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Angiotensin-1-Converting Enzyme Activity in the Spontaneously Hypertensive Rat (40855)

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Abstract. Spontaneously hypertensive rats of the Okamoto Wistar strain were found to have reduced angiotensin-1-converting enzyme activity in the serum, kidney, and anterior pituitary ($P < 0.01$) as compared to control Wistar Kyoto rats. The activity of this enzyme in lung, testis, epididymis, posterior pituitary, and hypothalamus did not differ between control and spontaneously hypertensive rats. Angiotensin-1-converting enzyme in the kidney of the control and the spontaneously hypertensive rats were immunologically identical. Mixing experiments showed that the depression of enzymatic activity in the kidney and the anterior pituitary could not be accounted for by the presence of an inhibitor in the extracts. We conclude that the concentrations of angiotensin-1-converting enzyme are altered from control levels in the spontaneously hypertensive rat and that regulation of this enzymatic activity may occur independently in different organs.

The spontaneously hypertensive rat (SHR) provides a useful animal model for the study of the role of the renin–angiotensin system in essential hypertension, since this animal exhibits a condition similar to essential hypertension in man (1, 2). Recent studies have demonstrated that levels of plasma renin, kidney renin (3), plasma aldosterone (4), and kidney angiotensinase (5) are all significantly below normal in the SHR. These studies suggest that the SHR may exhibit a generalized depression of the renin–angiotensin system.

Angiotensin-1-converting enzyme (EC 3.4.15.1) (ACE), the enzyme in the renin–angiotensin system responsible for the formation of the potent vasopressor, angiotensin II, from the decapeptide, angiotensin I, is a carboxydipeptidyl hydrolase and is present in serum and on the luminal surfaces of the endothelial cells lining the vasculature in all organs so far examined (6–8). In addition, ACE has been localized on the epithelial brush border cells of the gut (8) and on the cells of the renal proximal tubules (6, 7). Also, ACE is present in the brain and may be a component of a separate brain renin–angiotensin system (9–11). The enzyme is present in relatively high concentrations in specific areas of brain, such as the anterior pituitary, the posterior pituitary (12), the cerebellum (13), and the choroid plexus (14).

The concentration of ACE in serum has been shown by Campbell *et al.* (15) to be correlated with the capacity to develop hypertension-associated arterial disease in rabbits. ACE has further been implicated in hypertension since inhibition of the enzyme in rats (16–19) and humans (20) has been shown to be effective in causing dramatic and long-lasting reductions in blood pressures.

Experiments such as those cited above strongly implicate ACE in blood pressure control, but provide no information about tissue concentrations of the enzyme in hypertension and give no information as to whether any endogenous control mechanism for regulation of ACE exists. The work reported here was undertaken in order to examine the concentrations of ACE in various organs of both normal and spontaneously hypertensive rats by direct measurement rather than to look solely at inhibition of enzyme activity in intact animals as has been done by other workers. In particular, we were interested in measuring ACE concentrations in organs where enzyme levels are known to be high (lung,

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epididymis, testis), as well as in organs where enzyme concentration is lower but may have a direct bearing on blood pressure regulation (kidney, pituitary, hypothalamus). We were also interested in measuring serum levels of ACE as a possible correlate to hypertension.

Materials and Methods. Our studies were performed on 14-week-old male rats weighing 275–300 g (WKY, Wistar Kyoto rats, Charles River Breeding Laboratories) or 250–275 g (SHR, Okamoto strain of Wistar Kyoto rats, Charles River Breeding Laboratories). Five separate groups of WKY and SHR rats (each consisting of three to five animals) were used for assays of lung, testis, epididymis, kidney, and serum. For these experiments, animals were killed either by ether overdose (three groups) or by decapitation (two groups), and blood was collected by cardiac puncture and by drainage from the severed carotids. Only the decapitated animals were used as a source of brain tissue as will be described later. The various organs to be used were placed on ice and frozen immediately upon removal from the animals. Organs were stored at −20° until used. When thawed for use, the organs from each of the types of animals were minced and pooled separately for each group. The minced tissues were homogenized for 30 sec in 3 vol of 0.02 *M* potassium phosphate buffer, pH 8.3, at 0° using a Polytron sonic homogenizer.

The brains of the animals killed by decapitation were removed and dissected immediately. The anterior and posterior hypothalamus and the anterior and posterior pituitary from each animal were quickly frozen separately and stored at −20° until used. The hypothalamus was dissected using the following landmarks: anterior margin, a line connecting anterior commissure and anterior margin of the optic chiasm; dorsal margin, a line connecting the anterior commissure and the mammillary nucleus. The lateral margin was a plane 2 mm lateral to the midline. The anterior hypothalamus was separated from the posterior hypothalamus by a section extending from a point just behind the pituitary stalk, to bisect the dorsal cut. The stalk median eminence was included in the anterior fragment. Brain tissues were homogenzied separately for each animal in 0.1 to 0.2 ml of 0.02 *M* potassium phosphate buffer, pH 8.3, in a glass on glass hand-held homogenizer at 0°.

All homogenates were subjected to centrifugation at 500*g* for 30 min. For some experiments, the 500*g* supernatant fractions were frozen and thawed three times, in order to solubilize particle-bound enzyme in the 500*g* supernatant fraction, and then subjected to centrifugation at 15,000*g* for 30 min. Supernatant fractions were assayed for enzymatic activity by either the method of Piquilloud *et al.* (21) or of Cushman and Cheung (22) using synthetic hippurylhistidylleucine (Fox Laboratories) as substrate. The enzyme measured in these assays was completely inhibited by 10^{-4} EDTA and completely dependent on the presence of chloride ion in the reaction mixture. One munit of enzyme is the amount of enzyme that hydrolyzes 1 nmole of substrate in 1 min at 37°. Protein determinations were performed according to the method of Lowry *et al.* (23).

Radioimmunoassay (RIA) (of ACE) was performed according to the method described by Polsky-Cynkin and Fanburg (24). Rat lung ACE was purified by the method of Lanzillo and Fanburg (25).

Results. The results of measurements of enzymatic activity in homogenates of kidney, lung, testis, and epididymis and in serum are shown in Table I. Approximately 75% less angiotensin-1-converting enzyme activity was present in the kidneys and 40% less enzyme was present in the sera of the SHR than of the WKY rats. No significant differences were observed in the concentrations of enzyme found in lung, testis, and epididymis (in SHR compared to WKY rats).

Angiotensin-1-converting enzyme activity was also measured in the anterior and the posterior pituitary and in the anterior and the posterior hypothalamus of the SHR and WKY rats. These results are shown in Table II. Approximately 65% less enzyme was found in the anterior pituitaries of the SHR as compared to those of the WKY rats. The amounts of enzyme present in the posterior pituitary and in the hypothalamus

TABLE I. SPECIFIC ACTIVITIES OF ACE IN VARIOUS RAT TISSUES[a]

	Kidney	Serum	Lung	Testis	Epididymis
WKY	2.2 ± 0.3	109 ± 6	82 ± 6	85 ± 8	113 ± 6
SHR	0.55 ± 0.04[b]	66 ± 2[c]	102 ± 12	90 ± 7	105 ± 5

[a] The organ homogenates used for this experiment were the 500 *g* supernatants prepared as described under Methods. Assays were performed according to the method of Cushman and Cheung (22). Each number is the average for five separate groups (consisting of three to five animals each) ± the standard error. Results are expressed as munits/mg protein.

[b] Significantly less than WKY kidney homogenates, $P < 0.001$ by Student's *t* test.

[c] Significantly less than WKY serum, $P < 0.01$ by Student's *t* test.

did not differ significantly between the SHR and the WKY rats.

In order to examine the possibility that an endogenous inhibitor of ACE activity was present in SHR kidney or anterior pituitary extracts, lung extracts were mixed with kidney and with pituitary extracts from either WKY or SHR rats. The results of these crossover experiments are shown in Table III. When equal amounts of lung extract were mixed with either kidney or pituitary extract from either type of rat and allowed to stand for 90 min at room temperature prior to assay, the amount of enzymatic activity in the mixture was similar to the amounts added. Thus, no evidence for an endogenous inhibitor was found.

Radioimmunoassays were performed in order to compare (ACE) in SHR kidney with the enzyme in both normal kidney and normal lung. Our rationale was that if the enzyme in the SHR and WKY kidneys is immunologically identical, addition of equal amounts of enzyme activity would reflect addition of equal amounts of immunologically identical protein and would result in superimposable curves for inhibition of radioimmune binding by the two extracts. If this were not true, and one enzyme either had an intrinsically higher specific activity or differed from the other(s) in some immunochemically detectible manner, it would be detected by a displacement of the radioimmune inhibition curve. As can be seen from this experiment (Fig. 1), the curves were superimposable for WKY lung, WKY kidney, and SHR kidney.

TABLE II. SPECIFIC ACTIVITIES OF ACE IN VARIOUS AREAS OF RAT BRAIN[a]

	Pituitary		Hypothalamus	
	Anterior	Posterior	Anterior	Posterior
WKY	6.9 ± 0.6	9.3 ± 2.3	1.0 ± 0.2	1.2 ± 0.2
SHR	2.4 ± 0.7[b]	9.8 ± 1.0	1.3 ± 0.3	1.2 ± 0.1

[a] The homogenates used for this experiment were the 500 *g* supernatants prepared as described under Methods. Assays were performed according to the method of Piquilloud et al. (21). The numbers in the table represent the averages of seven animals ± standard error. Results are expressed as munits/mg protein.

[b] Significantly lower than the WKY anterior pituitary homogenates, $P < 0.01$ by Student's *t* test.

Discussion. Our results indicate that ACE concentration is depressed both in the serum and in certain organs of the SHR. The fact that the enzyme level is depressed selectively suggests the existence of a mechanism for exerting control over the amount of active enzyme present in specific organs such as kidney and anterior pituitary (as well as perhaps in other organs not examined). In this regard, it is relevant to note that Gavras *et al.* (26) reported that in normotensive salt-depleted dogs the ACE inhibitor SQ 20,881 caused regional blood flow to the adrenal, brain, kidney, and heart to increase by at least 64% whereas there was either no change or a decrease in blood flow to all other organs examined. These results, considered in conjunction with ours, suggest that regional changes in ACE activity may play a role in the regulation of blood flow to certain organs. Changes in kidney ACE activity might be particularly relevant to blood pressure regulation if intrarenal control of glomerular filtration rate and electrolyte excretion is mediated by the renin–angiotensin system (especially angiotensin II) as Hall *et al.* (27) and DeClue *et al.* (28) suggest.

TABLE III. ANGIOTENSIN-1-CONVERTING ENZYME ACTIVITY IN MIXED EXTRACTS[a]

Lung WKY	munits enzyme added in 0.1 ml: Kidney WKY	Kidney SHR	Pituitary WKY	Pituitary SHR	0.1 ml buffer added	Enzymatic activity: Total munits calculated (per tube)	Total munits measured (per assay)	Measured activity as % calculated
1. 101	—	—	—	—	+	101	81	81
2. —	15	—	—	—	+	15	13	89
3. —	—	3	—	—	+	3.2	2.0	63
4. 101	15	—	—	—	−	116	138	119
5. 101	—	3	—	—	−	104	124	119
6. —	—	—	4	—	+	4.0	3.0	75
7. —	—	—	—	1	+	1.2	1.0	83
8. 101	—	—	4	—	−	105	92	88
9. 101	—	—	—	1	−	102	100	98

[a] The lung homogenate used for this experiment was the 15,000 *g* supernatant and contained only soluble enzyme. The kidney and pituitary extracts were the 500 *g* supernatants and, therefore, contained both particle-bound and soluble ACE. All of the extracts were prepared as described under Methods. All extracts were mixed, either with buffer (0.02 *M* potassium phosphate, pH 8.3) or with a second extract in equal volume at the start of the experiment. The final volume for all mixtures was 0.2 ml. Each mixture was allowed to stand for 90 min at 23°. After the period of standing, appropriate aliquots of the mixtures were assayed according to the method of Piquilloud *et al.* (21). (The experiments were done in duplicate and comparable values were obtained. One set of determinations is reported.)

The results of our mixing experiments indicate that the decrease in ACE activity probably is not mediated by the presence of an endogenous inhibitor in the extracts. The fact that the RIA of the enzyme from kidneys of WKY and of SHR rats results in superimposable curves for inhibition of radioimmune binding when equal amounts of the two extracts are used strongly suggests that the structure and intrinsic specific activity of the enzyme are identical in the kidneys of these two strains of rats. It should be noted, however, that a change in intrinsic specific activity would shift the curve for inhibition of radioimmune binding to the left or to the right, and that a change in either immunologic cross-reaction or enzyme concentration could counteract that shift. It is conceivable, although unlikely, that such differences could result in superimposable curves for inhibition of radioimmune binding if the changes were of the appropriate magnitudes. Bearing this reservation in mind, it still appears likely that the difference that we observe in the activities of the kidney extracts of WKY and SHR rats is due to a difference in enzyme concentration. Regulation of ACE concentration in the rat may occur by means of altered protein turnover, but this possibility has not been explored. Also, it is not known whether the decreased ACE activity in SHR is genetically determined or whether it is secondary to the hypertensive disease state.

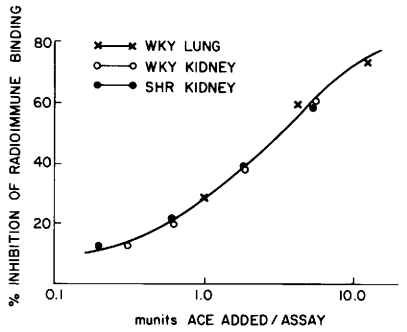

FIG. 1. The extracts used for this experiment were 15,000*g* supernatants. Radioimmunoassays of ACE were carried out as described previously (24). Milliunits of ACE as measured enzymatically for WKY lung and kidney and for SHR kidney are plotted against percentage inhibition of radioimmune binding.

A renin–angiotensin system analogous to that of blood and peripheral organs appears to be present in the hypothalamus (10, 11), and there is a selective distribution of angiotensin II containing nerve tracts in

the hypothalamus (29). In addition, changes in central angiotensinergic activity induced by direct injection of angiotensin II lead to increased blood pressure, but the effect of increased blood pressure on the central angiotensinergic system is not known. The change in pituitary enzyme activity may be a reflection of a feedback control mechanism that can be postulated to operate under conditions of increased blood pressure.

In addition to its role in activation of angiotensin I to angiotensin II, ACE is also capable of inactivating the potent vasodilator, bradykinin. Thus, the control of ACE may provide the body with a method for modulating blood pressure. For example, an increase in ACE activity could potentiate the vasopressor effect of angiotensin II by both increasing the circulating concentration of that peptide and decreasing circulating bradykinin. Alternatively, a decrease in ACE would decrease circulating angiotensin II concentration and increase circulating bradykinin concentration. The decrease in angiotensin II is the presumed mechanism for the decrease in blood pressure.

The hypertension disease state exhibited by the SHR appears to be similar to that of low renin hypertension and may, in fact, be similar to that of a much wider group of patients with essential hypertension, if renin levels are elevated in young hypertensives and fall with increasing age as suggested by workers such as McDonald *et al.* (30). The depressed level of ACE in the serum of SHR correlates with the results reported by Campbell *et al.* (15) for rabbits in whom the investigators induced hypertension secondary to single kidney perinephritis. Serum ACE decreased as blood pressure rose in these animals and the authors suggest that "depressed serum ACE activity is an important and perhaps an essential feature in the pathogenesis of hypertension associated arterial disease that occurs in the setting of decreased plasma renin activity." These results, in conjunction with ours, suggest that serum ACE levels may have clinical relevance to the patient with low renin hypertension.

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