Angiotensin converting enzyme activity is present in the endothelium-denuded aorta

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Angiotensin converting enzyme activity was measured in intact aortic rings utilizing the synthetic tripeptide [³H]-benzoyl-Phe-Ala-Pro as the substrate. Intact aortic rings possessed angiotensin converting enzyme activity which was blocked by captopril. Enzyme activity was reduced by approximately 30% after removal of the endothelium by chemical or mechanical methods. The remaining activity was also captopril-sensitive. These results suggest that in addition to endothelium, angiotensin converting enzyme activity is present in other vascular cells and may contribute to the metabolism of angiotensin I generated within the vessel wall.

Introduction Angiotensin converting enzyme (ACE) catalyzes the release of histidyl-leucine from the —COOH terminus of angiotensin I (AI) to yield the potent vasoactive octapeptide, angiotensin II. The ectoenzyme is primarily located on the luminal membrane of vascular endothelium cells where it hydrolyzes the circulating (blood-borne) hormones (Ryan *et al.*, 1976). Although it is not found within the endothelial cytoplasm (Catravas & Watkins, 1985) indirect evidence suggests that it may be present elsewhere within the vascular wall (Saye *et al.*, 1984; Dzau, 1986). In order to address this question directly, ACE activity was measured in intact and endothelium-denuded aortic rings *in vitro*. The results of the present study clearly indicate that significant ACE activity remains after removal of the endothelium and confirm a postulated role for this enzyme in the metabolism of locally (intra-wall) produced angiotensin I.

Methods Male Wistar-Kyoto (WKY) rats were anaesthetized with pentothal (50 mg kg⁻¹, weight). The thoracic aorta was removed and placed in oxygenated McEwen's solution of the following composition (mm): NaCl 130, NaHCO₃ 25, KCl 5.6, CaCl₂ 2.2, NaH₂PO₄ 1.2, glucose 11 and sucrose 13. It was then cleaned carefully and cut into rings (2–3 mm wide). The rings were either set up for tension experiments or examined for ACE activity.

The endothelium was removed either by infusing 3 ml sodium deoxycholate *in situ* (1 mg ml⁻¹ for about 30 s) Byfield *et al.*, 1986) or by gently scraping the endothelium away from the intima. To test for the successful removal of endothelium, samples for light microscopy were prepared by the method of Abrol *et al.* (1984) for silver staining.

ACE activity was determined by using [³H]-benzoyl-Phe-Ala-Pro ([³H]-BPAP) as the substrate (Catravas & Watkins, 1985). The enzyme removes Ala-Pro (in much the same way as it removes histidyl-leucine from angiotensin I). The product ([³H]-benzoyl-Phe) is quantitatively extracted from the acidified sample into toluene. In our system, 2 rings per sample were incubated in 1 ml HEPES buffer (0.1 M, pH 7.4, 37°C) containing 50 nCi [³H]-BPAP, for 15 min. A total of 15 samples was used for experiments with intact endothelium and a total of 10 samples for the experiments where the endothelium was removed. The aortic rings for these samples were taken from 6 rats. The reaction was stopped with the addition of captopril (200 µg ml⁻¹ final concentration) at 4°C and acidified by adding 0.12 N HCl. Product ([³H]-B-Phe) was extracted into Ventrex No. 2 (a toluene-containing scintillation mixture) by incubating in the dark for 72 h and radioactivity was measured in a scintillation spectrometer. Results are expressed as units of ACE activity per mg of wet weight of tissue. One unit is determined as the amount of enzyme converting 1% of the substrate in 1 min under first order reaction conditions. Appropriate controls included captopril to ensure the specificity of the reaction. Statistical analysis was done by Student's *t* test.

Results The successful removal of the endothelium was tested pharmacologically by examining the effect of acetylcholine (ACh 10⁻⁵ M) on aortic rings pre-contracted with noradrenaline (10⁻⁹ M). In the presence of endothelium ACh caused a substantial fall in tension whereas in the absence of endothelium it was totally ineffective. In addition histological examination (silver nitrate stain) of opened aortic rings showed that both gentle scraping (*n* = 4) and

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Figure 1 Angiotensin converting enzyme activity in the presence (+E) and absence (-E) of endothelium and with (solid column) and without (open column) captopril (1 mg ml^{-1}); $n = 15$ for +E; $n = 10$ for -E.

sodium deoxycholate ($n = 4$) infusion resulted in the successful removal of the endothelium.

In the presence of endothelium, ACE activity was found to be 0.76 ± 0.04 units per mg tissue wet weight ($n = 15$) (Figure 1). In endothelium-denuded vessels this activity was reduced partially but significantly ($P < 0.05$) to 0.52 ± 0.07 units per mg tissue wet weight ($n = 10$) (Figure 1). In both cases ACE activity was almost completely blocked by captopril ($4 \times 10^{-3} \text{ M}$ during the 15 min incubation). Thus a substantial amount of ACE activity remained in the medial and adventitial layer of the aorta. These findings were similar whether sodium deoxycholate infusion or gentle scraping techniques were used to remove the endothelium.

Discussion The substantial ACE activity remaining after endothelium removal suggests that this activity may not be limited to the vascular endothelium. This nonendothelial enzyme appears to be similar to the one found in endothelium in so far as it is blocked by the classical ACE inhibitor, captopril. It bears, therefore, no relation to the captopril-insensitive, chymototin-sensitive angiotensin II generating enzyme which was recently located in the adventitia of dog, monkey and human aorta (Okunishi *et al.*, 1987).

These findings are in agreement with those of

Peach and colleagues (Saye *et al.*, 1984) who recently reported that endothelium removal only attenuated, but did not abolish angiotensin-I-elicited contractile responses in rabbit aortic rings. It might be argued that under the present experimental conditions, the endothelium of the vasa vasorum is unmasked and this may be an important source of ACE activity. However, removal of endothelium from the aorta does not unmask any measurable endothelium-derived relaxing factor (EDRF) activity, the main source of which is the endothelial cell, as reflected in the total inhibition of the relaxing responses to ACh in endothelium-denuded vessels. In addition it is known that other endothelial cell markers, such as the surface blood group antigens, are absent from the underlying structures of the vessel wall (Szulman, 1960). It therefore appears probable that the endothelium of the vasa vasorum makes a negligible contribution to the overall endothelium-associated metabolic activities.

The locus of the ACE activity remaining after endothelium removal (approximately 70% of total) is still unclear. Interstitial fibroblasts might be a likely candidate, since investigators have reported significant levels of ACE activity as well as immunofluorescence localization of the enzyme in cultured fibroblasts from various species (Rubin *et al.*, 1982; Weinberg *et al.*, 1982). Of course, it should be emphasized that given the minimal fraction of total vessel weight associated with endothelium ($< 1\%$) the presently measured ACE activity still represents specific activity which is vastly higher than in any other cell type. There is considerable experimental evidence today suggesting that angiotensin II can be formed outside the vascular endothelium (Dzau, 1986; Swales & Heagerty, 1987; Story & Ziogas, 1987). Furthermore, all the necessary components of the renin-angiotensin system are believed to be located within the vascular wall where angiotensin II can be formed and act *in situ* (Desjardins-Giasson *et al.*, 1981). The physiological function of vascular wall angiotensin II is believed to be the facilitation of NA release from nerve endings as ACE inhibitors have been found to reduce its release from the rat mesentery (Kawasaki *et al.*, 1984). Circulating angiotensin I and bradykinin are believed to be hydrolysed by endothelial ACE (Ryan *et al.*, 1970).

In conclusion, removal of the endothelium from rat aortic segments causes a partial reduction in total ACE activity directly assayed in this tissue. These results indicate that ACE activity is not limited to the vascular endothelium and suggest a role for intrawall ACE in the hydrolysis of locally produced angiotensin I.

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