ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN BLOOD SERUM AND LUNG TISSUE OF SPONTANEOUSLY HYPERTENSIVE RATS

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Angiotensin-converting enzyme (ACE) is regarded as a key component linking the renin–angiotensin and kallikrein–kinin systems of pressure regulation and, consequently, as an important factor for the understanding of the pathophysiological mechanisms of development of arterial hypertension [8, 9]. However, direct investigations of ACE activity experimentally [2, 16] and in patients with essential hypertension [1, 5, 7, 17] have not yielded unequivocal results.

In the present investigation ACE activity was determined in the blood and lung tissue of normotensive and spontaneously hypertensive rats during their individual development. Considering the important role of the lung barrier in regulating activity of physiologically active substances [3], and also data on the localization of ACE in the endothelium of the pulmonary microcirculatory system, changes in ACE activity in the blood serum and lung tissue were compared.

EXPERIMENTAL METHOD

Experiments were carried out on 33 male spontaneously hypertensive rats (SHR) of the Okamoto-Aoki strain, of different ages: group 1) animals aged 9-13 weeks and weighing 215.0 ± 7.1 g; group 2) rats aged 6-8 months weighing 275.8 ± 13.2 g; group 3) rats aged 12-14 months weighing 338.3 ± 15.0 g. The control consisted of 34 normotensive rats (NR) of the Wistar-Kyoto strain, of the same age and body weight.

Blood pressure (BP) in the aorta was recorded directly by a "Barovar" strain gauge transducer through a catheter introduced through the left carotid artery. Arterial blood and lung tissue were taken from the anesthetized animals (thiopental sodium 100 mg/kg, chloral hydrate 100 mg/kg). The blood serum and lungs were frozen immediately after isolation and kept at −20°C until required for testing.

ACE activity in the serum and lung tissue homogenates was determined by the method in [11], in our own micromodification [4]. Hippuryl-histidyl-leucine (from Serva, West Germany) was used as the substrate. The histidyl-leucine formed by the action of ACE gives a fluorogen with *o*-phthalic dialdehyde (from Serva) in an alkaline medium, which can be estimated quantitatively by spectrofluorometry. The order of working with the material was as follows. After thawing the lungs were washed with physiological saline to remove blood and then homogenized in 0.02 M K-phosphate buffer, pH 8.3 After centrifugation for 10 min at 1000 g, protein in the supernatant fraction was determined by Lowry's method.

The incubation sample with a total volume of 250 µl consisted of 2 µl serum (or 1-2 µl of a 10% lung tissue homogenate containing 10 µg protein) in 0.1 M K-phosphate buffer, pH 8.3/0.3 M NaCl, with 10 µl of a 25 mM solution of hippuryl-histidyl-leucine for 15 min at 37°C. Simultaneously with the experimental samples a control sample and also a standard sample containing 10 µl of 0.516 mM histidyl-leucine solution were incubated. The enzyme reaction was stopped by addition of 1.45 ml of 0.28 N NaOH to the incubation mixture. Next,

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TABLE 1. Changes in ACE Activity in Blood Serum and Lungs of NR and SHR (M ± m)

Parameter studied	Group of animals	Age of animals		
		9–13 weeks (9/10)	6–8 months (12/12)	12–14 months (12/12)
BP (mean)	NR	94,4±4,5	94,6±2,7	93,2±4,8
	SHR	102,0±6,1	146,7±9,7***	154,2±4,4***
ACE activity:				
in serum, nmoles His-Leu/min/ml	NR	83,2±5,8	68,9±3,9**	63,9±5,6**
	SHR	27,3±2,7*	34,3±4,3*	29,5±3,7*
In lungs, nmoles His-Leu/min/mg protein	NR	32,3±4,5	38,9±4,5	46,4±3,3**
	SHR	42,9±1,7*	50,7±4,9*	36,8±2,6*

Legend. Number of experiments shown in parentheses (numerator — NR, denominator — SHR). *P < 0.05 compared with NR; **P < 0.05 compared with group 1; ***not identified in Russian original.

100 µl of a 2% solution of *o*-phthalic dialdehyde, made up in CH_3OH, was added to all the samples, followed 10 min later by 200 µl of 3 N HCl. After centrifugation the intensity of fluorescence in the supernatant was determined on an MPF-4 spectrofluorometer (Hitachi, Japan) at 500 nm (excitation wavelength 360 nm). Since the lungs, unlike other tissues, contain only very small quantities of leucine aminopeptidase, which hydrolyzes the resulting histidyl-leucine, ACE activity in these samples was determined without the addition of *n*-butanol [13].

EXPERIMENTAL RESULTS

Values of the mean BP in SHR differed appreciably from those in NR (Table 1). The difference in rats aged 6-8 months was 55.0%, compared with 65.4% in rats of group 3. Determination of ACE activity revealed considerable differences both between NR and SHR and also in the course of the animal's individual development. Serum ACE activity was considerably higher in NR than in SHR, and this difference (67.2%) was distinctly detectable in the rats of group 1, before the hemodynamic parameters had begun to differ significantly. Differences in ACE activity in lung tissue of NR and SHR were not so marked, although in the youngest age group (group 1) ACE activity was higher in SHR ($P < 0.05$), whereas in rats with established hypertension (group 3) their ACE activity was 22.0% lower than in NR ($P < 0.05$). Analysis of age changes revealed that in NR the serum ACE activity falls whereas its activity in lung tissue rises. In SHR this tendency, however, is not observed: Age changes in ACE activity were virtually absent in the blood serum, whereas in lung tissue the differences were phasic in character and were significant only between the animals of groups 2 and 3. The general conclusion can be drawn that whereas in NR the relationship between serum and tissue ACE correlates negatively with age, no such correlation is present in SHR. Moreover, the results indicate a generally depressed level of ACE activity in SHR of the older age group.

In the discussion of the importance of the renin–angiotensin system in the genesis of arterial hypertension it must be stressed that although this system is important in humoral regulation of BP, it is only one of its essential components. Investigation of ACE activity directs attention to a key factor in this regulation, namely the relationship between the depressor kallikrein–kinin system and the pressor renin-angiotensin system of the blood and tissues [9]. However, the results mentioned above and data in the literature suggest that ACE activity may reflect tendencies in the development of spontaneous hereditary hypertension. The marked difference in serum ACE activity in SHR and NR, and the low level of activity in the former in the early stage of development can be used in principle as a prognostic test of the development of the hypertensive state. The results agree with data obtained by other workers [16], who found lowered ACE activity in the serum, kidneys, and anterior lobe of the pituitary in 14-week-old SHR.

Pathogenetic changes in the development of hereditary spontaneous hypertension include a generalized disturbance of membrane permeability [6], a defect of the filtration function of the kidneys, increased aldosterone synthesis, and changes in function of the sympathico–adrenal system [10]. All these phenomena occur during the period of the animal's development before hemodynamic manifestations of the disturbance of BP regulation have begun to appear. The difference in serum ACE activity, which is detectable even in this period, can thus

probably serve as a manifestation of the complex multistage pathogenetic system of disturbance of BP regulation.

Relative activity of ACE in tissue and blood is determined by the rate of its synthesis in the endothelial cells and release of the enzyme into the surrounding medium. The serum enzyme level of activity reflects the state of function of the endothelium, and injury to the latter leads to release of ACE into the lumen of the vessel [12, 14].

It can be tentatively suggested that under physiological conditions the "excess" of synthesized ACE passes into the blood stream, and constitutes a functional reserve for regulation of converting activity and, correspondingly, regulation of the BP level.

A genetically determined disturbance of membrane permeability, which is one cause of the development of spontaneous hypertension [6], leads to disturbance of the ratio between tissue and "humoral" forms of ACE. The following pathway of development of this pathological mechanism can be postulated: a disturbance of permeability and the excessive inflow of monovalent cations, sodium in particular, inside the cell leads to a decrease in ACE secretion from the endothelium. This can probably explain the decrease in ACE activity in the serum while its tissue level rises in SHR of groups 1 and 2. Furthermore, according to data in the literature [15], an increased Na^+ concentration in the medium causes inhibition of ACE synthesis (activity). This phenomenon can explain why in the SHR of group 3 the ACE level was low not only in the serum, but also in the tissue.

Data on changes in ACE activity in NR during ontogeny may be important on their own account (no such studies have previously been undertaken). They indicate that profound age changes in rats at 12-14 months also are accompanied by an increase in ACE activity in the lung tissue, with a simultaneous decrease in its activity in the blood. The trend of these changes is similar to that observed in ethanol with established spontaneous hypertension.

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