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LOCAL INHIBITION OF CONVERTING ENZYME AND VASCULAR RESPONSES TO ANGIOTENSIN AND BRADYKININ IN THE HUMAN FOREARM

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SUMMARY

1. The function of angiotensin converting enzyme was investigated in twenty-four healthy men. Forearm blood flow was measured under basal conditions and during administration of enalaprilat (a converting enzyme inhibitor) and/or peptide substrates of converting enzyme into the left brachial artery. Blood flow was compared in the two arms.

2. Enalaprilat had no effect on basal blood flow. The concentration of enalaprilat in venous blood from the control arm was low, and plasma renin activity was not increased, indicating that systemic inhibition of converting enzyme did not occur.

3. Effects of angiotensin and of bradykinin, administered intra-arterially, were limited to the infused arm. Enalaprilat (13 nmol min^{-1}) inhibited converting enzyme in the infused arm, in which it caused approximately a 100-fold reduction in sensitivity to angiotensin I, while having no effect on the vasoconstriction caused by angiotensin II. Enalaprilat increased vasodilatation caused by bradykinin.

4. Aspirin, an inhibitor of cyclo-oxygenase, did not inhibit vasodilatation caused by bradykinin whether infused alone or with enalaprilat, indicating that these responses are not mediated by prostaglandins.

5. We conclude that under basal conditions neither conversion of angiotensin I to angiotensin II nor degradation of bradykinin determines resistance vessel tone in the human forearm. Converting enzyme may affect vascular tone in situations in which intravascular concentrations of peptides are increased over those present under basal conditions.

INTRODUCTION

Angiotensin converting enzyme (kininase II; EC 3.4.15.1) is a glycoprotein that catalyses the release of carboxy-terminal dipeptides from a variety of substrates. The enzyme was originally identified in plasma (Skeggs, Lentz, Kahn & Shumway, 1956), but is also present on the surface of endothelial cells (Ryan, Ryan, Schultz, Whitaker & Chung, 1975; Caldwell, Seegal, Hsu, Das & Soffer, 1976). Actions of this enzyme

in vitro include generation of the vasoconstrictor peptide angiotensin II from angiotensin I, and degradation of the vasodilator peptide bradykinin to inactive fragments (Lentz, Skeggs, Woods, Kahn & Shumway, 1956; Dorer, Kahn, Lentz, Levine & Skeggs, 1974).

Conversion of angiotensin I to angiotensin II occurs more rapidly in isolated blood-free organs perfused with Krebs solution than in plasma (Ng & Vane, 1967). Bradykinin inactivation during passage through the lungs and other vascular beds also occurs rapidly (Ferreira & Vane, 1967), suggesting that tissue-located converting enzyme may be important in controlling the activity of these peptides. However, a number of other enzymes are also capable of generating angiotensin II (reviewed by Campbell, 1987) and inactivating bradykinin (reviewed by Regoli & Barabé, 1980), so the physiological importance of converting enzyme remains uncertain. The first object of the present study was to investigate the role of converting enzyme in the forearm vasculature using enalaprilat, a potent and specific inhibitor of this enzyme (Cushman & Ondetti, 1980; Ulm, 1983).

Angiotensin II constricts vascular smooth muscle by an action on specific receptors (Mendelsohn, 1985), stimulation of which produces an increase in intracellular calcium by activation of the phosphoinositide signalling system (Brock, Rittenhouse, Powers, Ekstein, Gimbrone & Alexander, 1985). Angiotensin I may also stimulate these receptors, although the evidence suggests that it is much less potent than angiotensin II (Haber & Carlson, 1983). Another object of this study was to investigate whether angiotensin I stimulates angiotensin receptors directly or whether its action is entirely attributable to angiotensin II produced by the action of converting enzyme.

Bradykinin causes vasodilatation in the human forearm (Fox, Goldsmith, Kidd & Lewis, 1961), possibly by a direct action on receptors in vascular smooth muscle. However, it stimulates endothelial cells to produce the two vasodilators nitric oxide and the cyclo-oxygenase product prostacyclin (Hong 1980; Palmer, Ferrige & Moncada, 1987). It is therefore possible that some or all of the vasodilatation caused by bradykinin is mediated indirectly by these products. An additional object of the study was to determine the contribution of cyclo-oxygenase products to the response to bradykinin using the cyclo-oxygenase inhibitor aspirin (Vane, 1971).

METHODS

Twenty-four healthy male volunteers between 20 and 44 years of age (median 23 years) participated in these studies, which were conducted with the approval of the St George's Hospital Ethics Committee and with the informed consent of each volunteer. Studies were performed after subjects had rested supine in a quiet clinical laboratory for a minimum of 30 min. Room temperature (between 25 and 28 °C) was maintained within ± 1 °C for each study. The subjects consumed their normal diet.

Forearm blood flow was measured in both arms using venous occlusion plethysmography with temperature-compensated mercury-in-Silastic strain gauges (Whitney, 1953). Collecting cuff pressure was 40 mmHg and wrist cuff occlusion pressure was 200 mmHg. Flows were recorded for 10 s in every 15 s. The mean of the final five measurements of each recording period was used for analysis. The percentage change in forearm blood flow following drug administration was calculated as:

$$\left(\frac{F(\mathrm{i})_{\mathrm{d}}}{F(\mathrm{ni})_{\mathrm{d}}}-\frac{F(\mathrm{i})_{\mathrm{v}}}{F(\mathrm{ni})_{\mathrm{v}}}\right)\Big/\frac{F(\mathrm{i})_{\mathrm{v}}}{F(\mathrm{ni})_{\mathrm{v}}}\times 100\,\%,$$

where F(i) and F(ni) represent measured blood flows in the infused and non-infused arms respectively during periods of drug (d) and vehicle (v) administration. This method is essentially that used by Greenfield & Patterson (1954) to minimize the effects of variation in blood flow caused by minor external factors.

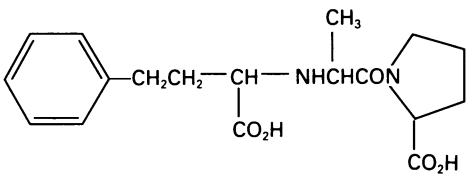
A 27 standard wire gauge unmounted steel cannula (Cooper's Needle Works, Aston Lane, Birmingham) was inserted into the left brachial artery using 1% lignocaine hydrochloride (Antigen Ltd., Ireland) to provide local anaesthesia. Drugs were dissolved in saline (0·9% NaCl; Travenol, Thetford, Norfolk) and infused at a constant rate of either 0·5 or 1·0 ml min^{-1} throughout the experiment by means of constant-rate infusion pumps (Harvard 944A). In experiments where two drugs were infused simultaneously, a Y-connector delayed mixing until the solutions entered the cannula.

Effect of angiotensin II on forearm blood flow

Six subjects were infused with saline for 10 min followed by eight incremental doses of angiotensin II (Calbiochem, USA) from 15 fmol min^{-1} to 512 pmol min^{-1}, each given for 6 min.

Effect of enalaprilat on forearm blood flow

Eight subjects received saline, followed by enalaprilat (a gift from Dr Warren Cooper of Merck, Sharpe and Dohme, Hoddesdon) at 13 nmol min^{-1}, each for 10 min. The chemical structure of enalaprilat is shown below.



Venous blood (5 ml) was drawn from an antecubital vein in the non-infused arm at the beginning and end of the study for measurement of plasma active renin concentration by the antibody-trapping method of Millar, Leckie, Morton, Jordan & Tree (1980).

Effect of enalaprilat on forearm blood flow during infusion of angiotensin I

Eight subjects received saline for 10 min, followed by angiotensin I (Calbiochem, USA) at 64 or 128 pmol min^{-1} for 60 min, and saline for a final 20 min. The initial dose of angiotensin I (64 pmol min^{-1}) was based on an earlier study (Webb & Collier, 1987), and if necessary was doubled to achieve a reduction in blood flow in the infused forearm of approximately 50%. After 10 min of angiotensin I infusion, enalaprilat (13 nmol min^{-1}) was added for 20 min. In three subjects a higher dose of enalaprilat (130 nmol min^{-1}) was infused for the second 10 min of this period. Plasma active renin was measured as in the previous experiment. At the end of the study venous blood (5 ml) was drawn from an antecubital vein in the right arm for estimation of serum enalaprilat concentration by the method of Kelly, Doyle, Donohue, Laher, Vandenburg, Currie & Cooper (1986). The use of this measurement when compared with serum concentrations during systemic administration makes the assumption that the distribution of enalaprilat between blood and tissues has reached equilibrium at the time of blood sampling.

Effect of enalaprilat on forearm blood flow during infusion of angiotensin I and angiotensin II

A paired comparison of the effects of enalaprilat on responses to angiotensin I and angiotensin II was made in six subjects. Each subject was studied on 2 days, separated by at least 1 week. On one day subjects received saline for 12 min followed by angiotensin II (4 and 16 pmol min^{-1}; each for 6 min) on each of two occasions separated by 40 min. Enalaprilat (13 nmol min^{-1}) was infused for 10 min before and throughout the second angiotensin II infusion. On the other day angiotensin I (16, then 64 pmol min^{-1}) was infused in place of angiotensin II. During enalaprilat infusion increasing doses of angiotensin I were given to achieve a reduction of blood flow comparable to that produced in the absence of enalaprilat. The order of days on which angiotensin I and angiotensin II were administered was governed by balanced randomization.

Plasma active renin was measured as before.

Effect of enalaprilat on forearm blood flow during infusion of bradykinin

A paired comparison of the effect of enalaprilat on the response to bradykinin was made in six subjects. Each subject was studied on 2 days, separated by at least 1 week. On one day subjects received saline for 12 min followed by bradykinin (Sigma Chemical Co., Poole, Dorset) (10, 30 and 100 pmol min^{-1}, each for 6 min) on each of two occasions separated by 24 min. On the other day the same protocol was followed apart from infusion of enalaprilat (13 nmol min^{-1}) for 6 min before and during the second bradykinin infusion. The order of the days with and without enalaprilat was governed by balanced randomization.

The same six subjects were studied on a later occasion after intravenous administration of aspirin (3·3 mmol; Aspégic, Egic-Joullié, France) 30 min before bradykinin infusion. Bradykinin and enalaprilat were infused as before. Venous blood (10 ml) was collected before and 30 min after aspirin and incubated in glass tubes at 37 °C for 1 h (Patrono, Ciabattoni, Pinca, Pugliese, Castrucci, De Salvo, Satta & Peskar, 1980). The serum was removed by centrifugation and stored at −20 °C for measurement of thromboxane B_2, as an index of platelet cyclo-oxygenase activity, using a previously described radioimmunoassay (Burch, Knapp & Halushka, 1979). The antiserum was a generous gift from Dr P. V. Halushka.

Analysis of results

Results are presented as mean ± standard error of the mean. Where appropriate, data were analysed using analysis of variance and differences considered significant when $P < 0·05$.

RESULTS

Effects of angiotensin II, bradykinin and enalaprilat

Neither enalaprilat nor angiotensin (I or II) produced subjective sensations, whereas bradykinin caused a sense of warmth and fullness in the infused forearm of some subjects. In the presence of enalaprilat the highest dose of bradykinin produced a marked local sensation of throbbing and warmth in all subjects, and discomfort in some.

Angiotensin II produced a dose-dependent decrease, and bradykinin a dose-dependent increase, of blood flow to the infused forearm. Blood flow in the control arm was unaffected (Fig. 1).

Enalaprilat (13 nmol min^{-1}) had no significant effect on resting blood flow. Mean values ($n = 8$) before and during enalaprilat were respectively 3·0 ± 0·5 and 3·3 ± 0·6 ml (100 ml)$^{-1}$ min^{-1} in the infused arm and 2·9 ± 0·6 and 3·4 ± 0·7 ml (100 ml)$^{-1}$ min^{-1} in the control arm. Plasma active renin concentration was 17·0 ± 1·7 μU ml^{-1} at the beginning of the study and 10·7 ± 1·3 μU ml^{-1} at the end.

Influence of enalaprilat on responses to angiotensin I

Angiotensin I (64–128 pmol min^{-1}) produced a rapid and sustained reduction of blood flow in the infused forearm ($n = 5$; Fig. 2). When enalaprilat (13 nmol min^{-1}) was administered together with the same dose of angiotensin I, blood flow reverted towards its initial level, increasing from 1·5 ± 0·3 to 3·2 ± 0·3 ml (100 ml)$^{-1}$ min^{-1} in 20 min. When enalaprilat was stopped, forearm blood flow again declined, returning gradually towards that during infusion of angiotensin I alone. Finally, when angiotensin I infusion was stopped, forearm blood flow again increased towards its initial level, from 1·8 ± 0·2 to 3·6 ± 0·5 ml (100 ml)$^{-1}$ min^{-1} within 20 min. Plasma active renin concentration was 13·2 ± 3·4 μU ml^{-1} at the beginning and

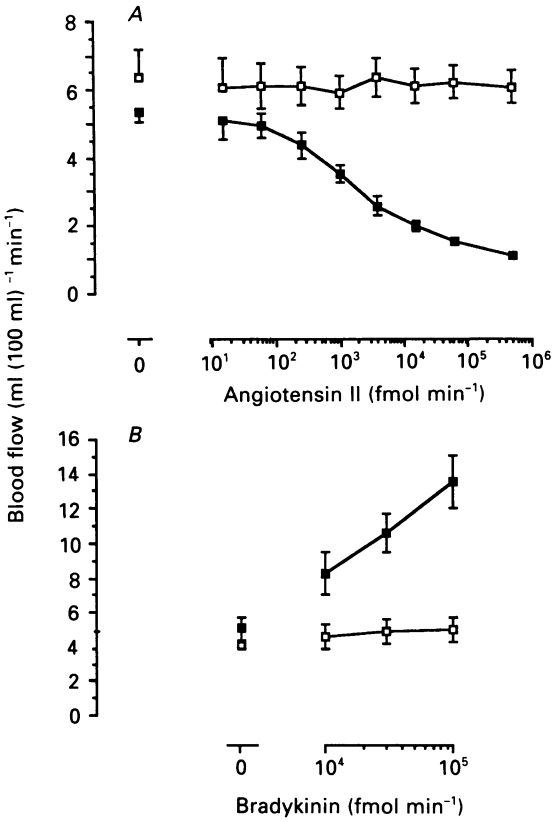


Fig. 1. Forearm blood flow in infused arm (■) and control arm (□) during infusion of increasing doses of angiotensin II (*A*) and bradykinin (*B*).

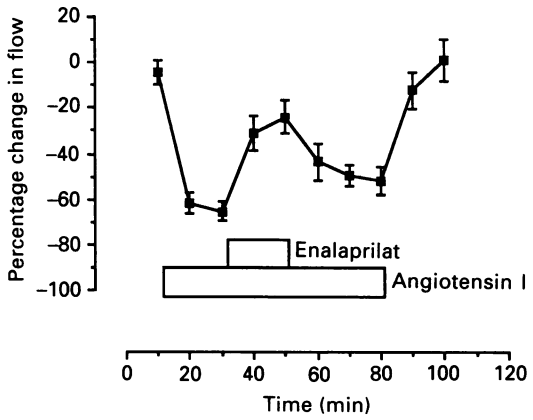


Fig. 2. Percentage change in forearm blood flow during infusion of saline, angiotensin I (16 or 128 pmol min^{-1}) and then further saline. Enalaprilat (13 nmol min^{-1}) was infused together with angiotensin I for 20 min.

$11{\cdot}8 \pm 1{\cdot}9$ μU ml^{-1} at the end of the study. Serum enalaprilat concentrations at the end of the study were all less than 1·6 nM.

There was no greater increase in blood flow during infusion of a higher dose of enalaprilat (130 nmol min^{-1}). Plasma active renin concentration was reduced from $16{\cdot}1 \pm 2{\cdot}0$ μU ml^{-1} at the beginning to $11{\cdot}8 \pm 1{\cdot}3$ μU ml^{-1} at the end of the study. Here the plasma enalaprilat concentration was $27{\cdot}6 \pm 5{\cdot}6$nM.

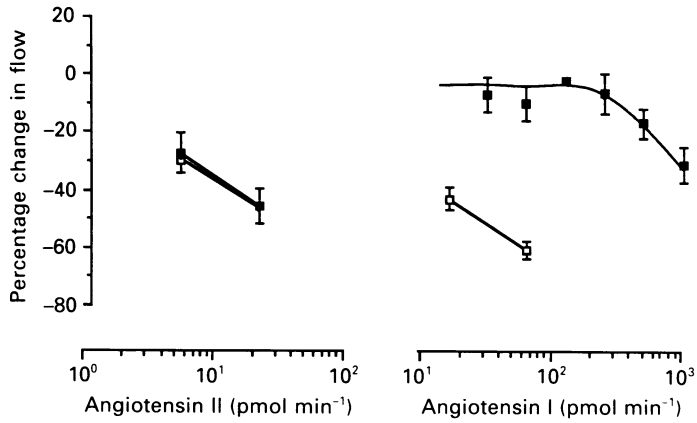


Fig. 3. Percentage changes in forearm blood flow during infusion of angiotensin alone (□) and together with enalaprilat (■: 13 nmol min^{-1}). Left panel shows responses to angiotensin II and right panel shows responses to angiotensin I.

Angiotensin II (4 and 16 pmol min^{-1}) reduced blood flow in the infused forearm from $2{\cdot}5 \pm 0{\cdot}5$ to $1{\cdot}7 \pm 0{\cdot}3$ and $1{\cdot}2 \pm 0{\cdot}1$ ml (100 ml)$^{-1}$ min^{-1} respectively (Fig. 3). When enalaprilat (13 nmol min^{-1}) was administered together with the same doses of angiotensin II the effect was similar, blood flow falling from $2{\cdot}7 \pm 0{\cdot}5$ to $1{\cdot}9 \pm 0{\cdot}3$ and $1{\cdot}5 \pm 0{\cdot}3$ ml (100 ml)$^{-1}$ min^{-1} with 4 and 16 pmol min^{-1} respectively (Fig. 3). Plasma active renin concentration was reduced from $16{\cdot}9 \pm 2{\cdot}4$ μU ml^{-1} at the beginning to $11{\cdot}6 \pm 2{\cdot}7$ μU ml^{-1} at the end of the study. Angiotensin I (16 and 64 pmol min^{-1}) reduced blood flow from $3{\cdot}4 \pm 0{\cdot}4$ to $1{\cdot}9 \pm 0{\cdot}2$ and $1{\cdot}3 \pm 0{\cdot}2$ ml (100 ml)$^{-1}$ min^{-1} respectively.

Angiotensin II was 2·5 times more potent than angiotensin I on a molar basis, as judged by the dose needed to cause a 45% reduction of flow (Fig. 3). When enalaprilat (13 nmol min^{-1}) was administered together with these same doses of angiotensin I (16 and 24 pmol min^{-1}), the effect of angiotensin I was blocked and there was no reduction in blood flow. However, the antagonism produced by enalaprilat was surmountable, a reduction in blood flow occurring when angiotensin I was infused at higher doses: from $3{\cdot}1 \pm 0{\cdot}4$ to $2{\cdot}5 \pm 0{\cdot}2$ ml (100 ml)$^{-1}$ min^{-1} at 1000 pmol min^{-1} (Fig. 3). Thus enalaprilat reduced the potency of angiotensin I 100-fold, but had no effect on responses to angiotensin II. Plasma active renin concentration was reduced from $16{\cdot}3 \pm 2{\cdot}4$ μU ml^{-1} at the beginning to $11{\cdot}0 \pm 2{\cdot}3$ μU ml^{-1} at the end of the study.

Influence of enalaprilat on the response to bradykinin and the effect of aspirin

Bradykinin increased blood flow in the infused forearm from 5·3±0·7 to 14·0±2·1 ml (100 ml)$^{-1}$ min^{-1} at the highest dose (100 pmol min^{-1}; $n = 6$). The second bradykinin infusion, 20 min later, produced less increase in blood flow, from 4·8±0·8 to 12·0±1·5 ml (100 ml)$^{-1}$ min^{-1} ($P < 0·01$; Figs 4 and 5*A*). Blood flow in the non-infused arm did not change (Fig. 4).

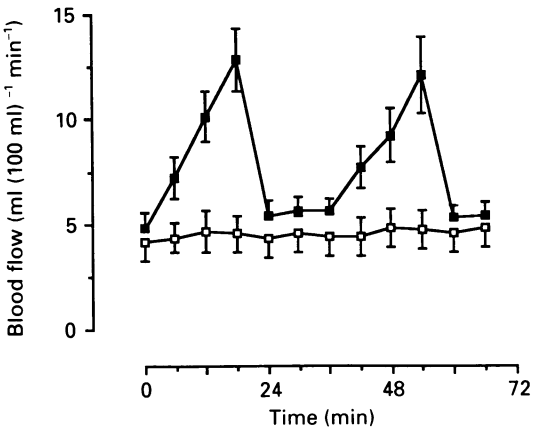


Fig. 4. Blood flow in infused (■) and control (□) forearm during infusion of saline (as vehicle) and two incremental infusions of bradykinin (10, 30 and 100 pmol min^{-1}) separated by 20 min.

When enalaprilat (13 nmol min^{-1}) was given together with the second bradykinin infusion, rather than a slightly diminished response, there was a considerably greater increase in blood flow (6·4±0·7 to 21·0±2·7 ml (100 ml)$^{-1}$ min^{-1}) than occurred during the first bradykinin infusion (4·8±0·8 to 12·9±1·5 ml (100 ml)$^{-1}$ min^{-1}; $P < 0·001$; Fig. 5*B*).

After treatment with aspirin, blood flow during infusion with bradykinin alone increased from 6·3±1·0 to 14·1±2·4 ml (100 ml)$^{-1}$ min^{-1}. When bradykinin was given together with enalaprilat blood flow increased from 8·6±2·0 to 26·9±5·8 ml (100 ml)$^{-1}$ min^{-1} (Fig. 5*C*). These responses were not significantly different from those obtained without aspirin ($P > 0·1$ and $P > 0·6$, respectively). Aspirin reduced serum thromboxane B_2 concentration from 119·4±25·0 ng ml^{-1} to less than 0·05 ng ml^{-1}.

DISCUSSION

These studies demonstrate that local inhibition of converting enzyme within the human forearm reduces arteriolar constriction in response to intra-arterial angiotensin I and increases the dilator response to intra-arterial bradykinin, but does not alter basal blood flow in healthy volunteers. Aspirin, in a dose that inhibits bradykinin-stimulated prostaglandin synthesis (Barrow, Cockcroft, Ritter & Salvidge, 1988), does not affect responses to bradykinin. The effects of angiotensin and bradykinin, administered intra-arterially in low doses, are limited to the infused

arm, no change being detectable in the control arm. Enalaprilat, in the doses used, markedly inhibits converting enzyme in the infused arm, evidenced by an approximately 100-fold reduction in sensitivity to angiotensin I. Inhibition is

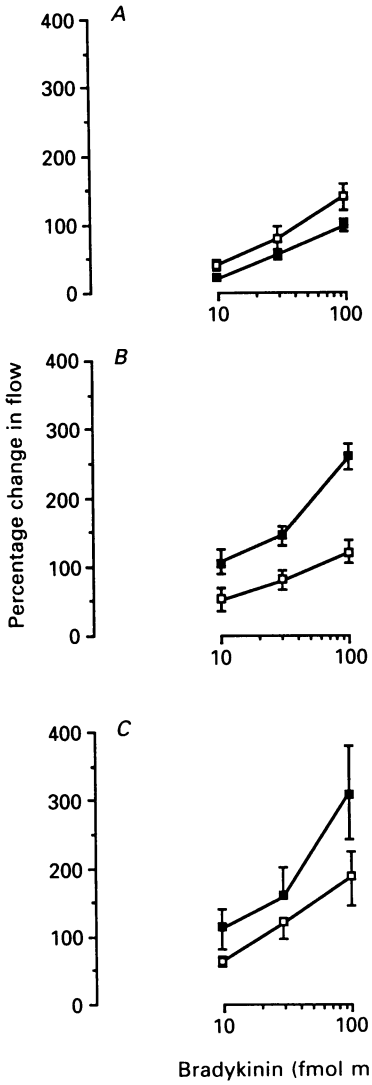


Fig. 5. Percentage change in forearm blood flow during first (□) and second (■) infusion of bradykinin together with vehicle (*A*) and enalaprilat (*B* and *C*, 13 nmol min^{-1}) during the second infusion. In *C* aspirin (3 mmol) was administered before the first bradykinin infusion.

apparently restricted to the infused arm, since there is no increase in plasma renin activity during enalaprilat administration, as would be caused by a systemically active dose of a converting enzyme inhibitor. Rather, there is a modest fall as expected during recumbency in an untreated subject. The conclusion that inhibition of converting enzyme is restricted to the infused arm is supported by the low

concentrations of enalaprilat present in venous blood sampled from the opposite arm at the end of enalaprilat infusion. These are less than one-twentieth the concentration associated with systemic inhibition of converting enzyme (Biollaz, Schelling, Jacot des Combes, Brunner, Desponds & Brunner, 1982).

Actions of converting enzyme

Converting enzyme is present in blood (Skeggs *et al.* 1956). However, conversion of angiotensin I to angiotensin II occurs slowly in blood *in vitro* (40 % in 60 s at 37 °C: Ng & Vane, 1967) compared to the transit time of the forearm arterial circulation *in vivo* (less than 30 s). Furthermore, when enalaprilat infusion is superimposed on a background infusion of angiotensin I, the redevelopment of constriction when enalaprilat is stopped but angiotensin I is continued is much slower to develop than the initial vasoconstriction to angiotensin I alone: approximately 8 min to half-maximal effect compared to less than 2 min (Fig. 2). The enzyme responsible for this effect cannot be restricted to circulating blood, which resides in the arm for only a few seconds. Converting enzyme has been demonstrated on the surface of endothelial cells (Ryan *et al.* 1975; Caldwell *et al.* 1976), and it is likely that this is the location of the enzyme responsible for modifying responses to peptides in the present studies, although we have no direct proof of this. Other enzymes may generate angiotensin II from renin substrate *in vitro* (Campbell, 1987), but our findings show that substantial generation of angiotensin II from angiotensin I by other enzymes is unlikely to occur *in vivo*, as the response to angiotensin I is so markedly attenuated by an inhibitor of converting enzyme.

Angiotensin I and angiotensin II each produce dose-dependent reductions in forearm blood flow. As these responses are confined to the infused arm they presumably reflect local alterations in vascular resistance. A semilogarithmic plot of the dose–response relationships of angiotensin II is sigmoid, responses occurring over a 10000-fold range in dose. The marked shift to the right of the dose–response relationship of angiotensin I caused by enalaprilat confirms the conclusion of Ng & Vane (1968) that its vasoconstrictor effect is mediated by generation of angiotensin II. The potency of angiotensin I is only slightly less than that of angiotensin II on a molar basis, implying that there is substantial conversion of angiotensin I to angiotensin II within a single passage through the arterial side of the forearm circulation. Indeed, the degree of conversion may be similar to that within the pulmonary circulation (Ng & Vane, 1967; Erdös, 1977). Thus peripheral vascular beds in man may be responsible for substantial generation of circulating angiotensin II.

Bradykinin caused an increase of blood flow restricted to the infused forearm, exhibiting a much steeper dose–response relationship than angiotensin II. Vascular responses to repeated doses of bradykinin *in vitro* exhibit tachyphylaxis (Needleman, Marshall & Sobel, 1975), and we found that responses to the second of two stepwise infusions of bradykinin are significantly reduced (Fig. 5*A*). The magnitude of this effect is small, and desensitization is overcome by co-administration of enalaprilat during the second infusion. This results in a substantial *increase* in the response to bradykinin (Fig. 5*B*). Previous studies have shown that converting enzyme inactivates bradykinin *in vitro* (Dorer *et al.* 1974), but so also do kininase I and other proteolytic enyzmes (reviewed by Regoli & Barabé, 1980), and indeed

bronchoconstriction in response to inhaled bradykinin is not influenced by systemic inhibition of converting enzyme (Dixon, Fuller & Barnes, 1987). By contrast, our present findings show that converting enzyme *is* important for the inactivation of bradykinin which has been administered intra-arterially. This difference may reflect the endothelial location of the enzyme (Ryan *et al.* 1975; Caldwell *et al.* 1976) which is presumably particularly favourable for the metabolism of intra-vascular peptides. It is possible that one function of endothelially located converting enzyme is to prevent kinins, formed intravascularly, from diffusing into tissues. This could have pathophysiological importance in disorders in which intravascular kinin concentrations may be increased, such as diffuse intravascular coagulation, septicaemia and hereditary angio-oedema.

The failure of inhibition of converting enzyme to affect basal forearm blood flow suggests that under basal conditions neither conversion of angiotensin I to angiotensin II, nor degradation of bradykinin, determines resistance vessel tone in this vascular bed. However, in situations where the sympathetic nervous system is stimulated, production of angiotensin II by converting enzyme may be sufficient to exert an important modulatory role in control of local blood flow. Indeed, subpressor doses of angiotensin II markedly enhance sympathetic constriction in the human forearm (Benjamin, Seidelin & Webb, 1988). Peripheral converting enzyme activity may also be important in conditions such as salt depletion where renin production is increased (Webb & Collier, 1987) or pathological conditions associated with increased intravascular concentrations of bradykinin.

Mechanism of bradykinin-induced vasodilatation

The mechanism whereby bradykinin causes vasodilatation is unknown, but probably varies from species to species and from one vascular bed to another. Bradykinin stimulates production of vasodilator prostaglandins by cells in culture including human endothelial cells (Hong, 1980). Intravenous infusions of bradykinin in man cause accumulation of prostaglandin break-down products in blood (Barrow, Dollery, Heavey, Hickling, Ritter & Vial, 1986; Barrow, Cockcroft, Dollery, Hickling & Ritter, 1987). Intravenous bradykinin administered as a bolus causes transient hypotension, the duration of which is shortened by cyclo-oxygenase inhibitors in rats and rabbits, suggesting that vasodilatation in these species is partly mediated by cyclo-oxygenase products (Lecomte & Troquet, 1960; Vargaftig, 1966; Damas & Deby, 1976; Blackwell & Flower, 1981). Bradykinin relaxes isolated arteries of dogs, cats and rabbits (Cherry, Furchgott, Zawadski & Jothianandan, 1982). Relaxation of cat and rabbit arteries is inhibited by cyclo-oxygenase inhibitors and is presumably due to prostaglandin release (Cherry *et al.* 1982; Förstermann, Hertting & Neufang, 1986) whereas relaxation of canine arteries by bradykinin appears to be mediated by endothelium-derived relaxing factor (Cherry *et al.* 1982) subsequently identified as nitric oxide (Palmer *et al.* 1987).

Aspirin (3·3 mmol intravenously) inhibits bradykinin-stimulated prostaglandin production in man by greater than 90% at 30 min (Barrow *et al.* 1988), and in the present study there was very marked suppression of serum thromboxane concentration. The finding that this dose of aspirin does not inhibit the vasodilator response to bradykinin in the human forearm therefore shows that prostaglandin

production does not mediate the vasodilatation caused by bradykinin in this vascular bed. We considered the possibility that the enhanced response to bradykinin following converting enzyme inhibition might result from bradykinin gaining access to, and stimulating prostaglandin synthesis in, deeper structures such as vascular smooth muscle. However, the enhancement by enalaprilat of bradykinin-induced vasodilatation was not prevented by aspirin. We conclude that bradykinin does not cause vasodilatation in the human forearm by stimulating prostaglandin production; it is possible that it acts by releasing nitric oxide from endothelium (Palmer *et al.* 1987) although in the absence, to date, of an effective inhibitor of this substance *in vivo* we are unable to examine this further.

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