REGIONAL CHANGES IN BRAIN ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RATS

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The discovery of virtually all the biochemical components of the renin-angiotensin system in brain tissue was the basis for the study of the role of this system in central regulation of the hemodynamics under normal and pathological conditions. From the abundant data on the distribution of activity of angiotensin-converting enzyme (ACE), the key factor of this system, in regions of the brain [3, 4, 8, 14], some idea can be formed of its neuronal localization, but the data are contradictory. The results of studies of the role of the renin-angiotensin system in the formation of pathological hypertensive states cannot be completely transposed to the system of central regulation of the circulation, for these biochemical system are known to possess considerable autonomy [12, 13]. There is increasing evidence of the stronger activity of the renin-angiotensin system of the brain in animals with experimental or spontaneous (hereditary) hypertension. Dissimilar changes in ACE activity have been found in individual brain zones in adult hypertensive rats [7, 9, 10].

The aim of this investigation was to study changes in ACE (dipeptidylcarboxypeptidase) activity in eight brain zones of rats during the development of spontaneous hypertension.

EXPERIMENTAL METHOD

Experiments were carried out on 38 male spontaneously hypertensive rats (SHR) of the Okamoto-Aoki line and 48 normotensive Wistar-Kyoto rats (NTR). The following age groups of animals were chosen: 2 months (weight 180-200 g), 3 months (200-220 g), 6 months (260-280 g), and 12 months (300-350 g). The arterial pressure (BP) was measured by the indirect method without anesthesia, by means of a tail cuff. The systolic pressure in the different groups of NTR varied within limits of 110-130 mm Hg (2 months), 130-150 mm Hg (3 months), 140-170 mm Hg (6 months), and 140-180 mm Hg (12 months). These values correspond to age changes in BP in NTR. In the analogous groups of SHR the systolic pressure varied within limits of 140-160 mm Hg (2 months), 170-200 mm Hg (3 months), 210-260 mm Hg (6 months), and 190-230 mm Hg (12 months). The material for testing was obtained from animals anesthetized with pentobarbital (5 mg/100 g body weight). The brain was perfused with ice-cold physiological saline through the left ventricle (perfusion of the brain through the carotid arteries does not remove all the blood from the cerebellum). After decapitation, the following regions were isolated in accordance with the scheme in [6]: pituitary gland, cerebellum, medulla, hypothalamus, thalamus, midbrain, hippocampus, and striatum. The material was preserved in plastic packs at -20°C until required for investigation of enzyme activity. ACE activity in brain tissue homogenates was determined by the method described previously, using hippuryl-distidyl-leucine [1] as the substrate. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

On the basis of the results the change in ACE activity was analyzed in each brain zone during individual development of the NTR, changes in ACE activity were compared in the brain zones of the SHR and NTR, and the distribution of ACE activity was studied in the brain as a whole in SHR and NTR of different ages.

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TABLE 1. Ratio between Specific ACE Activity in SHR and NTR during Development of Spontaneous Hypertension

Age of animals, months	Pituitary	Cerebellum	Medulla	Hypothalamus	Midbrain	Thalamus	Hippocampus	Striatum
2	9,01	3,71	14,2	5,63	10,07	1,89	5,08	2,52
3	1,91	1,75	1,52	1,11	0,92	1,05	1,73	2,15
6	1,56	1,60	1,08	0,50	0,92	1,06	0,82	0,74
12	0,93	1,43	0,73	0,52	0,36	0,21	1,22	1,25

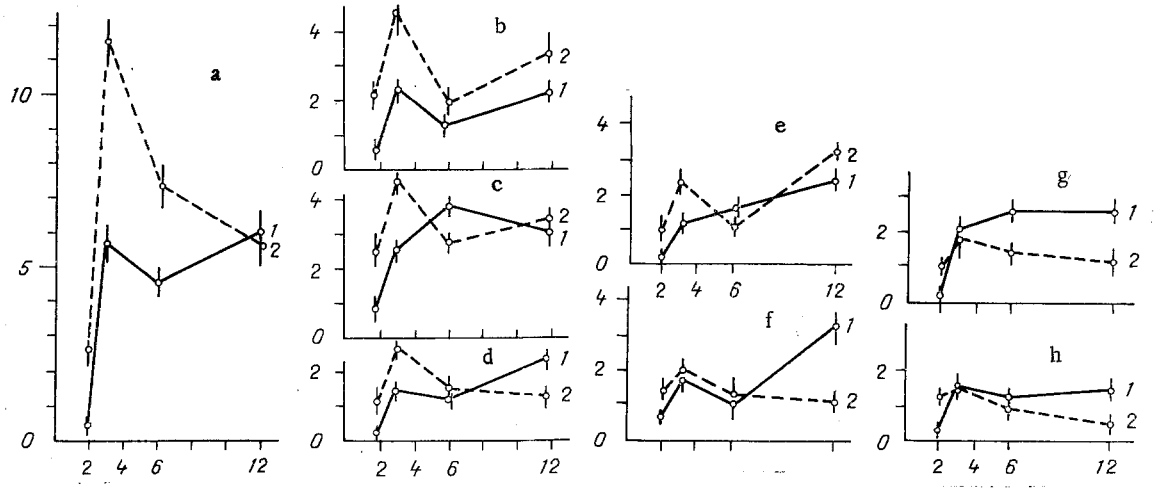

Fig. 1. Changes in ACE activity in brain zones of NTR (1) and SHR (2). Ordinate, specific activity of enzyme (in nmoles his-leu/min/mg protein); a) pituitary, b) cerebellum, c) striatum, d) medulla, e) hippocampus, f) thalamus, g) hypothalamus, h) midbrain.

It will be clear from Fig. 1 that in most parts of the brain of the NTR similar age changes in ACE activity were found. From 2 to 3 months of individual development of the animals this parameter increased considerably, to exceed the original values by 20.3 times for the pituitary, and by 17.6 and 12.6 times respectively for the medulla and midbrain. In other zones ACE activity increased by a lesser degree during this period. In the striatum, hypothalamus, and hippocampus the increase in ACE activity continued until the 6th month of the animal's development, but in most other zones of the brain and pituitary its level in the period from 3 to 12 months did not change significantly. Only a further rise in ACE activity in the hippocampus and thalamus was observed, so that in rats aged 1 year it was 5 and 10 times higher respectively than in rats aged 2 months and twice as high as in rats aged 3 months. Thus, when changes in ACE activity are evaluated during ontogenetic development of NTR, an initial age period, when in most parts of the brain and in the pituitary there is a considerable increase in enzyme activity, facilitating angiotensin II formation, must be specially distinguished. These data were obtained for the first time.

A similar age dynamics of ACE could also be observed in SHR. However, they differed from NTR in that the original level of ACE activity in SHR at the age of 2 months was already significantly higher than in NTR. Besides, a sharp increase in ACE activity took place in the SHR between the 2nd and 3rd months of development, so that it was considerably higher than ACE activity in NTR of the same age. It will be noted that this "flash" of ACE activity in SHR took place at a time before a stable hypertensive state was present in rats of the Okamoto-Aoki line, and the rise of their BP was negligible. Later in SHR, despite the presence of a persistent hypertensive state, ACE activity in all parts (except the hippocampus) decreased and did not differ significantly from that in NTR.

The coefficients of correlation between specific ACE activity in SHR and NTR, for the various brain zones, show (Table 1) how the ACE level in SHR and NTR differed at the age of 2 months, namely by 9 times in the pituitary, 10 times in the midbrain, and 14 times in the medulla. However, at all subsequent stages of development the ratio between their ACE activity was close to unity. One other fact must be mentioned: the SHR/NTR ratio in the midbrain,

thalamus, and hypothalamus of rats aged 12 months were shifted in the opposite direction: the level of ACE activity in these zones was significantly higher in NTR than in the hypertensive rats.

As a result of a more detailed analysis than that undertaken previously (as regards both brain zones and age trends) some idea could thus be obtained of the consecutive pattern of changes in ACE activity during the formation of spontaneous, hereditary hypertension. These data explain the contradictory results of the study of ACE activity in certain parts of the brain in rats in which the hypertensive state was already formed [5, 7, 9, 10]. The most important discovery in these experiments was the increase in ACE activity in SHR between the period of 2 and 3 months, when the rise of BP was hardly noticeable. The subsequent decrease in ACE activity against the background of stabilized hypertension (6-12 months) is evidence that its sharp increase in most brain zones and, in particular, in the pituitary, striatum, and cerebellum, in the initial age period is one factor inducing the development of hereditary hypertension. Our results are in agreement with data in [11], according to which intracerebral infusion of the ACE inhibitor captopril into Okamoto-Aoki rats aged 7 weeks may weaken the development of hypertension in them.

The writers previously studied changes in ACE activity in the blood and lung tissue of SHR aged 3, 6, and 12 months [1]. ACE activity in the blood serum at all stages of development was much lower in SHR than in NTR, but in the lung tissue of 3-month-old SHR it was not high enough for this to be interpreted as the essential pathogenetic mechanism of formation of hypertension. The results contained in the present investigation emphasize the fundamental importance of activity of systems of regulatory brain peptides and, in particular, of ACE, which leads to the formation of the important pressor factor angiotensin II.

Finally, an integrative assessment of ACE activity in the brain as a whole for SHR and NTR at different age periods is interesting. For NTR aged 3 and 12 months the distribution of activity of the enzyme among the different zones is relatively uniform. However, for NTR aged 6 months and SHR of all age groups ACE activity is distributed distinctly unevenly: higher values are found in the pituitary, cerebellum, and striatum than in the other zones. It will be noted that the highest ACE activity is concentrated in the pituitary gland, irrespective of the animal's age and functional state. This emphasizes yet again the special importance of this gland in the regulation of metabolism of physiologically active peptides.

ACE is a key enzyme in the formation of angiotensins II and III. However, the participation of this dipeptidylcarboxypeptidase in the metabolism of kinin peptides and enkephalins must also be emphasized. Since the role of these peptides in the central regulation of the hemodynamics now rests on a sufficiently firm basis [2], the results on regional changes in ACE activity in the brain during development of spontaneous hereditary hypertension, described above, can in principle be given a broader interpretation.

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