Demonstration of extrapulmonary activity of angiotensin converting enzyme in intact tissue preparations

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1 The activity of angiotensin converting enzyme (ACE) has been studied on functional parameters of intact isolated preparations of extrapulmonary tissues. The conversion of angiotensin I (A I) to angiotensin II (A II) and the cleavage of bradykinin (BK) were used as indicators of ACE activity. Captopril was employed as a specific inhibitor of ACE.

2 Captopril augmented the BK-induced contractions of the rat isolated uterus, the BK- and substance P-induced contractions of the guinea-pig ileum, and the BK-induced vasoconstriction in the isolated perfused ear of the rabbit. Degradation of BK by ACE was calculated to be 52% in the rat uterus and 75% in the rabbit perfused ear.

3 Captopril inhibited the A I-induced contractions of the rat isolated colon, the A I-induced vasoconstriction in the isolated perfused ear of the rabbit and the rise in blood pressure induced by i.a. injections of A I in pithed rats. Conversion of A I to A II was calculated to be 13% in the rat colon and 26% in the rabbit perfused ear.

4 From estimations of the A II activity (bioassay on the rat colon) in the effluent of the perfused ear of the rabbit after injections of A I into the arterial inflow cannula it was calculated that approximately one tenth of A I was converted to A II during a single passage through the ear (less than 15 s).

5 The present experiments suggest that the high activity of ACE in endothelium of blood vessels of extrapulmonary tissues may provide an additional (endothelium-dependent) local vasoconstrictor mechanism by the rapid formation of A II and inactivation of BK. The ACE activity in non-vascular smooth muscles, other than those of blood vessels, may also affect the physiological functions of these tissues.

Introduction

The angiotensin converting enzyme (ACE) catalyzes the conversion of angiotensin I (A I) to angiotensin II (A II) and inactivates bradykinin (BK). The antihypertensive effect of captopril has been mainly attributed to its action as an inhibitor of ACE and consequently to the prevention of the formation of A II. The inhibition of the cleavage of BK may also contribute to its antihypertensive effect (Carretero *et al.*, 1981; Lindsey *et al.*, 1987). Some of the side effects of ACE inhibitors, both beneficial and adverse, were also attributed to inhibition of BK cleavage (Wilkin *et al.*, 1980; Schölkens *et al.*, 1988). It is well established that the major site for the conversion of A I to A II by ACE is the vascular bed of the lung (Vane, 1974). By means of immunohistochemical and biochemical methods, ACE has also been demonstrated in other tissues such as kidneys, testes, and brush border membranes of the intestine (Huggins & Thampi, 1968; Roth *et al.*, 1969; Cushman & Cheung, 1971). In blood vessels the enzyme is localized in endothelial cells (Caldwell *et al.*, 1976). The physiological significance of ACE in extrapulmonary blood vessels has, however, been questioned (Ng & Vane, 1968; Oparil *et al.*, 1970; Marceau *et al.*, 1981; Whalley, 1987).

In order to gain information about a possible physiological role of extrapulmonary ACE, studies on intact tissue preparations appear to be more suitable than biochemical enzyme studies on tissue homogenates. Thus, in the present work pharmacological experiments on ACE activity in visceral smooth muscles, the isolated perfused ear of the rabbit and rat blood pressure were carried out with captopril as an inhibitor of ACE.

Methods

Smooth muscle preparations

Rat colon The ascending colon of Sprague-Dawley rats of either sex was cut longitudinally into halves and suspended in

Krebs solution containing indomethacin (5.6 μM) and propranolol (5.0 μM) at 32°C as in the method of Regoli & Vane (1964). Isotonic contractions were recorded with a resting tension of 0.5 g. A I or A II was added to the organ bath at intervals of 3 min; the bath fluid was changed 1 min after the addition of the angiotensins. When the colon was used for the bioassay of A II activity in effluents from the isolated perfused ear of the rabbit, captopril (46 μM) was added to the Krebs solution.

Rat uterus The uterus of Sprague-Dawley rats was used in which oestrous had been induced by s.c. injection of diethylstilboestrol 100 $\mu\text{g kg}^{-1}$, 16 h before cervical dislocation and exsanguination. The distal parts of the uterine horns were suspended at 32°C in De Jalón solution which contained indomethacin (2.8 μM) and atropine (0.35 μM). Isotonic contractions were recorded with a resting tension of 0.5 g. BK was added to the incubation medium at 5 min intervals and left in the organ bath for 1 min.

Guinea-pig ileum Pieces of ileum (1.5 cm) were suspended in Tyrode solution at 37°C. Isotonic contractions were recorded with a resting tension of 2.0 g. BK, substance P (SP), histamine or carbachol were added at intervals of 3 min and left in contact with the ileum for 1 min. The contractile responses were expressed as a percentage of the maximum response.

Isolated perfused ear of the rabbit

Vasoconstriction Rabbits of either sex were killed by an i.v. injection of an overdose of pentobarbitone sodium and the auricular arteries of both ears were cannulated. The ears were separated from the head and perfused with Tyrode solution at 37°C under constant pressure; the initial flow rate was adjusted to 6 ml min⁻¹. Noradrenaline (NA), BK, A I or A II were injected at intervals of 45–60 min. The venous outflow was measured with an electronic drop interval recorder. In each experiment captopril (3.8 μM) was added to the perfusion medium of one ear and the other ear was used as control.

Removal of the endothelium Rabbit ears were perfused as described above. The vasoconstrictor effects of NA (50 pmol),

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A I (100 pmol) and BK (10 pmol) were determined in the presence or absence of captopril (3.8 μM). Subsequently, the endothelium was removed by a modification of the method of Spokas & Folko (1984): air was pushed through the blood vessels with a 20 ml syringe, followed by a quick short perfusion with 20 ml Tyrode solution. Both procedures were repeated 3 times. Normal perfusion was then continued for at least 30 min. The injections of NA, A I and BK were then repeated.

Bioassay on the rat colon of angiotensin II contained in the effluent of the perfused rabbit ear For this purpose the rabbit ear was perfused as described above. However, the flow rate was kept constant at 1.5 ml min⁻¹ using a roller pump. Indomethacin (5.6 μM) was added to the Tyrode solution to eliminate the formation of prostaglandins which may interfere with the bioassay of A II on the rat colon. A I or A II were injected into the arterial inflow cannula. The venous effluent was collected in 1 min fractions before and during the 2 min period following the angiotensin injections. A II equivalent activity was then determined in these fractions by comparing the effects of 100–200 μl portions of the test samples with the effects of known amounts of A II on the rat colon.

Blood pressure in pithed rats

Male Sprague-Dawley rats were pretreated with hyoscine butylbromide (30 mg kg⁻¹, s.c.); 30 min later the rats were anaesthetized with ether and pithed (Shipley & Tilden, 1947); artificial respiration with a mixture of 95% O₂ and 5% CO₂ was applied and the rectal temperature was maintained at 37°C. Blood pressure was recorded in one carotid artery with a pressure transducer. The contralateral carotid artery was cannulated retrogradely and used for i.a. injections of A I and A II (in volumes of 0.1 ml). The cannula was rinsed with 0.2 ml of a 154 mM NaCl solution after each injection. Captopril (23 nmol min⁻¹, 0.05 ml min⁻¹) was infused into a jugular vein.

Physiological salt solutions

The composition of the salt solutions (in mM) was as follows: Krebs: NaCl 118.1, KCl 4.6, CaCl₂ 2.5, MgSO₄ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 15.5, glucose 10.0. De Jalón: NaCl 153.9, KCl 5.6, CaCl₂ 0.7, MgCl₂ 0.1, NaHCO₃ 5.9, glucose 3.0. Tyrode: NaCl 136.9, KCl 2.9, CaCl₂ 1.8, MgCl₂ 1.15, NaH₂PO₄ 0.4, NaHCO₃ 11.9, glucose 5.0. The solutions were gassed with a mixture of 95% O₂ and 5% CO₂.

Substances

The following substances were used: bradykinin, ⁵Ile-angiotensin I, noradrenaline (Sigma, U.S.A.); ⁵Ile-angiotensin II (Calbiochem-Behring, U.S.A.); substance P (SP) (Bachem, Switzerland); carbachol (Ebewe, Austria); histamine, diethylstilboestrol (Serva, F.R.G.); captopril (Squibb, Austria); atropine, indomethacin (Merck Sharp & Dohme, U.S.A.); (–)-propranolol (ICI, U.K.); pentobarbitone sodium (Nembutal; Ceva, F.R.G.); hyoscine butylbromide (Buscopan; Boehringer Ingelheim, F.R.G.).

Statistical analysis

The log-logit dose-response curves to BK, NA, A I and A II in the perfused ear of the rabbit, on the rat uterus and on the rat colon were calculated by the least squares method. The regression lines were tested for non-identity and non-parallelism by *F* tests (Zar, 1984). When the regression lines were found to be non-coincidental and parallel, the horizontal distance was calculated (Geigy, 1980) to estimate the extent of

degradation or conversion. These values are given with 95% confidence limits. All other values are given as means $\pm$ s.e.mean.

When individual groups of responses to the same dose of a drug in the absence and presence of captopril were compared the Mann-Whitney *U* test was used (see Figures 1, 4 and 5). To compare contractions of the guinea-pig ileum in response to drugs before and after the addition of captopril to the bath fluid, multiple nonparametric comparisons with a control (Zar, 1984) were made (see Figure 3). Drug-induced vasoconstrictions in the perfused ear of the rabbit before and after removal of the endothelium were compared by the Wilcoxon matched pairs signed rank test; vasoconstriction in the absence or presence of captopril was compared by the Mann-Whitney *U* test (see Figure 6). For the comparison of the hypertensive effects of A I and A II in pithed rats before and during an infusion of captopril the Wilcoxon-Wilcox test was used (see Figure 8).

Results

Smooth muscle preparations

Rat colon Concentration-response curves to A I and A II were established on the rat colon (Figure 1). After testing for parallelism and difference in location ($F_{(1;69)} = 312.8$; $P < 0.001$) the horizontal distance was determined as 0.89 (0.85–0.94) log units giving an estimate for the conversion of A I to A II of 13% (12%–14%) during the contact time of 1 min. The validity of this estimate requires that A I does not possess an intrinsic contractile activity. To test this requirement captopril (46 μM) was added to the bathing solution. In the presence of captopril A I in concentrations up to 125 nM no longer elicited contractions of the colon. Only when the concentration of A I was increased to 250 nM was a contraction observed and this was significantly ($P < 0.01$) smaller than that elicited by the same concentration of A I in the absence of captopril (see Figure 1).

Rat uterus Concentration-response curves for BK were established on the rat uterus in the absence and presence of captopril (46 μM) (Figure 2). The log-logit regression lines were

Figure 1 Rat isolated colon: concentration-response curves for angiotensin I (A I, $\diamond$) and angiotensin II (A II, $\square$); ($\blacklozenge$) contraction induced by A I (250 nM) in the presence of captopril (46 μM). The log-logit regression lines were calculated by the least squares method. Values are given as means with s.e.mean (vertical lines); where no s.e.mean is given it was smaller than the symbol. $n = 5$ –13. Significance of difference from value obtained in the absence of captopril: ** $P < 0.01$ (Mann-Whitney *U* test).

Figure 2 Rat isolated uterus: concentration-response curves to bradykinin (BK) in the absence (○) and presence (●) of captopril (46 μ M). Least squares regression analysis of the log-logit regression lines showed the regression lines to be non-coincident and no significant difference in the slopes could be detected. The difference in location was highly significant ($P < 0.01$); the values given represent mean values and vertical lines show s.e.mean; $n = 6$.

found to be parallel but with a significant difference in location ($F_{(1;45)} = 9.70$; $P < 0.01$). The horizontal distance between the regression lines of 0.32 (0.20–0.45) log units leads to an estimate of 52% (37%–65%) for the degradation of BK by ACE.

Guinea-pig ileum Test doses of BK (23 nM), SP (2.2 nM), carbachol (33 nM) or histamine (90 nM) were applied to the isolated guinea-pig ileum until reproducible contractions were obtained (Figure 3). Captopril was then added to the organ bath. Subsequent contractions induced by the test dose of BK were more than doubled ($P < 0.01$ and $P < 0.05$). For SP the increase in the response was much smaller but still significant ($P < 0.05$), following captopril. This confirms that SP is a poor substrate for ACE (Skidgel *et al.*, 1984). The effects of carbachol and histamine were not affected by captopril.

Figure 3 Guinea-pig isolated ileum: bradykinin (23 nM), substance P (2.2 nM), carbachol (33 nM) and histamine (90 nM) were added to the organ bath at regular intervals of 5 min. After a contact time of 1 min the tissue was washed by emptying and refilling the organ bath three times. When the response was constant, a single dose of captopril (46 μ M) was added at the points indicated by arrows and the additions of the contracting drugs were continued as previously. Magnitude of contractions is given as % of the maximum contraction. Means with s.e.mean shown by vertical lines; $n = 6$. Significance of difference from the last contraction before the addition of captopril: * $P < 0.05$; ** $P < 0.01$ (multiple nonparametric comparisons with a control).

Isolated perfused ear of the rabbit

Vasoconstriction in response to noradrenaline, angiotensins and bradykinin Isolated rabbit ears were perfused under constant pressure and dose-response curves were established for the vasoconstrictor effects of NA, A I and A II, and BK (Figures 4 and 5) in the presence and absence of captopril (3.8 μ M) in the perfusion fluid. The response to NA (Figure 4) was not affected by captopril ($F_{(2;74)} = 1.10$; $P > 0.10$).

For A II a linear dose-response relation in the absence of captopril was established (Figure 4). When captopril was present, the regression analysis showed a significant deviation from linearity ($F_{(1;24)} = 6.26$; $P < 0.05$) and no regression line was calculated. The effects of 100 pmol A II were significantly ($P < 0.05$) enhanced by captopril. The effects of higher doses of A II were not influenced by captopril. A few experiments with a dose of 50 pmol A II also suggested an augmentation by captopril (data not shown).

The dose-response curve of A I was linear and of a slope similar to that of A II, but with a significantly different location ($F_{(1;47)} = 21.33$; $P < 0.001$; Figure 4). The horizontal distance between the regression lines for A I and A II was 3.78 (2.85–5.10) log units, which suggests a conversion of 26% (20%–35%) of A I to A II during its passage through the ear. In the presence of captopril (3.8 μ M) A I was completely ineffective in doses up to 1 nmol; the vasoconstriction observed in response to 2 nmol A I was significantly smaller than that observed in the absence of captopril ($P < 0.05$) (Figure 4).

The reduction of the venous outflow from the perfused ear of the rabbit induced by BK is due to venoconstriction (Goldberg *et al.*, 1976). The log-logit dose-response regression lines for BK in the absence and presence of captopril (3.8 μ M) were parallel and non-coincident ($P < 0.001$). When captopril (3.8 μ M) was present in the perfusion medium, the dose-response curve to BK was shifted to the left by 0.61 (0.49–0.72) log units when compared with the curve obtained under control conditions (Figure 5). In the presence of captopril the effect of 50 pmol BK was larger than that of 200 pmol BK in the absence of captopril. It was calculated that degradation of BK by ACE in the perfused ear of the rabbit was 75% (68%–81%).

By injecting Evans blue it was found that the perfusion time of the rabbit ear under the experimental conditions used was less than 15 s.

Removal of the endothelium Using an immunofluorescence technique, Caldwell *et al.* (1976) have found that ACE is

Figure 4 Isolated perfused ear of the rabbit: vasoconstriction induced by angiotensin I (A I, ◇), angiotensin II (A II, □) and noradrenaline (NA, △) in the absence (open symbols) and presence (closed symbols) of captopril (3.8 μ M). The least squares method was used to calculate log-logit regression lines. In the presence of captopril no regression line was calculated for A II because of significant deviation from linearity ($P < 0.05$). Each symbol represents % reduction of venous outflow and vertical lines show s.e.mean; $n = 5$ –14. Significance of difference from control values: * $P < 0.05$ (Mann-Whitney *U* test).

Figure 5 Isolated perfused ear of the rabbit: vasoconstriction induced by bradykinin (BK) in the absence (○) and presence (●) of captopril ($3.8 \mu\text{M}$). Log-logit regression lines were calculated by the least squares method. Significance of difference from control values: $**P < 0.01$ (Mann-Whitney *U* test). Each symbol represents % reduction of venous outflow, vertical lines give s.e.mean; $n = 7-10$.

Figure 6 Isolated perfused ear of the rabbit: % reduction of venous outflow induced by i.a. injections of noradrenaline (NA), angiotensin I (A I) and bradykinin (BK), at doses given in pmol below the columns, in the absence (open columns) or presence (hatched columns) of captopril ($3.8 \mu\text{M}$). (a) Endothelium intact; (b) endothelium removed. Column heights represent mean values, vertical lines show s.e.mean; $n = 6-8$. Significance of difference from values obtained in the absence of captopril: $*P < 0.05$, $**P < 0.01$ (Mann-Whitney *U* test).

Figure 7 Bioassay on the rat colon of angiotensin II (A II) in the effluent of the perfused ear of the rabbit: 50 pmol A II or 500 pmol angiotensin I (A I) were injected i.a. into the rabbit ear which was perfused at a constant flow of 1.5 ml min^{-1} . The venous effluent was collected in 1 min fractions, from ears perfused with captopril ($3.8 \mu\text{M}$) either present (hatched columns) or absent (open columns). Captopril ($46 \mu\text{M}$) had been added to the bathing solution of the rat colon in order to prevent the conversion of any A I left in the test samples by angiotensin converting enzyme (ACE) present in the colon. Column height represents mean values, vertical lines show s.e.mean; $n = 5-8$. Significance of difference from controls: $**P < 0.01$ (Mann-Whitney *U* test).

Figure 8 Rise in mean arterial blood pressure induced by i.a. injections of angiotensin I (A I) and angiotensin II (A II) in pithed rats; doses (in pmol) given below the columns, before (open columns) and during an i.v. infusion of captopril (23 nmol min^{-1} , hatched columns). Column height represents mean values, vertical lines show s.e.mean; $n = 5$. Significance of difference from control values: $**P < 0.002$ (Wilcoxon-Wilcoxon test); NS, not significant.

almost exclusively localized in vascular endothelial cells. Therefore, we investigated the effect of captopril on the actions of NA, A I and BK in the rabbit ear before and after removal of the endothelium. From Figure 6 it can be seen that removal of the endothelium strongly enhanced the effects of NA, A I and BK ($P < 0.01$). Before removal of the endothelium captopril reduced the effect of A I and enhanced the potency of BK ($P < 0.05$) as seen previously (compare Figures 4 and 5). After removal of the endothelium the A I-induced vasoconstriction was still significantly inhibited by captopril ($P < 0.01$).

Bioassay on the rat colon of angiotensin II in the effluent of the rabbit perfused ear The results obtained with the ACE inhibitor captopril and the methods described above provided indirect evidence for the formation of A II and for the degradation of BK in several tissue preparations. For the quantitative estimation of the amount of A II formed from A I in the isolated perfused ear of the rabbit perfusion was carried out at a constant flow of 1.5 ml min^{-1} ; fractions of the effluent were collected over periods of 1 min and tested for A II activity on the rat isolated colon. Captopril was present in the bathing solution of the colon in order to prevent further conversion of any A I in the effluent by ACE present in the rat colon (compare Figure 1).

When 50 pmol A II were injected into the inflow cannula of the rabbit ear, the total amount was recovered in the effluents collected during the 1st and 2nd min after the injection (Figure 7). This excludes any significant degradation of A II during its passage through the rabbit ear. None of the samples collected before or during the 3rd min after the injections showed any activity on the rat colon. Addition of captopril to the perfusion medium of the ears did not alter the recovery of A II in the effluents (Figure 7).

When 500 pmol A I was injected into the circulation of the rabbit ear, the effluent collected during the 1st min after the injection contained 49 ± 10 pmol A II equivalent activity; 7 ± 3 pmol A II equivalent activity was found in the sample collected during the 2nd one min period after the injection (Figure 7). When captopril was present in the perfusion medium, the A II equivalent activity in the venous outflow was reduced to 14 ± 8 pmol in the 1st one min fraction; none was detectable thereafter. The amounts of A I required to elicit contractions of the rat colon (even in the presence of captopril compare Figure 1) were much larger than the amounts that could be expected to be present in the venous outflow of the perfused ear after the injection of A I. Therefore, the A II equivalent activity estimated on the rat colon

represents in all probability the amount of A II formed from A I during a single passage through the rabbit ear. It indicates a conversion of approximately one tenth.

Blood pressure in pithed rats

The pithed rat preparation was used because of its high sensitivity to pressor substances (Shipley & Tilden, 1947). Various doses of A I and A II were injected retrogradely into a carotid artery followed by a fast injection of 0.2 ml of a 154 mM NaCl solution. The peak of the rise in blood pressure induced by A I as well as by A II occurred already before the end of the saline injection. When captopril (23 nmol min^{-1}) was infused into a jugular vein starting 5 min before the next injection, the effect of A II was the same as under control conditions (Figure 8), that of A I was reduced by about 90% ($P < 0.01$). The effect elicited by 1000 pmol A I was comparable to that of 30 pmol A II in the absence of captopril. Thus, extensive conversion of A I to A II occurred in less than 12 s.

Discussion

Immunohistochemical and biochemical methods allow precise localization of ACE in tissues and the estimation of its activity in tissue homogenates. Its functional significance depends, however, on the availability of its substrates, A I or BK, which are supplied either through the blood stream or are formed in the same tissue where ACE is active. Although there is good evidence that most of the circulating A I is converted to A II by ACE in the lungs, some A I might reach the extrapulmonary circulation and may be converted to A II by ACE present in vascular endothelium in other tissues (Caldwell *et al.*, 1976). Some A I might also be converted to A II in extravascular tissues, e.g. in the brush border membranes of the intestine (Ward *et al.*, 1980). It has been demonstrated that angiotensinogen can be synthesized in extrahepatic tissues such as the aorta (Hellmann *et al.*, 1988). In addition, renin was found to be synthesized in aortic smooth muscles (Re *et al.*, 1982). Both these observations point to a possible local formation of A I. In the present study the specific ACE inhibitor captopril has been employed to gain information on the possible physiological function of ACE in extrapulmonary tissues.

In isolated smooth muscle preparations which contain brush border membranes such as the rat colon and the guinea-pig ileum (Figures 1 and 3), the conversion of A I to A II was found to occur at a fast rate. This was also the case in the rat uterus (Figure 2). Regoli & Barabé (1980), however, could not find a change in the response of the rat isolated uterus to BK after application of captopril.

During the short time required for the perfusion through the vascular bed of the rabbit isolated ear (less than 15 s), a considerable proportion of A I injected into the arterial inflow cannula was converted to A II as indicated by the degree of the ensuing vasoconstriction (Figure 4) and by the bioassay on the rat colon of A II equivalent activity in the venous effluent (Figure 7). These experiments do not allow a precise determination of the rate of conversion of A I to A II. However, the differences between the results obtained in control experi-

ments and in experiments in which ACE was inhibited by captopril suggest a conversion rate of 10 to 25% in less than 1 min. After the removal of the endothelium from the vessels in the ear some ACE activity was still present (Figure 6). This could be due to incomplete removal of the endothelium and/or to the presence of ACE activity in deeper layers of the vessel wall as suggested previously (Saye *et al.*, 1984; Story & Ziogas, 1986; Schölkens & Tilly, 1986; Pipili *et al.*, 1989).

Observations on the blood pressure of the pithed rat (Figure 8) did not allow a distinction between the conversion of A I to A II in the pulmonary circulation and that in peripheral vessels because of the short circulation time. As the immediate rise in blood pressure induced by A I i.a. was greatly inhibited by captopril, a considerable contribution of ACE in peripheral vessels seems to be likely.

In contrast to A I, BK is formed within the tissues. Its short half-life in the blood (less than 30 s; Saameli & Eskes, 1962; McCarthy *et al.*, 1965; Ferreira & Vane, 1967) indicates a rapid inactivation by ACE not only in pulmonary but possibly also in peripheral vessels (Griesbacher *et al.*, 1989). BK causes local arterial vasodilatation, venoconstriction, plasma extravasation, prostaglandin release and nociceptor stimulation. In the present study the effects of BK on the rat uterus (Figure 2), guinea pig ileum (Figure 3) and isolated perfused ear of the rabbit (Figure 5) were augmented by captopril. This is evidence for cleavage of BK by ACE in these tissues. In the rabbit ear, the apparent inactivation of BK (75%) by ACE was more pronounced than the conversion of A I to A II (see above); this finding is consistent with the results of Stewart *et al.* (1981) who found that BK is a better substrate for ACE than A I. In contrast, Whalley & Wahl (1983), working with cat cerebral arteries, found that ACE converts A I but does not inactivate BK. It is still controversial whether BK contributes to the regulation of vascular tone but experiments with a BK antagonist have indicated that BK could have a significant effect on vasomotor tone when ACE is inhibited (Schölkens *et al.*, 1988).

The results of the present investigation suggest that the ACE activity in extrapulmonary blood vessels and smooth muscles may be of physiological significance. If, under *in vivo* conditions, A I reaches extrapulmonary vessels or is formed there locally from angiotensinogen, it would be rapidly converted to A II by endothelial ACE. The rate of conversion may be modulated by factors such as thrombocyte adhesion, inflammatory plasma extravasation or other forms of endothelial irritation. In addition to endothelial-derived relaxing factor and endothelin, the local conversion of A I to A II may, thus, contribute to the local regulation of vasomotor tone.

Note added in proof

Recently, Schalekamp *et al.* (*Br. J. Clin. Pharmacol.*, 28, 105S–113S (1989)) reported that, in man, a major fraction of regionally produced angiotensin I appears to be formed locally, i.e. not in circulating plasma.

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References

- CALDWELL, P.R.B., SEEGAL, B.C., HSU, K.C., DAS, M. & SOFFER, R.L. (1976). Angiotensin converting enzyme: vascular endothelial localization. *Science*, **191**, 1050–1051.
- CARRETERO, O.A., SCICLI, G. & MAITRA, S.R. (1981). Role of kinins in the pharmacological effects of converting enzyme inhibitors. In *Angiotensin Converting Enzyme Inhibitors. Mechanism of Action and Clinical Applications*. ed. Horowitz, Z.P. pp. 105–121. Munich: Urban & Schwarzenberg.
- CUSHMAN, D.W. & CHEUNG, H.S. (1971). Concentrations of angiotensin-converting enzyme in tissues of the rat. *Biochim. Biophys. Acta*, **250**, 261–265.
- FERREIRA, S.H. & VANE, J.R. (1967). The disappearance of bradykinin and eledoisin in the circulation and vascular beds of the cat. *Br. J. Pharmacol. Chemother.*, **30**, 417–424.
- GEIGY (1980). *Scientific Tables Geigy (Statistics)*. 8th edition. Basle: Ciba-Geigy.
- GOLDBERG, M.R., CHAPNICK, B.M., JOINER, P.D., HYMAN, A.L. & KADOWITZ, P.J. (1976). Influence of prostaglandin synthesis on venoconstrictor responses to bradykinin. *J. Pharmacol. Exp. Ther.*, **198**, 357–365.
- GRIESBACHER, T., LEMBECK, F. & SARIA, A. (1989). Effects of the bradykinin antagonist B4310 on smooth muscles and blood pres-

- sure in the rat, and its enzymatic degradation. *Br. J. Pharmacol.*, **96**, 531–538.
- HELLMANN, W., SUZUKI, F., OHKUBO, H., NAKANISHI, S., LUDWIG, G. & GANTEN, D. (1988). Angiotensinogen gene expression in extrahepatic rat tissues: Application of a solution hybridization assay. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **338**, 327–331.
- HUGGINS, C.G. & THAMPI, N.S. (1968). A simple method for the determination of angiotensin I converting enzyme. *Life Sci.*, **7**, (Part II), 633–639.
- LINDSEY, C.J., BENDHACK, L.M. & PAIVA, A.C.M. (1987). Effects of teprotide, captopril and enalaprilat on arterial wall kininase and angiotensin converting activity. *J. Hypertension*, **5**, (Suppl. 2), S71–S76.
- MARCEAU, F., GENDREAU, M., BARABÉ, J., ST-PIERRE, S. & REGOLI, D. (1981). The degradation of bradykinin (Bk) and of des-Arg⁹-Bk in plasma. *Can. J. Physiol. Pharmacol.*, **59**, 131–138.
- MCCARTHY, D.A., POTTER, D.E. & NICOLAIDES, E.D. (1965). An *in vivo* estimation of the potencies and half-lives of synthetic bradykinin and kallidin. *J. Pharmacol. Exp. Ther.*, **148**, 117–122.
- NG, K.K.F. & VANE, J.R. (1968). Fate of angiotensin I in the circulation. *Nature*, **218**, 144–150.
- OPARIL, S., SANDERS, C.A. & HABER, E. (1970). *In-vivo* and *in-vitro* conversion of angiotensin I to angiotensin II in dog blood. *Circ. Res.*, **26**, 591–599.
- PIPILI, E., MANOLOPOULOS, V.G., CATRAVAS, J.D. & MARAGOU-DAKIS, M.E. (1989). Angiotensin converting enzyme activity is present in the endothelium-denuded aorta. *Br. J. Pharmacol.*, **98**, 333–335.
- RE, R., FALLON, J.T., DZAU, V., OUAY, S.C. & HABER, E. (1982). Renin synthesis by canine aortic smooth muscle cells in culture. *Life Sci.*, **30**, 99–106.
- REGOLI, D. & BARABÉ, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, **32**, 1–46.
- REGOLI, D. & VANE, J.R. (1964). A sensitive method for the assay of angiotensin. *Br. J. Pharmacol.*, **23**, 351–359.
- ROTH, M., WEITZMAN, A.F. & PIQUILLOU, Y. (1969). Converting enzyme content of different tissues of the rat. *Experientia*, **25**, 1247.
- SAAMELI, K. & ESKES, T.K.A.B. (1962). Bradykinin and cardiovascular system: estimation of half-life. *Am. J. Physiol.*, **203**, 261–265.
- SAYE, J.A., SINGER, H.A. & PEACH, M.J. (1984). Role of endothelium in conversion of angiotensin I to angiotensin II in rabbit aorta. *Hypertension*, **6**, 216–222.
- SCHÖLKENS, B.A., LINZ, W. & KÖNIG, W. (1988). Effects of the angiotensin converting enzyme inhibitor, ramipril, in isolated ischaemic rat heart are abolished by a bradykinin antagonist. *J. Hypertension*, **6** (Suppl. 4), S25–S28.
- SCHÖLKENS, B.A. & TILLY, H. (1986). Inhibition of converting enzyme in isolated arterial preparations with intact or disrupted endothelium. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **332** (Suppl.), R60.
- SHIPLEY, R.E. & TILDEN, J.H. (1947). A pithed rat preparation suitable for assaying pressor substances. *Proc. Soc. Exp. Biol. Med.*, **64**, 453–455.
- SKIDGEL, R.A., ENGELBRECHT, S., JOHNSON, A.R. & ERDÖS, E.G. (1984). Hydrolysis of substance P and neurotensin by converting enzyme and neutral endopeptidase. *Peptides*, **5**, 769–776.
- SPOKAS, E.G. & FOLCO, G.C. (1984). Intima-related vasodilatation of the perfused rat caudal artery. *Eur. J. Pharmacol.*, **100**, 211–217.
- STEWART, T.A., WEARE, J.A. & ERDÖS, E.G. (1981). Purification and characterization of human converting enzyme (kininase II). *Peptides*, **2**, 145–152.
- STORY, D.F. & ZIOGAS, J. (1986). Role of endothelium on the facilitatory effects of angiotensin I and angiotensin II on noradrenergic transmission in the caudal artery of the rat. *Br. J. Pharmacol.*, **87**, 249–255.
- VANE, J.R. (1974). The fate of angiotensin I. In *Angiotensin, Handb. Exp. Pharmacol.*, Vol. 37. ed. Page, I.H. & Bumpus, F.M. pp. 17–40. Berlin, Heidelberg, New York: Springer.
- WARD, P.E., SHERIDAN, M.A., HAMMON, K.J. & ERDÖS, E.G. (1980). Angiotensin I converting enzyme (kininase II) of brush border of human and swine intestine. *Biochem. Pharmacol.*, **29**, 1525–1529.
- WHALLEY, E.T. (1987). Metabolism of bradykinin and angiotensin I by human basilar artery and rabbit aorta. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **335**, 551–554.
- WHALLEY, E.T. & WAHL, M. (1983). The effect of kininase II inhibitors on the response of feline cerebral arteries to bradykinin and angiotensin. *Pflügers Arch. (Eur. J. Physiol.)*, **398**, 175–177.
- WILKIN, J.K., HAMMOND, J.J. & KIRKENDALL, W.M. (1980). The captopril-induced eruption. A possible mechanism: cutaneous kinin potentiation. *Arch. Dermatol.*, **116**, 902–905.
- ZAR, J.A. (1984). *Biostatistical Analysis*. 2nd edition. Englewood Cliffs: Prentice Hall.

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