatant was applied to a column of Sephadex G 6-10, equilibrated with 50 mM Tris-maleate buffer, pH 6.0. The eluate from this column, containing a protein peak, was applied to a column of di-iodothyroninesepharose 4B, equilibrated with 10 mM K-phosphate, pH 6.0. After efflux of the ballast proteins and oligomeric form of TH, the column was eluted with 10 mM K-phosphate buffer, pH 8.5. After emergence of the peak at pH 8.5, elution was carried out with water. The aqueous eluate was lyophilized and used for the work. This eluate contained TH with molecular mass of 36 kDa.

Monomolecular films of TH were obtained by the Langmuir—Schafer technology, by means of which monomolecular layers of amphiphilic molecules can be formed on the surface of water and transferred to a solid-phase carrier with surface tension of 20 mN/m. TH also was immobilized by a simple sorption method, by placing solid surfaces in a solution of the enzyme (1 mg/ml) for 30 min. As solid-phase surfaces (slabs measuring 9 × 9 mm) we used surfaces of gold, polycor,

*Deceased.

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