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SHORT COMMUNICATIONS

Endothelium removal does not abolish angiotensin converting enzyme activity from the mesenteric arterial bed of the rat

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Angiotensin converting enzyme (ACE) or kininase II cleaves the terminal dipeptide from angiotensin I to form the vasoactive angiotensin II. This enzyme is located mainly on the luminal membrane of vascular endothelium [1]. The presence of ACE on vascular endothelial cells of several organs [2] has led to the suggestion that this enzyme could provide a useful marker for endothelial cells in culture [3, 4]. There is now however, an increasing body of indirect evidence suggesting that ACE may be present elsewhere within the vascular wall [5, 6]. We have recently addressed this question directly by measuring ACE activity in endothelium denuded aortic rings [7] and we have shown that indeed considerable ACE activity was present in the vascular wall in the absence of endothelial cells. In the present study, ACE activity was determined in a perfused arterial preparation, namely the mesenteric arterial bed (MAB) of the rat, in the presence and absence of endothelium. Our results offer further evidence for the presence of ACE activity in non-endothelial vascular cell layers which may participate in the conversion of locally (within the wall) produced angiotensin I.

Materials and Methods

The perfused mesenteric arterial bed. Wistar–Kyoto rats 20–25 weeks old weighing 250–350 g were anesthetized with ethyl ether. The mesenteric arterial bed was subsequently cannulated and removed, as described by McGregor [8]. The preparation was then transferred to a thermostatically controlled perspex bed, maintained at 37° and perfused through the vessels with oxygenated (95% O_2, 5% CO_2) McEwen's solution of the following composition (mM: NaCl 130, $NaHCO_3$ 25, KCl 5.6, $CaCl_2$ 2.2, $NaHPO_4$ 1.2, glucose 11 and sucrose 13. The perfusion rate was 1 mL/min and was achieved by means of a peristaltic pump (LKB microperpex 2132). The vascular volume of the bed was approximately 0.25 mL.

Endothelium removal. The endothelium was removed by infusion of sodium deoxycholate (1 mg/mL in saline) for 40 sec [9]. The successful removal of endothelium was checked by the lack of relaxant effect of acetylcholine (0.03–3.0 μg, bolus injection) on the MAB precontracted with noradrenaline (NA) (1 μg/mL) and by histological examination following staining with toluidine blue and hematoxylin-eosin.

Measurement of ACE activity. Enzyme activity of the perfused intact or endothelium denuded MAB was determined by using [^{3}H]benzoyl-phe-ala-pro ([^{3}H]BPAP) as a substrate [10]. The enzyme cleaves ala-pro in much the same way as it removes histidyl-leucine from angiotensin I. The product ([^{3}H]benzoyl-phe) is quantitatively extracted from the acidified sample into toluene. The MAB was allowed to equilibrate for 30 min before the beginning of measuring ACE activity. [^{3}H]BPAP (=500,000 dpm, 20 Ci/mmol) was injected as a bolus (0.05 mL) into the inflow cannula and 1 min samples were collected for 5 min following the bolus, with an LKB fraction collector. Captopril was introduced into the perfusion fluid at a concentration of 1 mg/mL for 20 min before measurement of ACE activity was repeated in its presence. Thus, three determinations of ACE activity were performed in each preparation (N = 5) in the following order: one with intact endothelium, one with endothelium removed and one in the presence of captopril. A 30 min interval was allowed between successive assays. The 1.0-mL samples of perfusate were collected into tubes placed on ice and 200 μg/mL of captopril were added to each tube in order to terminate the enzymatic reaction. From each sample a volume of 0.5 mL was removed and acidified by adding 2.5 mL of 0.12 N HCl. The production ([^{3}H]B-phe) was extracted into Ventrex No. 2 (a toluene containing scintillation mixture) by incubating in the dark for 72 hr and radioactivity was determined in scintillation spectrometer. Another 0.1 mL of each sample was transferred into Lumagel (scintillation cocktail) and the exact total dpm of [^{3}H]BPAP injected was determined by measuring its radioactivity. Results are expressed as the per cent metabolism (% M) of BPAP: $\%M = 100 * [Y - \alpha X]/[X(\beta - \alpha)]$, as follows. If $X = {}^3$H-dpm in water phase, $Y = {}^3$H-dpm in toluene phase, $\alpha =$ fractional extraction of [^{3}H]BPAP into toluene and $\beta =$ fractional extraction of [^{3}H]B-phe into toluene, then: $X = A + B$, and $Y = \alpha A + \beta B$. Simultaneously solving these two equations results in: $B = (Y - \alpha X)/(\beta - \alpha)$; since $\%M = 100 \times B/X$, then $\%M = 100 * (Y - \alpha X)/X(\beta - \alpha)$. Statistical analysis was done by the one-way analysis of variance followed by Tukey's test. P values smaller than 0.05 were considered to be statistically significant.

Results and Discussion

In the presence of endothelium, Ach (0.03–3.0 μg) caused a dose related drop in the perfusion pressure of the MAB which was preconstricted by continuous infusion of NA (1 μg/mL) (Fig. 1a). In the absence of endothelium, Ach had no effect on perfusion pressure (Fig. 1b). His-

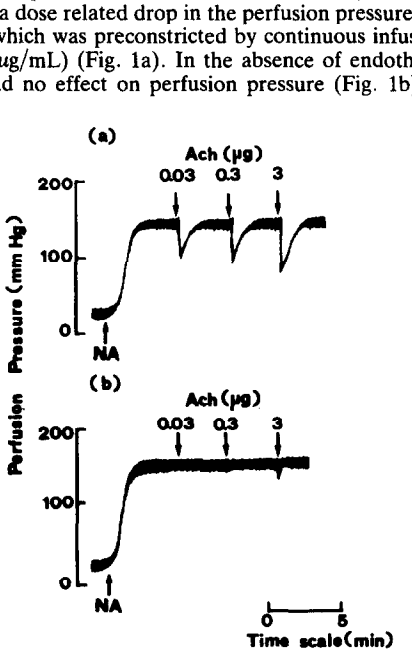

Fig. 1. Effect of Ach (0.03–3.0 μg) on the intact (a) and endothelium denuded (b) MAB preconstricted by continuous infusion of NA (1 μg/mL).

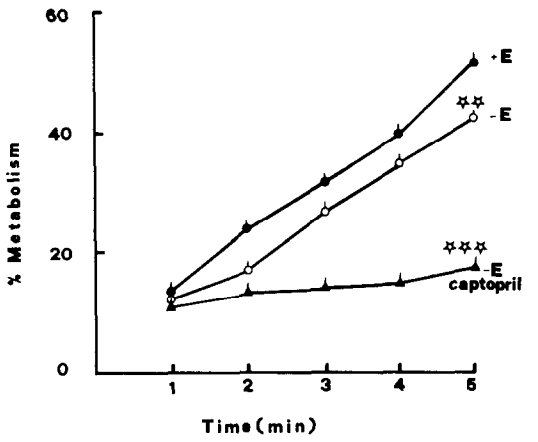

Fig. 2. ACE activity during a single passage of [^{3}H]BPAP through the mesenteric arterial bed, expressed as per cent metabolism of [^{3}H]BPAP, with intact endothelium (+E), after endothelium removal (−E) and after endothelium removal in the presence of captopril (−E, captopril). Points are the mean ± SE of five observations. (** $P < 0.02$, *** $P < 0.01$).

tological examination of the MAB following staining showed that deoxycholate infusion resulted in the removal of endothelium lining while leaving the underlying cell layers undamaged. The bed responded similarly to 1 μg/mL noradrenaline at the beginning as at the end of the experiment.

In the intact MAB, ACE activity was present in all of the 5 min samples collected after the [^{3}H]BPAP bolus. This activity increased with time and reached 51.1 ± 2.3% (5th min) of substrate metabolism. In the absence of endothelium, ACE activity was reduced to 42.2 ± 0.8% (5th min) of substrate metabolism. However, although the reduction was statistically significant, ($P < 0.22$) there was a substantial amount of ACE activity in the endothelium denuded MAB. In the presence of captopril (1 mg/mL) this activity was greatly and significantly ($P < 0.01$) reduced to 17.7 ± 1.25% (5th min) of substrate metabolism (Fig. 2).

These results provide additional direct evidence that ACE activity (as inferred from the metabolism of the ACE substrate) is not limited to the vascular endothelium but is also present in other vascular cell lavers. This ACE activity is similar to that found in the endothelium as it is blocked by the well known ACE inhibitor captopril. These findings are in agreement with our recent report that substantial captopril sensitive ACE activity was present in endothelium denuded rat aortic rings [7]. The perfused system in the present study however, provides information concerning the metabolism of angiotensin I by blood vessels which is closer to the physiological situation, than the rat aortic rings. The reason for this is that in the aortic rings the substrate may gain access through the cut ends of the vessel and from the adventitia side of the vascular wall. In the perfused mesenteric arterial bed, however, substrate reaches the vascular wall through the lumen as is the case in the physiological situation. The present results, therefore, suggest that while circulating angiotensin I is converted to angiotensin II by the endothelial ACE (since it does not leave the vascular space), locally produced angiotensin I may be a substrate for the enzyme in other cell layers within the blood vessel wall. This intra-wall ACE activity may be important in the regulation of vascular tone in pathological conditions such as high blood pressure and may present an additional target for captopril which is commonly used as an antihypertensive drug [11]. It is of interest, in this respect, that captopril has been shown to be effective in lowering blood pressure in hypertensive patients with low or normal plasma renin levels [11]. The vascular wall increases in thickness during hypertension [12]. If this hypertrophy were accompanied by an increase in the intra-wall ACE activity, then more angiotensin II might be expected to be formed. This may help explain the effectiveness of captopril in hypertensive patients with normal or low plasma renin.

In conclusion, the results of the present study support the suggestion that ACE may be a more ubiquitous enzyme than originally thought. In view of the high levels of ACE activity found after endothelium removal in both aortic strips as well as in the perfused mesenteric arterial bed of the rat, it is possible that this enzyme plays an important role in the intra-wall formation of angiotensin II and the regulation of blood vessel tone.

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Variation in hepatic membrane-bound catechol-*O*-methyltransferase activity in Fischer and Wistar–Furth strains of rat

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Catechol-*O*-methyltransferase (COMT) is one of the major enzymes involved in the inactivation of the catecholamine neurotransmitters dopamine, norepinephrine and epinephrine [1, 2]. Two distinct forms of COMT have been identified [3–7], a soluble and a membrane-bound species, possessing similar biochemical and kinetic properties [8, 9] although having different molecular weights on sodium dodecyl sulfate (SDS) gels of 23 and 26 kD respectively [10]. The major biochemical difference between the two forms of COMT is that the catecholamine substrates possess a considerably higher affinity for the membrane-bound form of the transferase by a factor of approximately 100 [2, 9, 11]. For example, the K_m value for dopamine binding to the human membrane-bound COMT is approximately 3 μM, whereas this value is 280 μM with the soluble enzyme species [2]. Despite this difference, the soluble enzyme has been regarded, in the past, as the predominant form of COMT responsible for the methylation of catecholamines. In contrast, prior studies [2] have revealed that membrane-bound COMT in human brain has a considerably greater capacity to degrade both dopamine and norepinephrine when these neurotransmitters are present at physiologically relevant concentrations of 10^{-5} M or less. These data suggest that membrane-bound COMT may, in fact, be the predominant form of the transferase responsible for methylation of the catecholamines dopamine and norepinephrine, at least, in human brain.

The soluble form of COMT has been reported to be regulated genetically in that two distinct allelic forms possessing different activities have been shown to exist in the human population [12]. Similarly, genetic differences for the soluble form of COMT have been demonstrated in various strains of rats [13–15]. In this regard, it was observed that the Wistar–Furth rat strain possesses almost twice the soluble COMT activity as that of the Fischer strain.

Given the fact that the membrane-bound form of COMT may be the major form of the transferase in human brain, it would be of interest to determine whether this form of COMT is also regulated genetically. Accordingly, in this paper we initially attempted to establish whether differences in activity of membrane-bound COMT in livers from Wistar–Furth and Fischer strains of rats are comparable to those observed with the soluble enzyme. To determine whether membrane-bound COMT is biochemically similar in these strains of rats, the kinetic constants for dopamine and the methyl donor, *S*-adenosyl methionine (SAM), were determined.

Materials and Methods

Livers from four male Wistar–Furth and four male Fischer rats were removed and homogenized separately with a polytron in 0.05 M potassium phosphate buffer, pH 7.4. The homogenates were centrifuged at 10,000 *g* for 10 min, and the resulting supernatant solution was recentrifuged at 100,000 *g* for 60 min to obtain the microsomal pellets. The microsomes were washed once in phosphate buffer, and the final microsomal fraction was resuspended in buffer and assayed immediately for membrane-bound COMT activity.

The activity of membrane-bound COMT was measured as previously described using [^{3}H]dopamine as the labeled substrate [2]. In brief, reaction mixtures containing 2.5 mM magnesium chloride, 1 mM pargyline (monoamine oxidase inhibitor), 1 mM SAM and 4 μM [^{3}H]dopamine (approx. 150,000 cpm/nmol) were incubated in a total volume of 0.3 mL of 50 mM potassium phosphate buffer, pH 7.4, for 40 min at 37°. To ensure that only membrane-bound COMT activity was measured in our microsomal preparation, a concentration of dopamine that was near its K_m value and approximately 100 times less than the K_m value for the soluble enzyme was chosen. The reactions were stopped with the addition of 0.6 mL of 0.5 M potassium borate, pH 10.0. A mixture (3 mL) of toluene:isoamyl alcohol (3:2, v/v) was added to the solutions which were vortexed for approximately 15 sec. After centrifugation, 1-mL aliquots of the organic phase were removed and assayed for radioactivity by liquid scintillation spectrometry. Reaction velocity was linear with time and protein concentration throughout the duration of the assay. K_m and V_{max} values for dopamine and SAM were determined by linear regression analysis from double-reciprocal plots.

3,4-Dihydroxyphenyl[7-^{3}H]ethylamine ([^{3}H]dopamine: 40 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Dopamine·HCl, *S*-adenosyl-L-methionine (chloride form, 85% pure), pargyline·HCl, and dithiothreitol were obtained from the Sigma Chemical Co. (St. Louis, MO). All other reagents were the highest commercial grades available.

Results and Discussion

The average activity of liver membrane-bound COMT in two separate experiments from Fischer and Wistar–Furth rats is indicated in Table 1. As demonstrated, the livers from Fischer rats contain approximately 60% of the membrane-bound COMT activity as that observed in the Wistar–