Br. J. Pharmacol. (1988), **94**, 449–462

Effects of enalapril on changes in cardiac output and organ vascular resistances induced by α_1- and α_2-adrenoceptor agonists in pithed normotensive rats

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1 Cardiac output, its distribution and regional vascular resistances were determined with tracer microspheres in pithed rats in the presence of the angiotensin converting enzyme inhibitor enalapril. The effects of enalapril on the cardiovascular responses elicited by either the α_1-adrenoceptor agonist phenylephrine or the α_2-adrenoceptor agonist xylazine were determined.

2 Enalapril decreased diastolic and mean blood pressure by decreasing cardiac index and total peripheral resistance. It induced vasodilatation in the kidney, epididimides, epididimidal fat and pancreas/mesentery. Vasoconstriction in the lungs, testes and liver was evident following enalapril administration as well as a decrease in the proportion of cardiac output passing to them, whilst the pancreas and mesentery received a greater proportion of the cardiac output. All the above effects of enalapril were reversed by infusion of angiotensin II at a rate of $75\,ng\,kg^{-1}\,min^{-1}$.

3 Xylazine increased blood pressure by increasing both cardiac output and total peripheral resistance. Enalapril did not affect the increase in cardiac output caused by xylazine but decreased the effect of the α_2-agonist on blood pressure by preventing the increase in total peripheral resistance. Inhibition by enalapril of xylazine-induced vasoconstriction in the kidneys, testes, fat and gastrointestinal tract contributed to the decrease in total peripheral resistance. Enalapril also inhibited xylazine-induced changes in cardiac output distribution to the liver, lungs and heart. All the above effects of enalapril were reversed by infusion of angiotensin II.

4 Enalapril decreased the sustained phase of the pressor response to an infusion of phenylephrine whilst having no effect on the initial peak pressor response to a bolus injection of phenylephrine. Phenylephrine increased both cardiac output and total peripheral resistance and enalapril abolished its effect on total peripheral resistance whilst having no effect on the increase in cardiac output. Enalapril inhibited phenylephrine-induced vasoconstriction in the testes, fat, muscle, spleen and gastrointestinal tract. Enalapril also inhibited phenylephrine-induced changes in cardiac output distribution to the lungs and liver. The infusion of angiotensin II did not fully reverse the inhibitory effect of enalapril either on the phenylephrine-induced increases in diastolic blood pressure or on the vasoconstriction in the fat, spleen and gastrointestinal tract, but did reverse all other effects of enalapril.

5 It is concluded that, although enalapril does not affect the increases in cardiac output caused by xylazine and phenylephrine, it inhibits the pressor responses to these agonists by reducing the resistance changes which they induce in several vascular beds. Vasoconstriction induced by xylazine may be mediated by circulating angiotensin II, as xylazine had a vasoconstrictor action in vascular beds devoid of α_2-adrenoceptors mediating vasoconstriction. It is also concluded that, since endogenous angiotensin II facilitates the sustained pressor responses to xylazine and phenylephrine infusion but not the peak response to a phenylephrine bolus, this action is on the calcium-dependent component of α-adrenoceptor activation. Further, since infusion of exogenous angiotensin II did not fully restore all the vasoconstrictor effects of phenylephrine following enalapril administration, angiotensin II of vascular origin may facilitate vasoconstriction induced by phenylephrine.

Introduction

Postjunctional α_1- and α_2-adrenoceptors mediate systemic pressor responses in the pithed rat (Docherty *et al.*, 1979; Drew & Whiting, 1979; Docherty & McGrath, 1980), and α-adrenoceptor-mediated increases in cardiac output contribute to these pressor responses (Kalkman *et al.*, 1984; Hiley

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& Thomas, 1987). Such responses to α_1- and α_2-adrenoceptor agonists can be differentially modulated by blood pH and blood gases (McGrath *et al.*, 1982; Grant *et al.*, 1985; Korstanje *et al.*, 1985; MacLean & Hiley, 1988) and also by calcium channel blockers (van Meel *et al.*, 1981; O'Brien *et al.*, 1985), although the differential influence of calcium channel blockers depends upon the time course of the pressor response rather than the subtype of the α-adrenoceptor involved (McGrath & O'Brien, 1987). In the absence of tonic sympathetic discharge in the pithed rat, the renin-angiotensin system is in a high state of activity (Vollmer *et al.*, 1984). Hence, the pithed rat has been used extensively to demonstrate the permissive role of angiotensin II on the cardiovascular responses to the activity of the sympathetic nervous system.

Thus, it has been shown that angiotensin converting enzyme inhibitors reduce vascular responses to both electrical stimulation of sympathetic efferent nerves and to exogenously administered noradrenaline or α-adrenoceptor agonists (Antonaccio & Kerwin, 1981; Clough *et al.*, 1982; Hatton & Clough, 1982; de Jonge *et al.*, 1983; Richer *et al.*, 1984). It has also been suggested that angiotensin converting enzyme inhibition differentially affects the pressor responses to α_1- and α_2-adrenoceptor stimulation (de Jonge *et al.*, 1982; Timmermans *et al.*, 1982), although there is evidence that this may also depend on the duration of the response and not the adrenoceptor subtype (O'Brien *et al.*, 1985).

The use of tracer microspheres enables extensive evaluation of the contribution of cardiac output and organ vascular resistances to pressor responses elicited by pressor agents. We have therefore used this methodology in order to evaluate, more fully, the influence of an angiotensin converting enzyme inhibitor (enalapril) on the changes in cardiovascular performance and resistance in the different vascular beds which contribute to the systemic pressor responses to phenylephrine and xylazine.

Methods

Determination of cardiac output and its distribution

Male Wistar rats weighing 250–300 g (Bantin & Kingman Ltd, Hull) were pithed under halothane anaesthesia by passing a 16 gauge steel needle through the orbit, through the foramen magnum and down into the spinal canal. Immediately after pithing, the rats were respired with air through a tracheal cannula by means of a respiratory pump (BioScience, Sheerness, U.K.) operating at 54 cycles min^{-1} with a volume of 20 ml kg^{-1}.

The right femoral artery was cannulated and connected to a Bell & Howell type 4-422-0001 transducer to measure systemic arterial blood pressure which was recorded on a Grass 7D polygraph. The left femoral artery was also cannulated and connected to a Braun Perfusor IV pump (Melsungen, F.R.G.) for the withdrawal of blood. With the aid of pressure monitoring, a cannula was passed down the right common carotid artery into the left ventricle. Drugs were administered through a cannula placed in the left external jugular vein and, when a sustained response had been obtained, 60000–80000 ^{113}Sn-labelled microspheres ($15 \pm 3\,\mu m$; NEN, Boston, MA), suspended by ultrasonication in saline containing 0.01% Tween 80, were injected into the ventricle over 20 s. Blood was withdrawn from the left femoral artery at a rate of 0.5 ml min^{-1} during and for 70 s after the microsphere injection.

Cardiac output and tissue blood flow were determined as described by McDevitt & Nies (1976). Organ vascular resistances were calculated from organ blood flows using the mean arterial pressure at the mid-point of the microsphere injection as inflow pressure and zero as venous outflow pressure. Blood gases and pH levels were analysed in all animals by removing 125 μl of blood from the right femoral artery before drug administration. This was analysed with a Corning 166 micro blood gas analyser. It has previously been shown that blood gases and pH do not change in the period between sampling and the end of the experiment after the microsphere injection (Hiley & Thomas, 1987). The values obtained in this study were as follows ($n = 80$): pH = 7.43 ± 0.01, $Paco_2$ = 30.5 ± 0.5, Pao_2 = 78.5 ± 0.9; there were no significant differences in blood gases or pH between the 10 groups of animals used in this study.

Drugs

All drugs were administered intravenously in 0.9% saline. All bolus injections were in volumes of 0.5 ml and all infusions at a rate of 0.1 ml min^{-1}.

No drugs were administered until blood pressure had been stable for 10 min after pithing (usually a total of 15–20 min was required). At this time a bolus injection was given, this was followed by a 10 min infusion before administration of a second bolus. This second bolus was followed by a further infusion during which the microsphere injection was made. The details are as follows.

Effects of enalapril alone The first bolus injection was of either 2 mg kg^{-1} enalapril (the generous gift of Merck, Sharpe & Dohme, Harlow, Essex) or the

saline vehicle. The first infusion was of either saline or angiotensin II (75 ng min^{-1} kg^{-1}; Sigma, Poole, Dorset). The second bolus was of saline and, after this, the saline or angiotensin II infusions were resumed.

Effects of enalapril on the responses to xylazine The first injection was of either saline or enalapril (2 mg kg^{-1}) and this was followed by infusion of saline or angiotensin II (75 ng min^{-1} kg^{-1}). The second injection was of saline or xylazine (0.5 mg; the kind gift of Bayer U.K., Newbury, Berkshire). The subsequent infusion was of xylazine (100 μg min^{-1}), saline or xylazine with angiotensin II (75 ng min^{-1} kg^{-1}). The saline control group was the same as that used to determine the effects of enalapril alone.

Effects of enalapril on the responses to phenylephrine The first injection was of either propranolol (3 mg kg^{-1}; the gift of ICI Pharmaceuticals, Macclesfield, Cheshire) or 3 mg kg^{-1} propranolol and 2 mg kg^{-1} enalapril (the propranolol was used to block any β-adrenoceptor effects of phenylephrine). The first infusion was of saline or 75 ng kg^{-1} min^{-1} angiotensin II. The second bolus injection was 5 μg phenylephrine (Sigma, Poole, Dorset) or saline and was followed by infusion of either saline, phenylephrine or phenylephrine with angiotensin II (75 ng min^{-1} kg^{-1}). Phenylephrine was infused at 100 μg min^{-1}.

In the groups given angiotensin II, the interruption of this infusion during the second bolus injection was allowed for by including angiotensin II (100 pg) with the agonist or saline.

Pressor responses to 100 ng angiotensin I (Sigma, Poole, Dorset) were determined before and after administration of enalapril.

Statistical comparison

All results are given as the mean ± s.e. mean. The statistically significant differences between groups were assessed by analysis of variance followed by the least significant difference procedure (Snedecor & Cochran, 1980).

Results

Cardiovascular effects of enalapril alone

Enalapril (2 mg kg^{-1}) abolished the pressor response to 100 ng angiotensin I, indicating inhibition of angiotensin II formation.

Table 1 shows the effects of enalapril on cardiovascular haemodynamics and organ vascular resistances; it can be seen that enalapril decreased diastolic and mean blood pressures by 28% and 31%, respectively. The decrease in blood pressure was due to the combined effect of decreases in both cardiac index (18%) and total peripheral resistance (18%). There were no significant changes in either heart rate or stroke volume following enalapril administration but inspection of the results presented in Table 1 suggests the most likely origin of this decrease in cardiac output to be stroke volume, the value of which is 18% less than the saline control. The effects of enalapril on blood pressure, cardiac index and total peripheral resistance were all fully reversed by angiotensin II. This rate of infusion of angiotensin II into rats given enalapril did not produce a significantly different systemic blood pressure from that in the control animals (Table 1).

Table 1 also shows that enalapril brought about vasodilatation in the kidneys (where vascular resistance was 22% lower than the saline control group), epididimides (33% lower resistance), epididimidal fat (a 73% decrease) and the pancreas and mesentery (62% lower). Following the infusion of angiotensin II, the resistances in these vascular beds returned to levels very close to those observed in the saline control group. Whilst enalapril itself did not affect the vascular resistance in the stomach, in the presence of angiotensin II there was an increase in resistance over that in the saline treated group. It should be noted that enalapril caused increases in the vascular resistance of the testes (48%) and liver (63%) as well as increasing the apparent vascular resistance of the lungs by 280%.

Figure 1 shows the effect of enalapril on the distribution of cardiac output to the organs. It can be seen that enalapril brought about a 92% increase in the proportion of cardiac output received by the pancreas and mesentery. The enalapril-induced vasoconstriction in the lungs, testes and liver, combined with the decreased resistances in other organs approximately halved the proportion of the cardiac output passing to the lungs, testes and liver (reduced by 55%, 59% and 52%, respectively). All the effects of enalapril on cardiac output distribution were completely reversed by the chosen dose of angiotensin II infused.

The effect of enalapril on the cardiovascular changes induced by xylazine

The bolus injection of xylazine induced a pressor response which did not decline and was maintained at a constant level during the subsequent infusion of the agonist. Table 2 shows that xylazine increased both diastolic and mean arterial blood pressures by 95% and this was due to the combined effects of increases both in cardiac index (34%) and in total peripheral resistance (41%). The increase in cardiac

Table 1 The effects of enalapril on haemodynamics and organ vascular resistances

	Saline and saline infusion	*Enalapril and saline infusion*	*Enalapril and angiotensin II infusion*
Diastolic blood pressure (mmHg)	49 ± 1	49 ± 1	53 ± 2
Change in diastolic blood pressure (mmHg)	−1 ± 1	−15 ± 1***	−2 ± 2
Mean arterial pressure (mmHg)	57 ± 2	57 ± 2	63 ± 3
Change in mean arterial pressure (mmHg)	−1 ± 1	−16 ± 1***	−2 ± 2
Heart rate (beats min^{-1})	371 ± 10	369 ± 9	370 ± 21
Change in heart rate (beats min^{-1})	1 ± 4	−1 ± 1	−3 ± 2
Cardiac index (ml min^{-1} 100 g body wt)	11.9 ± 0.6	9.8 ± 0.5*	11.4 ± 0.6
Stroke volume (μl)	93 ± 3	76 ± 7	89 ± 8
Total peripheral resistance (mmHg ml^{-1} min 100 g body wt)	4.9 ± 0.3	4.0 ± 0.3*	5.4 ± 0.2
Organ vascular resistances (mmHg ml^{-1} min g)			
Heart	43.0 ± 3.8	32.6 ± 5.0	39.5 ± 3.0
Lungs	96.7 ± 14.3	372 ± 129*	118 ± 29
Kidneys	26.0 ± 2.0	20.4 ± 1.3*	32.0 ± 1.0
Testes	294 ± 22	436 ± 15***	345 ± 32
Epididimides	641 ± 78	432 ± 30*	633 ± 64
Fat	3520 ± 632	944 ± 96**	2409 ± 679
Skeletal muscle	478 ± 46	464 ± 64	579 ± 56
Skin	1553 ± 668	1494 ± 363	1048 ± 164
Liver	542 ± 47	883 ± 106**	680 ± 64
Spleen	94.4 ± 9.8	98.8 ± 10.1	120 ± 15
Stomach	149 ± 21	130 ± 10	203 ± 19*
Small intestine	58.1 ± 5.1	53.5 ± 3.1	57.4 ± 2.9
Large intestine	93.8 ± 9.9	76.6 ± 10.7	86.5 ± 5.3
Pancreas and mesentery	201 ± 24	77.0 ± 3.4***	217 ± 22

For all groups, $n = 8$. Significant differences between the saline and the two enalapril groups were determined by analysis of variance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
The values of heart rate, diastolic and mean arterial pressures are those before administration of any drug. The values for changes are those at the mid-point of the microsphere injection. Resistance calculations assume central venous pressure to be zero and total peripheral resistance was calculated using the mean arterial pressure at the mid-point of the microsphere injection.

index was due solely to an increase in stroke volume as heart rate was not changed. Enalapril inhibited the xylazine-induced increases in diastolic and mean arterial pressure by 46% and 44%, respectively. The inhibition by enalapril of the pressor effect of xylazine was due to total peripheral resistance being 36% lower in those animals receiving both drugs compared to those given xylazine alone. Enalapril did not affect the xylazine-induced increase in cardiac index.

It can also be seen from Table 2 that xylazine promoted vasoconstriction in the kidneys (vascular resistance increased by 60%), testes (70%), fat (110%), muscle (88%), spleen (148%), stomach (76%), small intestine (86%), large intestine (64%) and pancreas and mesentery (131%), and decreased hepatic vascular resistance by 53%.

Prior administration of enalapril considerably reduced these responses to xylazine. With the exceptions of the skeletal muscle, liver, spleen and pancreas/mesentery, organ vascular resistances in the presence of enalapril and xylazine were significantly lower in those tissues which experienced an increase with xylazine alone. Indeed, the only organ resistances significantly different between the xylazine/enalapril and the saline control groups were those in

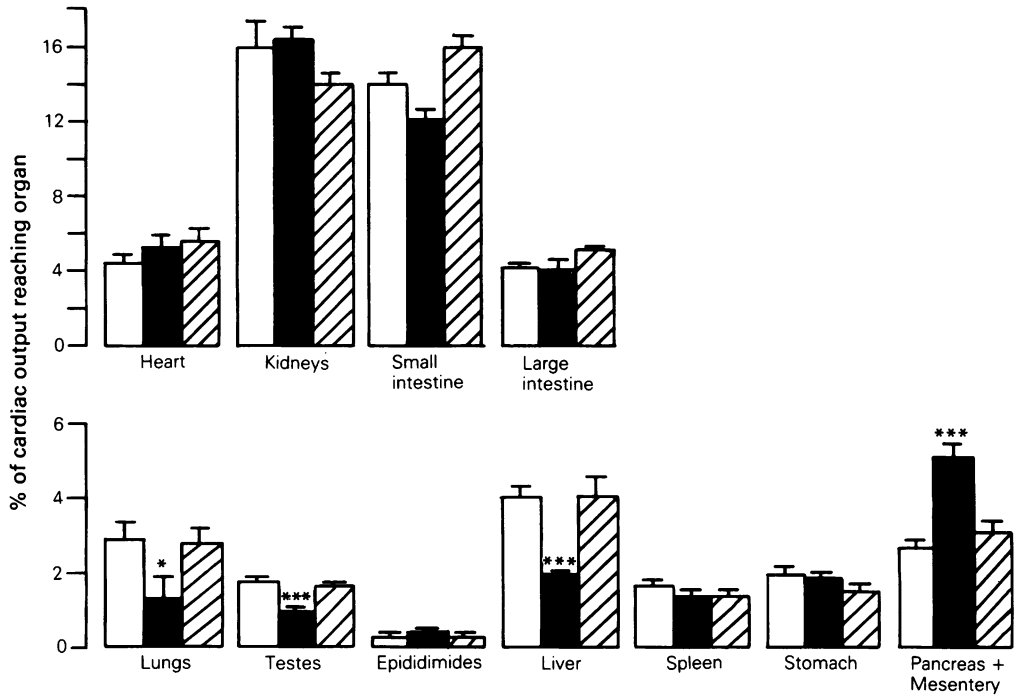

Figure 1 Effects of enalapril on the distribution of cardiac output to the organs in the pithed rat. The open columns represent the values obtained in animals given saline alone; the solid columns represent the results in rats given $2\,mg\,kg^{-1}$ enalapril and infused with saline; and the hatched columns give the data obtained in rats given $2\,mg\,kg^{-1}$ enalapril and then infused with angiotensin II ($75\,ng\,kg^{-1}\,min^{-1}$). $n = 8$ for each group. Statistical comparison between the enalapril groups and the saline group was by analysis of variance: $^{*}P < 0.05$; $^{***}P < 0.001$.

the heart (where it was 31% lower than the controls; $P < 0.05$), epididimides (33% lower than the saline group; $P < 0.05$), liver (37% lower than control; $P < 0.001$) and spleen (resistance 145% higher than control; $P < 0.001$). Thus, only in the skeletal muscle, spleen and pancreas/mesentery did enalapril have no significant influence on the increases in vascular resistance induced by xylazine. However, in the presence of this agonist and enalapril there was a 64% decrease in skin vascular resistance where no change had been seen with enalapril alone (see Table 1), but the vasodilator action of the enalapril/xylazine combination in the epididimides had also been evident after enalapril administration alone. During the angiotensin II infusion, none of the vascular resistances in the presence of xylazine and enalapril were significantly different from those observed in the presence of xylazine alone. Therefore all the changes induced by enalapril in the cardiovascular responses to xylazine administration were fully reversed by the chosen dose of angiotensin II infused.

Figure 2 shows the effects of xylazine on the percentages of cardiac output passing to the organs and also the influence of enalapril on the xylazine-induced changes. Xylazine decreased the proportion of cardiac output distributed to the testes (by 24%), spleen (by 46%) and pancreas/mesentery (by 50%), presumably as a result of its vasoconstrictor action in these vascular beds. It also doubled the distribution of cardiac output to the liver largely due to the decrease in hepatic arterial vascular resistance. Xylazine also increased the fraction passing to the epididimides (200%) and lungs (134%), probably because of vasoconstriction within other vascular beds since there was no evidence of vasodilatation in these two organs. However, it should be noted that the microspheres trapped in the lungs represent not only those passing directly through the bronchial arteries but also those passing through arteriovenous anastomoses. These effects of xylazine on liver and lungs were inhibited by enalapril (45% and 61% reductions, respectively). Enalapril also reversed the increase by xylazine of the percentage of cardiac output sent to the heart, reducing this by 26% such that it was no longer significantly different from

Table 2 The effects of enalapril on xylazine-induced changes in haemodynamics and organ vascular resistances

	Saline and saline infusion	*Xylazine and saline infusion*	*Xylazine and enalapril and saline infusion*	*Xylazine and enalapril and angiotensin II infusion*
Diastolic blood pressure (mmHg)	49 ± 1	54 ± 3	34 ± 2**	54 ± 2
Change in diastolic blood pressure (mmHg)	−1 ± 1	52 ± 6†††	28 ± 3***	45 ± 5
Mean arterial pressure (mmHg)	57 ± 2	62 ± 2	42 ± 2**	62 ± 2
Change in mean arterial pressure (mmHg)	−1 ± 1	59 ± 6†††	33 ± 4***	56 ± 5
Heart rate (beats min^{-1})	371 ± 10	344 ± 13	370 ± 9	369 ± 11
Change in heart rate (beats min^{-1})	1 ± 4	−6 ± 8	−11 ± 8	3 ± 9
Cardiac index (ml min^{-1} 100 g body wt)	11.9 ± 0.6	15.9 ± 0.6††	17.1 ± 0.5	18.6 ± 1.6*
Stroke volume (μl)	93 ± 3	131 ± 6††	129 ± 4	134 ± 13
Total peripheral resistance (mmHg ml^{-1} min 100 g body wt)	4.9 ± 0.3	6.9 ± 0.5††	4.4 ± 0.3***	6.8 ± 0.6
Organ vascular resistances (mmHg ml^{-1} min g)				
Heart	43.0 ± 3.8	33.8 ± 4.4	29.6 ± 3	33.0 ± 2.4
Lungs	96.7 ± 14.3	59.3 ± 8.6	109 ± 19*	56.2 ± 11.3
Kidneys	26.0 ± 2.0	43.2 ± 2.8††	18.1 ± 2.0***	47.8 ± 6.3
Testes	294 ± 22	501 ± 36†††	294 ± 18***	584 ± 65
Epididimides	641 ± 78	642 ± 67	430 ± 20*	640 ± 73
Fat	3520 ± 632	7410 ± 1129†	2137 ± 435**	5845 ± 1773
Skeletal muscle	478 ± 46	900 ± 101††	651 ± 94	990 ± 145
Skin	1553 ± 668	1738 ± 184	619 ± 32*	1558 ± 281
Liver	542 ± 47	255 ± 17†††	341 ± 40	274 ± 38
Spleen	94.4 ± 9.8	234 ± 17†††	231 ± 23	228 ± 23
Stomach	149 ± 21	262 ± 31†	123 ± 14**	264 ± 55
Small intestine	58.1 ± 5.1	108 ± 11†††	56.4 ± 6.6***	85.4 ± 10.1
Large intestine	93.8 ± 9.9	154 ± 23†	101 ± 11*	154 ± 21
Pancreas and mesentery	201 ± 24	464 ± 106†	295 ± 73	522 ± 100

For all groups, $n = 8$.
For clarity, the saline and saline infusion group from Table 1 has been included here. Significant differences between that group and the xylazine + saline infusion group were assessed by analysis of variance: †$P < 0.05$; ††$P < 0.01$; †††$P < 0.001$.
Statistical significance between the xylazine and enalapril groups and the xylazine and saline group was also assessed by analysis of variance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
The values of heart rate, diastolic and mean arterial pressures are those obtained after administration of enalapril or its saline vehicle. Changes are those between these values and those at the mid-point of the microsphere injection.

control. The increased distributions to the kidneys (51% higher than with xylazine alone and 35% greater than the controls) and small intestine (22% and 12% greater than xylazine alone and controls, respectively), observed in the presence of both xylazine and enalapril, were due to the comparative vasodilator effect of enalapril on these organs under these circumstances (see Table 2). All the above effects of enalapril on the changes in distribution caused by xylazine were reversed by simultaneous infusion of angiotensin II.

The effect of enalapril on cardiovascular changes induced by phenylephrine

The bolus injection of phenylephrine elicited a large transient peak pressor response which declined rapidly before the subsequent infusion of phenylephrine (which was started immediately after the bolus injection) produced a stable, though lesser, pressor response which was maintained for the 70 s period between microsphere injection and termination of the experiment. Table 3 shows the values obtained

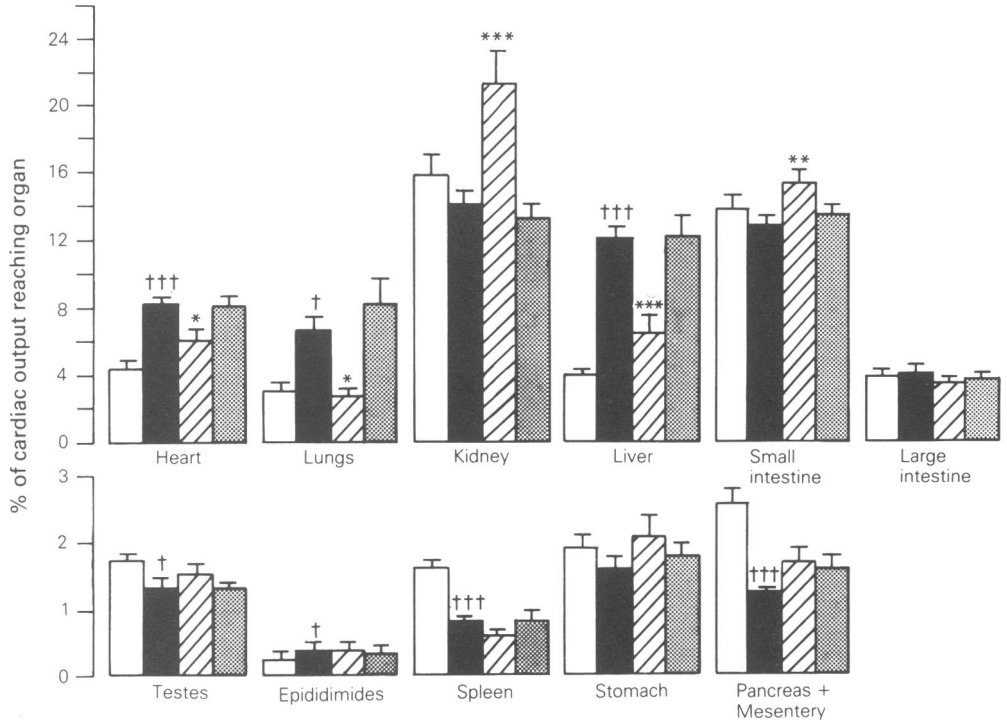

Figure 2 Effects of enalapril on the changes in cardiac output distribution induced by xylazine. The open columns give the results obtained in rats given saline alone; the solid columns give the data from animals given xylazine alone; the hatched columns are the results from rats given 2 mg kg^{-1} enalapril before xylazine (0.5 mg bolus followed by an infusion of 0.1 mg min^{-1}); and the stippled columns the data from rats given enalapril and then infused with angiotensin II (75 ng kg^{-1} min^{-1}) before and during the xylazine administration. $n = 8$ for each group. Statistical differences were assessed by analysis of variance: between the saline control group and the xylazine alone group; †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$: between the xylazine alone group and the groups given enalapril; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

for the prolonged pressor response, during which there were increases in diastolic and mean arterial pressures of 89% and 81%, respectively, relative to the pre-phenylephrine values. This sustained pressor response was due to increases in both cardiac index (50%) and total peripheral resistance (39%); the increased cardiac index was due to a 38% increase in stroke volume. Enalapril inhibited the phenylephrine-induced increase in diastolic blood pressure by 75% and the increase in mean arterial pressure by 59%. As enalapril did not influence the phenylephrine-induced increase in cardiac index, these effects on blood pressure were due to a 30% lower total peripheral resistance in those animals given phenylephrine and enalapril compared to those given the α-agonist alone. The chosen rate of infusion of angiotensin II reversed all the above effects of enalapril, except that there was not complete restoration of the response in diastolic blood pressure.

The effect of enalapril on the peak pressor response to the phenylephrine bolus injection was also investigated. With phenylephrine alone the peak response to the bolus injection was an increase in mean arterial pressure of 69.8 ± 5.5 mmHg ($n = 8$). In those rats pretreated with enalapril, the peak pressor response was 62.3 ± 6.4 mmHg ($n = 8$) and when angiotensin II was given as well as enalapril, there was an increase of 62.1 ± 7.0 mmHg ($n = 8$). There were no significant differences between these values and so it may be concluded that enalapril only inhibited the prolonged pressor response to phenylephrine infusion.

Table 3 also shows that phenylephrine induced greatly differing degrees of vasoconstriction in the kidneys, testes, fat, muscle, spleen, small intestine,

Table 3 The effects of enalapril on phenylephrine-induced changes in haemodynamics and organ vascular resistance in the presence of propranolol (3 mg kg^{-1})

	Saline and saline infusion	*Phenylephrine and saline infusion*	*Phenylephrine and enalapril and saline infusion*	*Phenylephrine and enalapril and angiotensin II infusion*
Diastolic blood pressure (mmHg)	49 ± 2	46 ± 3	39 ± 2*	53 ± 4
Change in diastolic blood pressure (mmHg)	−1 ± 1	41 ± 4†††	11 ± 1***	28 ± 6*
Mean arterial pressure (mmHg)	59 ± 2	57 ± 2	49 ± 2*	61 ± 4
Change in mean arterial pressure (mmHg)	1 ± 1	46 ± 7†††	19 ± 3***	37 ± 7
Heart rate (beats min^{-1})	331 ± 19	326 ± 10	359 ± 20	323 ± 19
Change in heart rate (beats min^{-1})	2 ± 4	9 ± 9	−1 ± 5	18 ± 6
Cardiac index (ml min^{-1} 100 g body wt)	13.9 ± 0.7	20.9 ± 0.9†††	19.4 ± 1.2	20.7 ± 1.6
Stroke volume (μl)	123 ± 9	170 ± 6††	151 ± 10	175 ± 17
Total peripheral resistance (mmHg ml^{-1} min 100 g body wt)	3.6 ± 0.1	5.0 ± 0.3††	3.5 ± 0.2**	4.8 ± 0.4
Organ vascular resistances (mmHg ml^{-1} min g)				
Heart	30.9 ± 4.1	31.9 ± 5.0	23.9 ± 3.7	27.3 ± 3.2
Lungs	101 ± 25	43.1 ± 8.0	232 ± 90**	65.8 ± 11.3
Kidneys	23.2 ± 1.1	28.7 ± 1.9†	15.5 ± 0.9***	29.8 ± 2.8
Testes	223 ± 11	377 ± 3.5†††	284 ± 16*	347 ± 41
Epididimides	433 ± 40	566 ± 64	404 ± 57	569 ± 71
Fat	1919 ± 300	6773 ± 1120†††	1702 ± 376***	3902 ± 928*
Skeletal muscle	376 ± 34	786 ± 70†††	486 ± 59**	741 ± 98
Skin	811 ± 106	834 ± 90	903 ± 173	744 ± 119
Liver	452 ± 44	251 ± 18††	457 ± 69**	344 ± 37
Spleen	72.0 ± 8.0	128 ± 9†††	98.6 ± 8.5*	90.1 ± 8.7**
Stomach	127 ± 9	215 ± 28††	114 ± 5***	168 ± 17
Small intestine	43.8 ± 2.8	63.4 ± 5.8††	30.4 ± 2.0***	46.9 ± 4.7**
Large intestine	67.8 ± 4.7	97.7 ± 10.9†	53.6 ± 7.5***	69.9 ± 7.3*
Pancreas and mesentery	133 ± 9	292 ± 42†††	96.6 ± 10.5***	190 ± 25**

For all groups, $n = 8$.
Statistical differences between the saline and saline infusion group and the phenylephrine and saline infusion groups were determined by analysis of variance: †$P < 0.05$; ††$P < 0.01$; †††$P < 0.001$.
Statistical significance between the phenylephrine and enalapril groups and the phenylephrine and saline group was also assessed by analysis of variance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
The values of heart rate, diastolic and mean arterial blood pressures were obtained after the administration of propranolol and either saline or enalapril. Changes are those occurring between these values and the mid-point of the microsphere injection.

large intestine and pancreas/mesentery (increases in resistance of 24%, 69%, 253%, 410%, 78%, 69%, 45%, 44% and 120% respectively, relative to the saline control group). Enalapril inhibited the phenylephrine-induced vasoconstriction in all these organs, respectively by 46%, 25%, 75%, 38%, 23%, 47%, 52%, 45% and 67% relative to those in rats given phenylephrine alone. Indeed, in the kidneys, resistance in the phenylephrine/enalapril group was significantly below that in the saline group by 33% ($P < 0.01$). There were also increases in apparent lung vascular resistance (of 438%) and in hepatic arterial resistance to flow (of 82%) following phenylephrine and enalapril administration compared to phenylephrine alone. The effects of enalapril on vascular resistances in the lungs, kidneys, testes,

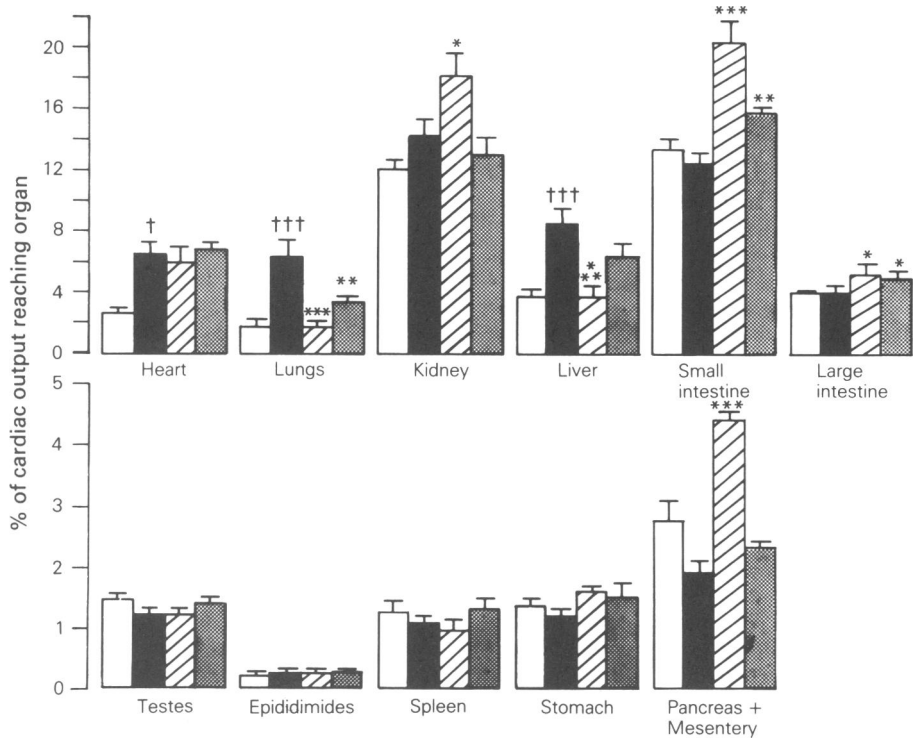

Figure 3 Effects of enalapril on the changes in cardiac output distribution induced by phenylephrine. All animals had received 3 mg kg^{-1} propranolol. The open columns show the results from rats given saline; the solid columns are the data from the group given phenylephrine (5 μg bolus followed by infusion of 0.1 mg min^{-1}); the hatched columns are results from the rats given 2 mg kg^{-1} enalapril before the phenylephrine; and the stippled columns are the data from the group given enalapril and then infused with angiotensin II (75 ng kg^{-1} min^{-1}) before and during the administration of phenylephrine. $n = 8$ for all groups. Statistical differences were assessed by analysis of variance: between the saline group and the phenylephrine alone group; †$P < 0.05$, †††$P < 0.001$: between the phenylephrine alone group and the two enalapril groups; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

muscle and liver were more or less fully reversed by angiotensin II, whilst the changes in the fat, small intestine, large intestine and pancreas/mesentery were not. Also the angiotensin II did not influence the enalapril-induced decrease in splenic vascular resistance that occurred in the presence of the α_1-adrenoceptor agonist.

Since there were no changes in resistance of the heart and lungs (Table 3), it would seem that vasoconstriction of other vascular beds by phenylephrine induced the increases in the proportions of cardiac output passing to the heart (47%) and lungs (214%), but the increase in distribution to the liver (45%) was due, at least in part, to a decrease in hepatic arterial resistance (Figure 3). Enalapril reversed the phenylephrine-induced increases in the proportion of cardiac output sent to the lungs and liver. It also increased the fractions of the cardiac output received by the kidneys, small intestine, large intestine and pancreas/mesentery due to its relative vasodilator actions in these vascular beds in the presence of phenylephrine. The angiotensin II infusion reversed the effects of enalapril on the phenylephrine-induced changes in cardiac output distribution to the liver, kidneys and pancreas/mesentery, but did not reverse the effects of enalapril on the fractions passing to the lungs, small intestine and large intestine. In the case of the small intestine, however, the response to phenylephrine in the presence of both angiotensin II and enalapril was significantly different from those obtained with phenylephrine alone ($P < 0.05$) and phenylephrine after enalapril ($P < 0.01$).

Discussion

The effects of enalapril on the cardiovascular system

In the absence of a tonic sympathetic discharge, the pithed rat must depend to some degree on the ele-

vated state of the renin-angiotensin system for maintenance of blood pressure. Our results show that enalapril decreased diastolic and mean blood pressures by 30%. This indicates that circulating angiotensin II contributes to the maintenance of basal blood pressure, although other vasopressor humoral agents (e.g. adrenal medullary catecholamines) and intrinsic myogenic tone must contribute considerably. Enalapril was used in this study as a potent inhibitor of angiotensin converting enzyme (Cohen *et al.*, 1983). Inspection of Table 1 shows that the chosen rate of infusion of angiotensin II restored all the haemodynamic parameters to values very close to those obtained in the saline control group with the exception of vascular resistance in the stomach. The fact that such identity of values could be obtained with angiotensin II alone gives strong support to the contention that the reduction in systemic blood pressure by enalapril was due solely to its inhibiting angiotensin II formation.

Enalapril reduced blood pressure by decreasing both cardiac output and total peripheral resistance, the decrease in cardiac output being most likely due to a decrease in stroke volume. Enalapril-induced vasodilatation in the kidneys, epididimides, fat and pancreas/mesentery contributed to the decrease in total peripheral resistance, and all of these resistance changes were returned to pre-enalapril levels by angiotensin II. As the kidneys and gastrointestinal tract normally receive a large proportion of the cardiac output (Wade & Bishop, 1962), even small vascular resistance changes in these organs will result in profound changes in total peripheral resistance. Indeed, it has been shown, both *in vivo* and *in vitro*, that the vasoconstrictor effect of angiotensin II is greatest in these vascular beds (for review, see Greenway, 1981).

It is interesting to note that there was increased hepatic arterial vascular resistance following enalapril administration. This is possibly a reflection of the enalapril-induced decrease in resistance within the gastrointestinal tract of 25%. Taken as a whole (stomach, small intestine, large intestine and pancreas and mesentery), the vascular resistance in the gastrointestinal tract fell from 99.2 ± 9.8 to 74.6 ± 4.5 mmHg ml^{-1} min g with enalapril. Sato *et al.* (1977) showed that the two vascular inputs into the liver interact, apparently by purely mechanical processes, such that increases or decreases in hepatic portal venous supply are at least in part compensated by changes in hepatic arterial inflow resistance; thus, a fall in resistance to portal inflow resulted in an increase in hepatic arterial resistance. Hence, the apparent vasoconstrictor activity of enalapril in the hepatic artery may be due to the removal of the influence of angiotensin II from the arterioles of the organs draining into the hepatic portal vein.

Enalapril also increased the apparent vascular resistance of the lungs and this effect was also observed in the presence of xylazine and phenylephrine. The calculated change in resistance derived in part from reductions in the number of microspheres trapped in the lungs and it must be remembered that microspheres reach the lungs by passing not only through the bronchial arteries but also peripheral arteriovenous shunts. These latter spheres become trapped after returning through the venous system and the right heart. Thus, a decrease in the proportion of microspheres trapped in the lungs may be due either to increased vascular resistance to bronchial artery flow or to a decrease in shunting; it is therefore possible that angiotensin II may facilitate opening of such shunts.

The vasodilatation by enalapril of the pancreatic and mesenteric vascular beds promoted a correspondingly large increase in the percentage of cardiac output passing to this vasculature. This effect was inhibited by angiotensin II. It is widely accepted that angiotensin II facilitates the vasoconstrictor effects of the sympathetic nervous system (e.g. Clough *et al.*, 1982). Our investigations show that, even in the absence of sympathetic drive, angiotensin II may exert a tonic vasoconstriction on such organs as the kidneys, gastrointestinal tract, epididimides and fat, and is capable of modulating total peripheral resistance through vasoconstriction in these vascular beds. Endogenous angiotensin II also facilitates basal adrenal medullary catecholamine release from sympathetically denervated adrenal glands and inhibition of the renin-angiotensin system inhibits such release (MacLean & Ungar, 1986). Thus the vasodilator effects of enalapril in the pithed rat may also reflect a decrease in basal adrenal catecholamine release due to removal of the permissive effect of angiotensin II on the adrenals. The results also indicate that endogenous angiotensin II maintains cardiac output in the pithed rat either by supporting cardiac contractility or, more likely, through maintenance of venous return by decreasing venous capacitance (Greenway, 1981).

The effect of enalapril on cardiovascular changes induced by phenylephrine and xylazine

There is much evidence that, in the pithed rat, circulating angiotensin II exerts a permissive effect on pressor responses to either sympathetic nerve stimulation or exogenously administered noradrenaline (Antonaccio & Kerwin, 1981; Clough *et al.*, 1982; Hatton & Clough, 1982; Richer *et al.*, 1986). There has been speculation as to whether or not angiotensin converting enzyme inhibitors selectively inhibit that part of the vasoconstrictor effect of noradrenaline which is mediated by α_2-adrenoceptors.

Such an interaction has been supported by Timmermans *et al.* (1982) and de Jonge *et al.* (1982). There is, however, evidence to suggest that the effects of these inhibitors do not depend on the adrenoceptor subtype but on the duration of the pressor response elicited by α-adrenoceptor agonists (Grant & McGrath, 1984a; O'Brien *et al.*, 1985).

The results of the present study show that enalapril inhibited the stable, prolonged pressor responses to both xylazine and phenylephrine. It did not, however, depress the initial transient peak pressor response to the phenylephrine bolus. This lends support to the view that susceptibility of α-agonist pressor responses to depression by such inhibitors depends upon the duration of the response and angiotensin II exerts a permissive effect on such pressor responses. McGrath & O'Brien (1987) have shown a similar phenomenon with the calcium channel blocker nifedipine, in that the second, slower, component of pressor responses to α_1- and α_2-adrenoceptor agonists was blocked by nifedipine, whilst the initial peak responses to a range of α_1-adrenoceptor agonists were nifedipine-resistant. In the pithed rat, the pressor response to angiotensin II can be blocked by nifedipine (Grant & McGrath, 1984b) and so part of the inhibitory effect of nifedipine on the prolonged pressor response to α-agonists may be due to interference with a permissive effect of angiotensin II.

The effects of phenylephrine and xylazine on cardiac output, its distribution and tissue blood flows, as determined using radioactive microspheres have been extensively discussed previously (Hicks & Waldron, 1983; Waldron & Hicks, 1985; Hiley & Thomas, 1987). Phenylephrine and xylazine increase blood pressure by increasing both cardiac output (by increasing stroke volume) and total peripheral resistance. Enalapril reduced the pressor responses to both these agonists by reducing the total peripheral resistance increments without affecting cardiac output. Kaufman & Vollmer (1985) demonstrated similar effects for captopril and saralasin; these agents inhibited the increase in total peripheral resistance, elicited by sympathetic nerve stimulation in the pithed rat, without reducing the increases in cardiac output. This indicates that pressor responses evoked by stimulation of either or both α_1- and α_2-adrenoceptors are facilitated by circulating angiotensin II acting on peripheral resistance vessel beds, as opposed to an action on cardiac output.

Enalapril inhibited the increase in total peripheral resistance induced by xylazine through inhibiting vasoconstriction in such organs as the kidneys, testes, fat, stomach, small intestine and large intestine, without affecting vasoconstriction in the muscle or spleen. Its action as a vasodilator in the epididimides was also evident in the presence of xylazine. Alone, neither enalapril nor xylazine had any effect on skin vascular resistance but combined they induced a decrease which was reversed by angiotensin II. This suggests that, although endogenous angiotensin II itself does not exert an overt tonic vasoconstriction in the skin, in the presence of xylazine such an effect was uncovered, though it should be noted that angiotensin II has been shown to be a constrictor of the skin vasculature (Greenway, 1981).

Xylazine caused vasoconstriction in the pancreas/mesentery and following enalapril administration, vascular resistance here was not significantly different from pre-xylazine levels. Angiotensin II infusion restored the xylazine response in the presence of enalapril to that observed with xylazine alone. Similar changes took place in the kidneys, testes, fat, skeletal muscle, stomach and the large and small intestines. Enalapril itself was shown to decrease vascular resistances in the kidneys, fat and pancreas/mesentery, which suggests that endogenous angiotensin II constricts these beds. Also, angiotensin II infusion caused vasoconstriction in the stomach (Table 1). Thus, angiotensin II and xylazine have parallel effects in several vascular beds and this suggests that at least part of the inhibitory effect of enalapril may be due to the direct removal of the action of the peptide on the vascular smooth muscle, but it does not exclude the possibility of a permissive role for angiotensin II on the effects of xylazine on these vascular beds.

Angiotensin II does facilitate the effects of adrenergic transmission in the rat mesenteric bed (Malik & Nasjletti, 1976; Zimmerman, 1978; Campbell & Jackson, 1979), and vasoconstriction of the rat mesenteric bed induced by exogenous noradrenaline can be inhibited by angiotensin II converting enzyme inhibitors (Clough *et al.*, 1982). Several *in vitro* and *in situ* studies on the perfused rat mesenteric bed have failed to show a direct vasoconstrictor action of α_2-adrenoceptor agonists and the pressor response to noradrenaline in this vascular bed is largely due to activation of α_1-adrenoceptors (Fiotakis & Pipili, 1983; Yamamoto *et al.*, 1984; Nichols, 1985; Nichols & Hiley, 1985). The dose of xylazine used in this study caused vasoconstriction in the kidneys, which have been shown to have postjunctional α_2-adrenoceptors (U'Prichard & Snyder, 1979; McPherson & Summers, 1981). Renal vasoconstriction has been shown to induce renin release (Coote *et al.*, 1972), hence where xylazine-induced vasoconstriction occurs in the apparent absence of functional α_2-adrenoceptors, its effects may be mediated by increased levels of circulating angiotensin II. Enalapril did not itself decrease vascular resistance in the testes (here it actually increased with enalapril), small or large intestine, yet it inhibited xylazine-induced vasoconstriction in these organs and so the effects of

xylazine on these vascular beds may be due to its increasing circulating angiotensin II.

Enalapril also antagonized the xylazine-induced increase in the proportion of cardiac output passing to the liver and lungs, perhaps by its indirect vasoconstrictor action in these organs. In addition, xylazine increased the fractions of cardiac output sent to the heart and epididimides but, since vascular resistances in these two organs were not changed, this redistribution in their favour must be due to vasoconstriction in other vascular beds, including the testes, spleen and pancreas/mesentery. Enalapril prevented the redistribution of cardiac output to the cardiac circulation by xylazine. There appeared to be a consequent redistribution of cardiac output to the kidneys and small intestine due to vasodilatation in these organs. All the above effects of enalapril were reversed by the infusion of angiotensin II and exemplify the ability of endogenous angiotensin II to influence the distribution of cardiac output.

The inhibition of enalapril of the increase in total peripheral resistance induced by phenylephrine was due to reduction of phenylephrine-induced vasoconstriction in the testes, fat, muscle, spleen and gastrointestinal tract. In the fat and pancreas/mesentery, the vasodilator effect of enalapril may also have contributed to its inhibitory effects. The reduction of phenylephrine-induced vasoconstriction in the testes, muscle and stomach were all fully reversed by angiotensin II, indicating a facilitatory role of endogenous angiotensin II on vasoconstriction induced by phenylephrine. The effects of enalapril on the vasoconstriction in the fat, small intestine, large intestine and pancreas/mesentery caused by phenylephrine were not fully reversed by angiotensin II, nor was splenic vascular resistance affected by its infusion. The failure of the angiotensin II infusion to reverse these effects of enalapril is reflected in its failure to restore completely the decreased diastolic blood pressure (Table 3).

It has been reported previously that exogenous angiotensin II fails to reverse fully angiotensin II converting enzyme inhibitor-induced reductions in pressor responses to both exogenous noradrenaline and sympathetic nerve stimulation (Bull & Drew, 1984; Grant & McGrath, 1984b). These authors suggested that angiotensin II converting enzyme inhibitors decrease the levels of angiotensin II synthesized in vascular smooth muscle (Gould *et al.*, 1964; Rosenthal *et al.*, 1969; Ganten *et al.*, 1970) and that infusion of angiotensin II may not readily replace these vascular sources of angiotensin II. The dose of phenylephrine used in our study did not induce renal vasoconstriction and does not directly induce renin release from the kidney (Hesse & Johns, 1985). Hence angiotensin II produced and stored in vascular smooth muscle may exert a permissive effect on vasoconstriction induced by phenylephrine, as well as pre-existing circulating angiotensin II, and this may account for the failure of the angiotensin II infusion to reverse fully the effects of enalapril on phenylephrine-induced vasoconstriction.

Enalapril reversed the phenylephrine-induced increase in the proportion of cardiac output received by the lungs and hepatic artery. The difficulties in interpreting changes in these regions have already been commented upon. It did not, however, influence the increase in the fraction of cardiac output passing to the heart. The relative vasodilator action of enalapril observed in the kidneys, small intestine, large intestine and pancreas/mesentery after administration of phenylephrine encouraged an increase in the proportions of cardiac output passing to these organs. The angiotensin II infusion failed to compensate fully for the effects of enalapril on cardiac output distribution to the lungs, small intestine and large intestine, reflecting its failure to counteract fully the effects of enalapril on vascular resistance.

The results of this study indicate that, in the pithed rat, circulating angiotensin II maintains blood pressure by maintaining both total peripheral resistance and cardiac output. It exerts a tonic vasoconstrictor effect in several vascular beds including the renal, epididimidal, fat and gastrointestinal vascular beds. The data do not indicate a selective effect of enalapril on xylazine- or phenylephrine-induced changes in cardiovascular haemodynamics and suggest that angiotensin II maintains increases in total peripheral resistance, induced by these agonists, by facilitating vasoconstriction in several vascular beds. Differences exist in the ability of angiotensin II infusions to reverse the effects of enalapril on vasoconstriction induced by these agonists. This suggests that vasoconstriction induced by xylazine may largely depend upon the facilitatory action of circulating angiotensin II, whilst vasoconstriction induced by phenylephrine may also be modulated by locally synthesized vascular smooth muscle angiotensin II.

This work was supported by a grant from the Wellcome Trust.

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(Received October 6, 1987
Accepted January 14, 1988)