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Concentrations of angiotensin-converting enzyme in tissues of the rat

D.W. CUSHMAN and H.S. CHEUNG
Squibb Institute for Medical Research, New Brunswick, N.J. 08903 (U.S.A.)
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SUMMARY

Angiotensin-converting enzyme, the peptidase which transforms inactive decapeptide, angiotensin I, to pressor octapeptide, angiotensin II, was measured in homogenates of 25 rat tissues, using a spectrophotometric assay for hydrolysis of hippuryl-L-histidyl-L-leucine. Most of the tissues studied contained measurable converting enzyme activities that were dependent upon added Cl^-, stimulated by added Co^{2+}, and inhibited by the specific pentapeptide inhibitor from the venom of *Bothrops jararaca*, pyrrolidone carboxylyl-L-lysyl-L-tryptophyl-L-alanyl-L-proline. High specific activities of converting enzyme were found in lung and in segments of the digestive tract, but the highest activities were in testis and epididymis, associated with tubular fluids, but not in sperm cells. The converting enzyme activity of rat testis increased markedly during maturation in intact, but not in hypophysectomized, rats.

Angiotensin I, a decapeptide generated by action of the enzyme renin on a glycoprotein substrate, is converted to the pressor octapeptide, angiotensin II, by enzymic removal of the C-terminal histidylleucine residue. The peptidase responsible for this 'conversion' was first identified, and isolated from horse plasma, by Skeggs *et al.*[1,2], who named it angiotensin-converting enzyme. Ng and Vane[3,4] later showed that, in dogs, conversion *in vivo* of circulating angiotensin I to angiotensin II is most rapid in the vascular beds of the lung. Stanley and Biron[5] confirmed the importance of lung as a site of angiotensin conversion by demonstrating that this activity was greatly reduced in dogs with cardiopulmonary bypasses; the lowered rate of conversion, however, was still higher than that in blood alone. Recently, conversion of angiotensin I to II has been reported to occur in the renal vasculature of humans[6] and dogs[7-10], and in the vascular beds of the intestine[11] and hind limb[9] of the dog.

Although angiotensin-converting enzyme has been isolated from plasma[1,2,12-17], lung[14,17-20], and kidney[17,21-23], it is difficult to determine the content of this physiologically important enzyme in tissue extracts directly. Both angiotensins are

degraded by other peptidases present in tissue homogenates, and the bioassay methods usually employed to measure angiotensin conversion lack specificity and precision. Thus, Page and Bumpus[24] were able to demonstrate only qualitatively the conversion of angiotensin I to biologically active angiotensin II by homogenates of heart, liver, aorta, uterus, and ileum, and Huggins and Thampi[25] measured activities from several tissues only after acid and $(NH_4)_2SO_4$ fractionation of the tissue homogenates. Roth and co-workers[26], however, were able to determine activities of angiotensin-converting enzyme directly in homogenates of nine tissues of the rat, using a fluorimetric assay to measure histidylleucine formed by action of the enzyme on a synthetic tripeptide, benzyloxycarbonyl-L-phenylalanyl-L-histidyl-L-leucine. Aiken and Vane[27] measured angiotensin conversion directly in isolated intact arterial tissues, using a bioassay in conjunction with a pentapeptide[28-30] that specifically inhibits angiotensin-converting enzyme.

We have measured angiotensin-converting enzyme activities in a large number of rat tissue homogenates, using a spectrophotometric assay[20] of hippuric acid, which is formed by action of angiotensin-converting enzyme on hippuryl-L-histidyl-L-leucine, a tripeptide substrate that is not hydrolyzed by most other tissue peptidases. To further ensure that the spectrophotometric assay specifically measures angiotensin-converting enzyme, we have, whenever a tissue contained sufficient activity, demonstrated dependence of the activity on added Cl^- (refs. 1,2,12–14, 17–20), stimulation of the activity by added Co^{2+} (ref. 20), and inhibition of the activity by the specific inhibitor of angiotensin-converting enzyme, pyrrolidone carboxylyl-L-lysyl-L-tryptophyl-L-alanyl-L proline[20,27-30]. This pentapeptide, one of several isolable from the venom of *Bothrops jararaca*, has been named BPP5a (bradykinin-potentiating peptide) by Stewart *et al.*[30]

Organs were excised from ether-anesthetized mature Sprague-Dawley rats of both sexes. Lungs, livers, hearts and kidneys were perfused with 155 mM saline, and these and other tissues were washed with saline and frozen until used; blood was collected by heart puncture. A sample of each tissue was obtained from each of five rats. Tissue samples were diced and homogenized in 10 vol. of 50 mM potassium phosphate buffer, pH 8.3, using a motor-driven Teflon pestle in a Potter-Elvehjem-type homogenizer. After centrifugation of each sample at 5 000 × *g* for 10 min, the supernatant fluids were dialyzed for three 12-h intervals against 20 vol. of the same buffer. Protein concentrations were determined by the biuret method[31]. Each dialyzed homogenate was assayed with the standard incubation mixture[20] and three modifications thereof: omission of NaCl (300 mM), addition of $Co(NO_3)_2$ (1 mM), and addition of the pentapeptide inhibitor (0.1 mM).

Specific activities of angiotensin-converting enzyme in homogenates of 25 tissues of the rat are shown in Table I. The amounts of protein extracted from most of the tissues were between 25 and 50 mg per g wet tissue weight; the only important exception was duodenum, from which only 11.8 ± 1.1 mg/g was extracted. All but two of the tissues studied contained detectable angiotensin-converting enzyme activities, most of which were inhibited at least 90% by the pentapeptide or by lack of Cl^-. The extent of stimulation by Co^{2+} varied from tissue to tissue, probably depending upon the concentrations of endogenous metal ions. Activities found in homogenates of uterus,

TABLE I

ACTIVITIES OF ANGIOTENSIN-CONVERTING ENZYME IN RAT TISSUES

Tissue	*Specific activity**	*Percent inhibition*		*Percent activation*
		*With pentapeptide***	*Without NaCl*	*With $Co(NO_3)_2$*
Epididymis	122 ± 12	99 ± 0.3	97 ± 0.7	44 ± 15
Testis	85 ± 4.3	99 ± 0.4	99 ± 0.3	36 ± 5
Lung	48 ± 5.0	98 ± 0.4	98 ± 0.7	56 ± 16
Duodenum	8.6 ± 1.0	92 ± 3.7	94 ± 3.4	118 ± 25
Pancreas	7.4 ± 1.2	80 ± 7.7	93 ± 6.1	−21 ± 22
Jejunum	6.0 ± 1.1	97 ± 2.0	95 ± 2.0	152 ± 28
Ovary	3.4 ± 0.5	97 ± 0.9	91 ± 1.5	67 ± 17
Ileum	3.3 ± 0.6	99 ± 0.4	96 ± 3.0	141 ± 38
Aorta	2.9 ± 1.0	97 ± 1.7	85 ± 4.1	93 ± 68
Stomach	2.7 ± 0.4	95 ± 3.7	89 ± 8.8	74 ± 27
Uterus	2.2 ± 0.2	71 ± 5.9	49 ± 12	58 ± 5
Seminal vesicle	2.0 ± 0.1	91 ± 8.0	99 ± 0.2	132 ± 27
Thymus	1.9 ± 0.3	62 ± 7.5	62 ± 3.2	145 ± 22
Thyroid	1.7 ± 0.4	58 ± 13	55 ± 10	107 ± 42
Serum	1.6 ± 0.1	97 ± 1.3	97 ± 1.3	36 ± 18
Brain	1.5 ± 0.2	†	†	†
Colon	1.2 ± 0.1			
Submaxillary	1.0 ± 0.1			
Spleen	0.9 ± 0.2			
Kidney	0.6 ± 0.2			
Adrenal	0.5 ± 0.1			
Heart	0.5 ± 0.3			
Diaphragm	0.3 ± 0.2			
Gastrocnemius	<0.2			
Liver	<0.2			

* munits/mg protein (mean ± S.E., $n = 5$).

** Pyrrolidone carboxylyl–Lys–Trp–Ala–Pro. This specific inhibitor of angiotensin-converting enzyme also potentiates the biological activities of bradykinin, and was named BPP5a by Stewart *et al.*[30].

† Activities were too low to permit accurate measurement of inhibition.

thymus and thyroid were only partially inhibited by omission of Cl^- or by addition of the pentapeptide inhibitor, and the activity of rat pancreas homogenate was incompletely inhibited by the pentapeptide, and inhibited, rather than activated, by Co^{2+}. Part of the activity measured in homogenates of these four tissues (uterus, thymus, thyroid and pancreas) may be due to peptidases other than the angiotensin-converting enzyme.

To our knowledge, the presence of angiotensin-converting enzyme in testis or epididymis has not previously been reported; in the rat, however, these tissues contain the highest specific activities that we have measured (Table I). The angiotensin-converting enzyme of these two tissues is found predominantly in the non-sedimenting fraction of the tubular content. It is possible that the enzyme is associated with sperm cells, but readily released into the tubular fluid. Female sex organs and other male sex organs, including prostate, penis and vas deferens, not shown in Table I, contained only low levels of angiotensin-converting enzyme; human seminal plasma contained 47 munits/mg. The concentration of angiotensin-converting enzyme in testes of immature rats

(23 days old) was only 0.96 ± 0.13 munits/mg, whereas lungs of immature rats contained normal levels. Angiotensin-converting enzyme activity in testes rises between 40 and 50 days after birth in normal, but not in hypophysectomized rats; this rise parallels the onset of formation of mature sperm cells and the increase in activity of testicular hyaluronidase[32], an enzyme known to be present in sperm cells. The high concentration of angiotensin-converting enzyme in testicular and seminal fluids suggests a role for this enzyme in spermatogenesis or in such functions as sperm motility or fertilization. The results of further studies on testicular angiotensin-converting enzyme will be the subject of a future publication.

The high concentrations of angiotensin-converting enzyme that we have found in lung and various segments of the digestive tract (Table I) are consistent with the findings in numerous studies of conversion in intact, perfused organs, or *in vivo*[3-11,27]. Our results are almost identical to those obtained for nine tissues of the rat by Roth *et al.*[26], but, for the most part, are in disagreement with those of Huggins and Thampi[25], who found much lower activities in general, and only small differences in the activities from lung, liver, diaphragm and heart. The preliminary acidification and $(NH_4)_2SO_4$ fractionation employed by the latter authors could have resulted in a low yield of the enzyme. The activity that we found in thoracic aorta is consistent with the results of Aiken and Vane[27], who measured angiotensin-converting enzyme contents of a variety of intact arterial tissues. The same authors[9] have suggested that significant conversion of angiotensin I takes place in the hind limb of dogs, whereas others[4,7] have failed to observe such conversion. Since we found no angiotensin-converting enzyme in gastrocnemius muscle, hind limb conversion of angiotensin I, if it occurs significantly in the rat, might be expected to be associated with such non-muscular tissue as skin.

Our results, and those of others[7,26], offer no support for the hypothesis[24,33] that the liver is the site of synthesis of angiotensin-converting enzyme. Although we and others[26] have found low activity of angiotensin-converting enzyme in rat kidney, this finding does not rule out, in this organism, an intrarenal role for angiotensin-converting enzyme, as suggested by Thurau *et al.*[34] and Leyssac[35]; the low activity that is present in rat kidney may be quite significant if the enzyme is localized in a site such as the afferent arteriole. Various workers[7-10] have demonstrated significant conversion of angiotensin I to angiotensin II in the renal vascular beds of the dog.

We have measured only total concentrations of angiotensin-converting enzyme in a variety of tissues. Such measurements may, in many cases, provide a good indication of the ability of the intact tissue to convert angiotensin I to angiotensin II under normal physiological conditions; in fact, many of our results are in agreement with those of studies on conversion of angiotensin I in intact tissues. Some of the enzymes released by cell destruction, however, may not normally be suitably located in the cell to participate in the conversion of angiotensin I. Obviously, a great many tissues have at least the potential for carrying out conversion of angiotensin I to II; such local conversions may play important roles in the physiology of the organism. The angiotensin-converting enzyme activities found in various tissues of the rat appear to be similar in their properties. Study of a pure angiotensin-converting enzyme from one source, such as lung, should aid our understanding of the properties of the similar enzymes from other important sources, such as testis, plasma, kidney and intestine.

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