ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN THE VASCULAR TISSUE FROM RABBITS

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ABSTRACT

The mean tissue angiotensin-converting enzyme activity in the aorta, inferior vena cava, renal artery, and pulmonary artery were 0.651 ± 0.044 , 0.910 ± 0.116 , 1.537 ± 0.268 , and 2.92 ± 0.364 SE units/cm², respectively. The enzyme activity was significantly higher in the pulmonary artery ($P < 0.001$) and renal artery ($P < 0.005$) than in the aorta. The different distribution of the enzyme in each vessel might lead to a difference of the metabolic activity of the vasoactive peptide in each organ.

KEY WORDS angiotensin-converting enzyme / vascular tissue / peptide metabolism

Angiotensin-converting enzyme (EC 3.4.15.1) is a dipeptidyl peptidase which converts angiotensin I to angiotensin II (10). Ryan *et al.* (9) and Caldwell *et al.* (3) have shown that the enzyme is localized in the vascular endothelial cells. Endothelial cells grown in tissue culture also contained the enzyme activity (5). Ng and Vane (7) reported that the conversion of circulating angiotensin I to angiotensin II took place mainly in the lung. But conversion was also found in the peripheral circulation (2).

In this paper, we measured angiotensin-converting enzyme activity in the aorta, inferior vena cava, renal artery, and pulmonary artery to study the distribution and the physiological role of the enzyme in the vessels.

MATERIALS AND METHODS

Hippuryl-His-Leucine was purchased from Protein Research Foundation, Osaka, Japan. Captopril (SQ 14225) was presented kindly by Sankyo, Japan. All other chemicals were obtained from Wako Pure Chemical Industries, Japan.

Preparation of the Materials

Nine female rabbits, weighing 2-2.5 kg, were used.

The animal was killed by bleeding through the cannulated carotid artery. The aorta, renal artery, main pulmonary artery, and inferior vena cava were isolated immediately and washed with excessive cold saline. Each vessel was trimmed gently with the dissecting forceps to remove all the connective tissue and rinsed with cold saline to remove the blood component. After measurement of the weight and luminal surface square of each vessel, the tissue sample was chopped into small pieces and suspended in 25 mM borate sodium carbonate buffer, pH 8.3. The suspension was homogenized at 4°C for 2 min with polytron set 6 (Kinematica, GmbtH, Luzen, Switzerland). The homogenate was centrifuged at 1,500 *g* for 10 min. The supernatant was used as an enzyme material.

Preparation of the Particulate Fractions from Vessel and Lung

For the check of contamination by the blood component in the sample materials, the partic-

ulate fractions from vessel and lung were prepared by the modified method of Wei *et al.* (11).

The pooled aortae and pooled pulmonary arteries from 3 rabbits were used for this experiment. The supernatants of 1,500 *g* centrifugation were prepared with the method described above except for the use of 25 mM borate sodium carbonate buffer, pH 8.3, containing 0.25 M sucrose. The fractions were centrifuged at 100,000 *g* for 60 min and separated into the supernatant and the pellet (the particulate fraction). The ratio of angiotensin-converting enzyme activity of the supernatant and the pellet was shown in Fig. 1; 63% of the activity obtained from the aorta and 54% obtained from the pulmonary artery were in the particulate fraction. The supernatant contained about 40% of the total enzyme activity. To determine whether this 40% of the total enzyme activity was contamination by the blood component or the result of mechanical solubilization with polytron, we prepared the particulate fraction from rabbit lung with the same method described above. The particulate fraction was resuspended in 25 mM borate sodium carbonate buffer, pH 8.3, containing 0.25 M sucrose and recentrifuged at 100,000 *g* for 60 min. The washed particulate fraction was suspended in 2 volumes of the same buffer and homogenized with polytron set 6 for 2 min at 4°C. Then the homogenate was centrifuged at 100,000 *g* for 60 min. The ratio of the enzyme activity of the supernatant and the pellet is shown in Fig. 1. The experiment was repeated at three different protein concentrations (0.76, 0.27, and 0.13 mg protein/ml) of the par-

ticulate fraction and the same data were obtained. About 30% of the total enzyme activity was solubilized mechanically with polytron. These data showed that almost 90% of the total activity of the samples was from the particulate fraction and that contamination by the blood component was negligible.

Assay of Angiotensin-Converting Enzyme

This was carried out by the modified method of Cushman and Cheung (4), using Hippuryl-His-Leucine as substrate. One unit of the enzyme activity was defined as that amount of the enzyme which hydrolyzed 1 nmol of the substrate for 1 min at 37°C. Since the enzyme activity from tissue homogenates and the plasmas was inhibited completely by Captopril (10^{-6} M) and showed chloride ion dependency (8, 4), the activity was identified as due to angiotensin-converting enzyme.

Protein concentrations in samples were determined by the method of Lowry *et al.* (6).

RESULTS

The mean tissue angiotensin-converting enzyme activity in the aorta, inferior vena cava, renal artery, and pulmonary artery was 0.651 ± 0.044 , 0.910 ± 0.116 , 1.537 ± 0.268 , and 2.92 ± 0.364 SE units/cm², respectively (Fig. 2). The enzyme activity was significantly higher in the pulmonary artery ($P < 0.001$) and renal artery

Fig. 1 A: Percent of total angiotensin-converting enzyme activity recovered in the particulate fraction from the aorta (Ao) and the pulmonary artery (PA). B: Percent of total angiotensin-converting enzyme activity recovered in the particulate fraction from rabbit lung. Experiments 1, 2, and 3 were done at three different protein concentrations (Exp. 1, 0.76 mg/ml; Exp. 2, 0.27 mg/ml; Exp. 3, 0.13 mg/ml).

Fig. 2 Mean angiotensin-converting enzyme activity in vessel walls. * $P < 0.005$, ** $P < 0.001$: significant difference from that in aorta. PA: pulmonary artery; RA: renal artery; VC: inferior vena cava; Ao: aorta

($P < 0.005$) than in the aorta. The pulmonary artery showed the highest activity.

We measured plasma angiotensin-converting enzyme at the same time. There was no correlation in enzyme activity between vessels and plasmas. Moreover, the experiment using the preparation of the particulate fractions from vessels and lung showed that contamination by the blood component in the tissue preparation was negligible.

DISCUSSION

We were able to confirm that vessels such as the aorta, inferior vena cava, renal artery, and pulmonary artery contained angiotensin-converting enzyme in the particulate fraction and that the enzyme activity in the vessels did not come from the blood component. Our data showed that the enzyme content was the highest in the pulmonary artery, followed by the renal artery, inferior vena cava, and aorta. The different distribution of the enzyme might lead to difference in the peptide metabolism in each organ, such as in the renin-angiotensin and kallikrein-kinin systems. Barret *et al.* (1) reported that each vessel contained renin activity but had a different distribution of the enzyme. The role of the renin-angiotensin converting enzyme system in the vessel walls remains to be solved.

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