Differential effects of captopril on regional haemodynamic responses to angiotensin I and bradykinin in conscious rats

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1 Conscious, Long Evans rats were chronically instrumented with pulsed Doppler flow probes and intravascular catheters to allow regional haemodynamic (coeliac, mesenteric and hindquarters vascular beds) responses to i.v. bradykinin to be assessed in the absence and presence of captopril and of ganglion blockade (with mecamylamine).

2 Bradykinin (3 nmol kg^{-1} , i.v. bolus) had no effect on mean arterial blood pressure, although it caused hyperaemic vasodilatation in the coeliac, mesenteric and hindquarters vascular beds. Following administration of captopril at a dose (28 nmol kg^{-1}) which had no effect on responses to angiotensin I, the hypotensive and coeliac and mesenteric vasodilator responses to bradykinin were enhanced. However, there was a temporal dissociation between these events indicating that changes in cardiac output must have been contributing to the changes in mean arterial blood pressure.

3 Captopril at a higher dose (280 nmol kg^{-1}) caused reversible inhibition of the pressor and coeliac and mesenteric vasoconstrictor effects of angiotensin I, but the inhibition of the mesenteric vascular responses was significantly less than that of the coeliac vascular responses. Under the same conditions, the mesenteric vasodilator effects of bradykinin were less enhanced than the coeliac vasodilator effects, consistent with greater inhibition of angiotensin-converting enzyme (i.e., kininase II) in the coeliac than in the mesenteric vascular bed. But, since the hypotensive action of bradykinin was markedly enhanced in these circumstances, the possibility existed that baroreflex responses influenced the haemodynamic effects of bradykinin. However, assessment of the haemodynamic changes following bradykinin administration (bolus or infusion) in the presence of ganglion blockade showed that only the hindquarters vasodilator response to bradykinin was enhanced, while the coeliac and mesenteric vasodilator responses were diminished. Thus, additional factors must have been influencing the latter responses.

4 The results show that inhibition of angiotensin-converting enzyme (kininase II) can have differential effects on the regional haemodynamic responses to angiotensin I and bradykinin. The results provide a striking illustration of our previous assertion that the measurement of arterial blood pressure alone cannot provide sufficient information to allow interpretation either of the effects of vasoactive substances, or of the influence of drugs thereupon.

Keywords: Bradykinin; angiotensin I; captopril; regional haemodynamics

Introduction

The traditional picture of the renin-angiotensin system (RAS) is one in which release of renin from the granular cells of the juxtaglomerular apparatus stimulates the production of angiotensin I (AI) from circulating angiotensinogen, and the AI is then converted into angiotensin II (AII) by the action of angiotensin-converting enzyme (ACE) in the pulmonary vascular bed. However, it is now known that all the components of the RAS may be located within individual tissues (see Campbell, 1987; Dzau, 1988, for reviews), and hence the regional haemodynamic responses to systemic, i.v. administration of exogenous AI could be due to its local conversion to AII, in addition to its pulmonary conversion. If this were the case, then pretreatment with an ACE inhibitor, particularly at submaximal doses, might not influence the regional haemodynamic effects of exogenous AI uniformly, especially if the extent of the local production of AII, from exogenous AI, differed in different vascular beds, and/or the degree of ACE inhibition was not the same in all vascular beds. However, if the exogenous AI were converted into AII in the pulmonary vascular bed, and the AII then exerted systemic effects, treatment with an ACE inhibitor should reduce all the regional haemodynamic actions of AI proportionately. Thus, our first objective was to determine which of these two possibilities pertains *in vivo*. Since the renal vascular bed is so markedly influenced by the renal RAS, it is feasible that the

functional consequences of the activity of any local vascular RAS in the kidney would be difficult to detect, and hence measurement of renal blood flow might not help to answer the question we were asking. To avoid this potential problem, and in order to obtain maximum information from each experiment, we compared the effects of exogenous AI, given i.v., on the coeliac, mesenteric and hindquarters vascular beds, in the absence and in the presence of the ACE inhibitor, captopril, at doses submaximal for ACE inhibition (Muller *et al.*, 1990). We judged the use of such doses of captopril would optimise our chances of detecting regional differences in the effects of the drug.

ACE is kininase II, and bradykinin is a better substrate for this enzyme than is AI, since its K_m is about 10^{-7} M compared to AI, which has a K_m about 10^{-5} M (see Erdős, 1975). Thus, in the presence of an ACE inhibitor, the regional haemodynamic effects of AI and bradykinin might be influenced to different extents, even though additional enzymes might be involved in the degradation of bradykinin (see Erdős, 1975; Regoli & Barabé, 1980). Furthermore, for the reasons given above, treatment with an ACE inhibitor could have a regionally differentiated influence on the haemodynamic responses to exogenous bradykinin. So, our second objective was to delineate the regional haemodynamic responses to exogenous bradykinin, given i.v., in the absence and presence of the submaximal doses of captopril, in the same animals that received AI.

The results of this experiment indicated that the haemo-

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dynamic effects of bradykinin in the presence of captopril might have been influenced by reflex mechanisms. Therefore, in a separate experiment we assessed regional haemodynamic responses to i.v. bolus injection and infusion of bradykinin in the absence and presence of the ganglion blocker, mecamlamine, and also in the presence of mecamlamine and captopril.

Methods

All experiments were carried out on male, Long Evans rats (350–450 g) bred in Nottingham. Under anaesthesia (sodium methohexitone, 60 mg kg⁻¹ i.p., supplemented as required) pulsed Doppler probes (Haywood *et al.*, 1981) were sutured around the coeliac and superior mesenteric arteries and the distal abdominal aorta (to monitor hindquarters flow) (Gardiner *et al.*, 1990a). Probe wires were fed subcutaneously to emerge at the back of the neck, where they were anchored by a suture. An i.m. injection of ampicillin (7 mg kg⁻¹; Penbritin, Beecham) was given before animals were returned to individual home cages with free access to food and water. At least 7 days after probe implantation, rats were anaesthetized (sodium methohexitone, 40 mg kg⁻¹ i.p.) and had an intra-arterial (distal abdominal aorta via ventral caudal artery) and 3 intravenous (right jugular vein) catheters implanted. The catheters ran subcutaneously to emerge at the same site as the wires from the pulsed Doppler probes. The latter were soldered into a microconnector (Microtech Inc., Boothwyn, USA) that was held in a clamp attached to a harness worn by the rat. A flexible spring, through which the catheters ran, was connected to the harness, and the lead connection to the probes was taped to the spring. The spring assembly was supported by a counter-balanced lever system that allowed the rat unrestrained movement. Only animals with acceptable signals (signal:noise > 20:1) from all 3 probes were entered into experimental protocols, and only after they had had their intravascular catheters implanted for at least 24 h. During the experiments individual rats were kept in their home cages with free access to food and water.

Experiment 1: Effects of captopril on responses to angiotensin I and bradykinin

In this experiment we assessed the haemodynamic effects of bolus i.v. injections of AI (250 pmol kg⁻¹) and bradykinin (3 nmol kg⁻¹) before and about 10 and 60 min after bolus i.v. injection of captopril (28 or 280 nmol kg⁻¹); these doses were chosen as being submaximal for ACE inhibition (Muller *et al.*, 1990). Animals ($n = 9$) received AI and bradykinin in that order (because of the prolonged effects of the latter in the presence of captopril). The challenges before and after captopril were carried out on separate days for each dose of captopril, but the lower dose of captopril was always given first, in order to avoid any possible carry-over effects.

Experiment 2: Effects of mecamlamine and mecamlamine plus captopril on responses to bradykinin

In this experiment, a separate group of rats ($n = 7$) received i.v. bolus injections (3 nmol kg⁻¹) and 3 min infusions (36 nmol kg⁻¹ min⁻¹) of bradykinin before and 20 min after the onset of mecamlamine administration (50 µmol kg⁻¹ h⁻¹; Gardiner *et al.*, 1992) and again about 10 min after the i.v. bolus injection of captopril (280 nmol kg⁻¹) in the presence of mecamlamine.

Drugs and peptides

AI and bradykinin (Bachem, UK) were dissolved in isotonic (0.9%) NaCl containing 1% bovine serum albumin (Sigma, UK). Captopril (Squibb, UK) was dissolved in isotonic saline and buffered to pH 7.0 with NaOH. Mecamlamine hydro-

chloride (Sigma, UK) was dissolved in isotonic saline. All bolus injections were given in 0.1 ml flushed in with 0.1 ml saline; mecamlamine infusions were given at 0.3 ml h⁻¹, and bradykinin infusions were given at 0.15 ml min⁻¹. Administration of vehicle at these volumes and rates had no significant cardiovascular effects.

Data analysis

Continuous recordings (on a Gould ES 1000 system) were made of phasic and mean arterial blood pressure (MAP), instantaneous heart rate and phasic and mean Doppler shift signals with a modified (Gardiner *et al.*, 1990b) VF-1 main-frame (Crystal Biotech, Holliston, USA). The mean Doppler shift signals were taken as indices of flow (Gardiner *et al.*, 1990a), and vascular conductances were calculated by dividing the mean Doppler shift signal by the mean arterial blood pressure (Gardiner *et al.*, 1992). Within an experimental run, responses to any intervention were assessed by Friedman's test (Theodorsson-Norheim, 1987) applied to changes relative to baseline. Comparison of responses, in the same group under different experimental conditions, was made using Friedman's test applied to areas under or over curves (AUC and AOC, respectively); resting values were compared with the Kruskal-Wallis test. A P value < 0.05 was taken as significant.

Results

Experiment 1: Effects of captopril on responses to angiotensin I and bradykinin

Responses to angiotensin I Since the responses to AI were so rapid in onset and offset, only maximum changes were measured. AI caused a marked rise in MAP (56 ± 2 mmHg) (Figure 1) and a substantial bradycardia (-104 ± 7 beats min⁻¹). There were reductions in coeliac (-2.2 ± 0.4 kHz) and mesenteric (-3.2 ± 0.4 kHz) flows, in association with marked, and similar falls in vascular conductances (coeliac = -43 ± 3 ; mesenteric = -40 ± 4 [kHz mmHg⁻¹] 10^3) (Figure 1); hindquarters flow and vascular conductance showed significantly smaller reductions (flow = -0.6 ± 2 kHz; conductance = -17 ± 3 [kHz mmHg⁻¹] 10^3) (Figure 1).

The lower dose of captopril (28 nmol kg⁻¹) had no effects on the regional haemodynamic responses to AI (Figure 1).

Prior to administration of the higher dose of captopril (280 nmol kg⁻¹), the regional haemodynamic responses to AI were not different from those before or after administration of the lower dose of captopril (Figure 1). However, about 10 min after the administration of the higher dose of captopril, AI evoked a significantly reduced pressor effect (16 ± 1 mmHg) (Figure 1), and bradycardia (-37 ± 7 beats min⁻¹). There were no significant reductions in coeliac or hindquarters flows, although mesenteric flow still showed a marked reduction. Thus, although the falls in coeliac and mesenteric vascular conductances (coeliac = -7 ± 2 ; mesenteric = -27 ± 5 [kHz mmHg⁻¹] 10^3) were significantly attenuated, the response in the latter bed was significantly greater than in the former. There was no hindquarters vasoconstriction (Figure 1).

About 1 h after the higher dose of captopril, the rise in MAP (50 ± 1 mmHg) (Figure 1), and the bradycardia (-90 ± 6 beats min⁻¹) and the reductions in coeliac, mesenteric and hindquarters flows (coeliac = -1.6 ± 0.3 ; mesenteric = -3.2 ± 0.4 ; hindquarters = -0.3 ± 0.2 kHz) and vascular conductances (coeliac = -38 ± 4 ; mesenteric = -39 ± 4 ; hindquarters = -13 ± 2 [kHz mmHg⁻¹] 10^3) in response to AI were not different from those before captopril (Figure 1).

Responses to bradykinin Bradykinin caused a slight rise in MAP (maximum = 8 ± 4 mmHg; AUC, 12 ± 3 mmHg min),

Figure 1 Cardiovascular responses (maxima) to bolus i.v. injection of angiotensin I (250 pmol kg^{-1} at arrow) in the same conscious Long Evans rats ($n = 9$) in the absence ($\bullet$) and about 10 min ($\circ$) and 1 h (Δ) after administration of captopril at a dose of 28 nmol kg^{-1} (left-hand panels) or 280 nmol kg^{-1} (right-hand panels). Values are mean and bars show s.e. mean; for clarity statistics are given in the text.

but a marked tachycardia (maximum = $116 \pm 8 \text{ beats min}^{-1}$ at 0.25 min after injection, falling to $46 \pm 14 \text{ beats min}^{-1}$ after 2 min; AUC, $130 \pm 22 \text{ beats}$) (Figure 2). There were early increases in coeliac and mesenteric flows (AUC, coeliac = 1.6 ± 0.5 ; mesenteric = $2.6 \pm 0.7 \text{ kHz min}$) and a later increase in hindquarters flow (AUC = $2.6 \pm 0.5 \text{ kHz min}$) (Figure 2). The changes in vascular conductances showed a similar pattern (AUC, coeliac = 13 ± 4 ; mesenteric = 21 ± 6 ; hindquarters = $20 \pm 3 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) (Figure 2).

About 10 min after the lower dose (28 nmol kg^{-1}) of captopril, bradykinin caused a fall in MAP (nadir = $-16 \pm 4 \text{ mmHg}$; AOC, $12 \pm 3 \text{ mmHg min}$) and marked tachycardia (maximum $120 \pm 8 \text{ beats min}^{-1}$; AUC, 148 ± 18

beats). The change in MAP was significantly different from that elicited by bradykinin before captopril (Figure 2), but the tachycardia was not; there were increases in coeliac, mesenteric and hindquarters flows as before, although the patterns of change differed (Figure 2); we did not compare these formally because of the differences in MAP. However, the coeliac and mesenteric vasodilator responses to bradykinin were significantly enhanced (AUC, coeliac = 42 ± 8 ; mesenteric = $49 \pm 12 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) whereas the hindquarters vasodilatation was not changed significantly (AUC = $25 \pm 6 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) (Figure 2).

About 1 h after the lower dose of captopril, the changes in MAP (AUC, $9 \pm 3 \text{ mmHg min}$) (Figure 2) and heart rate (maximum = $104 \pm 12 \text{ beats min}^{-1}$, AUC = $115 \pm 11 \text{ beats}$) in response to bradykinin were not different from those before captopril, but the change in MAP was significantly different from that seen 10 min after captopril (Figure 2). The coeliac vasodilatation (AUC = $16 \pm 4 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) and the mesenteric vasodilatation (AUC = $31 \pm 9 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) were less than those seen following bradykinin administration 10 min after captopril, and not different from the responses prior to captopril. As before, the hindquarters vasodilator response to bradykinin (AUC, $15 \pm 3 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) was unchanged (Figure 2).

Before administration of the higher dose (280 nmol kg^{-1}) of captopril the responses to bradykinin (MAP, AOC $7 \pm 2 \text{ mmHg min}$; heart rate, AUC $103 \pm 25 \text{ beats}$; coeliac flow, AUC, 1.6 ± 0.5 ; mesenteric flow, AUC 2.6 ± 0.8 ; hindquarters flow, AUC $2.2 \pm 0.2 \text{ kHz min}$; coeliac conductance, AUC 18 ± 5 ; mesenteric conductance, AUC 27 ± 8 ; hindquarters conductance, AUC $21 \pm 3 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) were not different from those seen before administration of the lower dose of captopril (Figure 2). However, about 10 min after the higher dose of captopril the bradykinin-induced fall in MAP was markedly increased (nadir = $-48 \pm 2 \text{ mmHg}$; AOC = $46 \pm 4 \text{ mmHg min}$), but the tachycardia was unchanged (maximum = $97 \pm 15 \text{ beats min}^{-1}$, AUC = $84 \pm 16 \text{ beats}$). As after the lower dose of captopril, the patterns of change in regional blood flows in response to bradykinin were altered, there being significant reductions in coeliac and mesenteric blood flows (Figure 2), and no change in hindquarters. Although the flows were not formally compared, the calculated vascular conductances showed significant enhancement of coeliac (AUC = $35 \pm 8 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$), but not of the mesenteric (AUC = $23 \pm 6 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$), or hindquarters (AUC = $29 \pm 5 \text{ kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) vasodilations (Figure 2).

About 1 h after the higher dose of captopril the hypotensive response to bradykinin was still enhanced (nadir = $-28 \pm 5 \text{ mmHg}$; AOC = $20 \pm 5 \text{ mmHg min}$), but significantly less so than the response 10 min after captopril (Figure 2). However, the tachycardia was still not changed significantly (maximum = $111 \pm 11 \text{ beats min}^{-1}$, AUC = $177 \pm 20 \text{ beats}$). Coeliac, mesenteric and hindquarters flows increased in response to bradykinin (Figure 2) and the vasodilations in the coeliac (AUC = $45 \pm 8 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) and mesenteric ($54 \pm 9 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$) vascular beds were significantly greater than the control responses. The increase in mesenteric vascular conductance was also significantly greater than that seen following bradykinin administration about 10 min after the higher dose of captopril. Although there was a tendency towards enhancement of the hindquarters vasodilator response to bradykinin (AUC = $34 \pm 9 \text{ [kHz mmHg}^{-1}\text{]}10^3 \text{ min}$), this did not reach significance.

Experiment 2: Effects of mecamlamine and mecamlamine plus captopril on responses to bradykinin

Cardiovascular status prior to administration of bradykinin in each phase of this experiment is shown in Table 1. The hypotensive effect of mecamlamine was accompanied by coeliac and hindquarters vasodilatation, but a mesenteric

Figure 2 Cardiovascular responses to bolus i.v. injection of bradykinin (3 nmol kg^{-1} at arrow) in the same conscious Long Evans rats ($n=9$) in the absence ($\bullet$) and about 10 min ($\circ$) and 1 h (Δ) after administration of captopril at a dose of 28 nmol kg^{-1} (left-hand panels) or 280 nmol kg^{-1} (right-hand panels). Values are mean and bars show s.e.mean; for clarity statistics are given in the text.

vasoconstriction. The values given in the presence of mecamylamine and captopril were obtained at least 10 min after the onset of bradykinin infusion in the presence of mecamylamine and so do not show the acute haemodynamic responses to ACE inhibition.

Responses to bolus injections of bradykinin In this group of animals there was a small, but significant, hypotensive response to bolus injection of bradykinin in the untreated state (Table 2), and this effect was accompanied by tachycardia and coeliac, mesenteric and hindquarters vasodilatations (Table 2).

In the presence of mecamylamine, the hypotensive, and hindquarters vasodilator effects of bradykinin were enhanced, but the tachycardia was reduced and the coeliac and mesenteric vasodilator responses were not changed significantly (Table 2). In the presence of mecamylamine and

captopril there was an augmentation of the tachycardic effect of bradykinin, but all other changes were not different from those seen in the presence of mecamylamine alone (Table 2).

Responses to infusion of bradykinin Infusion of bradykinin elicited substantial hypotensive, tachycardic and coeliac, mesenteric and hindquarters vasodilator responses (Table 3). In the presence of mecamylamine, the hypotensive effect of bradykinin was significantly increased, and the tachycardia was significantly reduced; the coeliac, mesenteric and hindquarters vasodilator responses were not significantly different from those in the absence of mecamylamine (Table 3). During co-administration of mecamylamine and captopril there was further enhancement of the hypotensive effect of bradykinin; the tachycardia was also increased significantly relative to that seen in the presence of mecamylamine alone, but it was still less than the control response (Table 3). The hind-

Table 1 Resting cardiovascular variables in the same, conscious, Long Evans rats ($n = 7$) under control conditions, in the presence of mecamlamine and in the presence of mecamlamine and captopril

		Control	Mecamylamine	Mecamylamine + captopril
Heart rate (beats min^{-1})		320 ± 7	$282 \pm 11^*$	$287 \pm 11^*$
Mean arterial blood pressure (mmHg)		107 ± 1	$84 \pm 3^*$	$90 \pm 5^*$
Flow (kHz)	Coeliac	7.4 ± 0.7	6.6 ± 0.7	7.0 ± 0.8
	Mesenteric	5.8 ± 0.3	3.3 ± 0.1	3.2 ± 0.2
	Hindquarters	3.2 ± 0.3	3.1 ± 0.3	3.3 ± 0.5
Vascular conductance	Coeliac	69 ± 6	$79 \pm 9^*$	$77 \pm 8^*$
[(kHz mmHg $^{-1}$) 10^3]	Mesenteric	54 ± 3	$39 \pm 2^*$	$36 \pm 3^*$
	Hindquarters	29 ± 3	$38 \pm 6^*$	$38 \pm 9^*$

Values are mean $\pm$ s.e.mean.

* $P < 0.05$ versus control values.

Flows were not compared under the different experimental conditions because of the marked differences in mean arterial blood pressure.

Table 2 Cardiovascular changes (areas under or over curves to 5 min) after bolus injection of bradykinin (3 nmol kg^{-1}) in the same, conscious, Long Evans rats ($n = 7$) under control conditions, in the presence of mecamlamine and in the presence of mecamlamine and captopril

		Control	Mecamylamine	Mecamylamine + captopril
Heart rate (AUC; beats)		$180 \pm 32^*$	$138 \pm 32^{**}$	$324 \pm 59^{**b}$
Mean arterial blood pressure (AOC; mmHg min)		$-19 \pm 4^*$	$-65 \pm 18^{**}$	$-63 \pm 13^{**}$
Flow (AUC; kHz min)	Coeliac	8.7 ± 1.5	5.4 ± 1.3	3.1 ± 1.0
	Mesenteric	4.2 ± 1.0	2.9 ± 0.7	2.5 ± 0.9
	Hindquarters	5.0 ± 1.2	6.7 ± 2.3	7.5 ± 1.5
Conductance (AUC; [(kHz mmHg $^{-1}$) 10^3)min)	Coeliac	$88 \pm 18^*$	$105 \pm 27^*$	$45 \pm 12^*$
	Mesenteric	$48 \pm 10^*$	$52 \pm 12^*$	$44 \pm 14^*$
	Hindquarters	$52 \pm 12^*$	$116 \pm 28^{**}$	$119 \pm 17^{**}$

Values are mean $\pm$ s.e.mean.

* $P < 0.05$ versus baseline; ** $P < 0.05$ versus control; $^bP < 0.05$ versus mecamlamine.

Flow changes were not compared under the different experimental conditions because of the marked differences in the mean arterial blood pressure changes.

Table 3 Cardiovascular changes (areas under or over curves) during 3 min infusions of bradykinin ($36 \text{ nmol kg}^{-1} \text{ min}^{-1}$) in the same, conscious Long Evans rats ($n = 7$) under control conditions, in the presence of mecamlamine and in the presence of mecamlamine and captopril

		Control	Mecamylamine	Mecamylamine + captopril
Heart rate (AUC; beats)		$303 \pm 25^*$	$165 \pm 12^{**}$	$214 \pm 20^{**b}$
Mean arterial blood pressure (AOC; mmHg min)		$-33 \pm 12^*$	$-60 \pm 9^{**}$	$-117 \pm 19^{**b}$
Flow (AUC; kHz min)	Coeliac	8.5 ± 1.0	3.0 ± 0.8	0.2 ± 0.1
	Mesenteric	8.8 ± 0.9	6.6 ± 1.2	1.9 ± 1.4
	Hindquarters	5.7 ± 1.3	4.2 ± 0.8	2.0 ± 0.7
Conductance (AUC; [(kHz mmHg $^{-1}$) 10^3)min)	Coeliac	$106 \pm 9^*$	$101 \pm 13^*$	$54 \pm 11^{**b}$
	Mesenteric	$103 \pm 9^*$	$132 \pm 17^*$	$75 \pm 26^{**b}$
	Hindquarters	$71 \pm 20^*$	$96 \pm 18^*$	$95 \pm 20^*$

Values are mean $\pm$ s.e.mean.

* $P < 0.05$ versus baseline; ** $P < 0.05$ versus control; $^bP < 0.05$ versus mecamlamine.

Flow changes were not compared under the different experimental conditions because of the marked differences in the mean arterial blood pressure changes.

quarters vasodilator effects of bradykinin in the presence of mecamlamine and captopril were not significantly different from those in the presence of mecamlamine alone, or under control conditions, but the increases in coeliac and mesenteric vascular conductance were significantly less than under both those conditions (Table 3).

Discussion

In the present work we used lower doses of captopril than are frequently employed (see Muller *et al.*, 1990) and assessed responses to AI and bradykinin soon after (about 10 min), and around 1 h subsequent to administering the ACE

inhibitor. We considered this protocol would optimise our ability to detect subtle changes in the regional haemodynamic effects of AI and bradykinin, under the influence of captopril. We also attempted to assess any possible involvement of reflex changes in the responses to bradykinin by measuring its effects in ganglion-blocked animals in the absence and presence of captopril.

The lower dose of captopril (28 nmol kg^{-1}), while having no influence on responses to bolus injection of AI, caused significant, but reversible, enhancement of the hypotensive and coeliac and mesenteric vasodilator effects of a bolus injection of bradykinin. This observation is entirely consistent with the greater affinity of bradykinin than AI for ACE (Erdős, 1975). However, the enhancement of the hypotensive response to bradykinin did not coincide with the peak of its coeliac and mesenteric hyperaemic vasodilator effects (which occurred later). One possible explanation of the enhanced hypotensive effect of bradykinin after captopril is that there was a reduction in cardiac output under these conditions, probably because inhibition of pulmonary ACE allowed a larger proportion of the administered bradykinin to gain access to the heart. Certainly, the proportionately similar increase in the effects of bradykinin on coeliac and mesenteric vascular beds after administration of the lower dose of captopril argues in favour of the effect of captopril being due to its action in the pulmonary vascular bed rather than on ACE in the coeliac and mesenteric vascular beds. The failure of captopril to enhance the hindquarters vasodilator effect of bradykinin could have been attributable to interference with adrenaline release (MacLean & Ungar, 1986) which underlies this event (Gardiner *et al.*, 1992). However, additional factors were probably involved (see below).

Soon (about 10 min) after the higher dose (280 nmol kg^{-1}) of captopril there was substantial inhibition of the AI-induced pressor and coeliac vasoconstrictor effects, but less marked reduction of the mesenteric vasoconstriction. Since there was no apparent difference in the sensitivity of the coeliac and mesenteric vascular beds to AI under control conditions, the most likely explanation of the differential antagonism by captopril of the response to AI is that this was due to greater inhibition of ACE in the coeliac than mesenteric vascular bed; clearly, an action confined to inhibition of ACE in the pulmonary vascular bed could not have produced the picture seen. It is notable that the coeliac vasodilator effect of bradykinin was also enhanced soon after the higher dose of captopril, whereas the mesenteric vasodilator effect was not. Hence, these results are also consistent with greater inhibition of ACE in the coeliac than the mesenteric vascular bed. However, this interpretation is too simplistic, since the lower dose of captopril clearly enhanced the mesenteric vasodilator effects of bradykinin (see above), and 60 min after the higher dose of captopril the coeliac and mesenteric vasodilator effects of bradykinin were both enhanced. One explanation of this paradox is that the marked hypotensive effect of bradykinin soon after the higher dose of captopril triggered baroreflex responses that offset the mesenteric vasodilator action of bradykinin (see below).

By 60 min after captopril, all effects of AI were back to control levels, while those to bradykinin were not. Once again, this is consistent with the greater affinity of bradykinin than AI for ACE. Moreover, the dissociation between the augmentation of the hypotensive effect of bradykinin (which was greatest 10 min after the higher dose of captopril) and its coeliac and mesenteric vasodilator effects (which were greatest 60 min after captopril) is in line with the proposition that baroreflex responses to the hypotension acted to suppress the regional vasodilations. However, this cannot be the full explanation; we suspect that changes in cardiac out-

put must have been contributing differently to the fall in MAP in the various experimental circumstances, otherwise there should have been a direct relationship between the magnitudes and time courses of the hypotensive and regional vasodilator responses to bradykinin.

The use of ganglion blockade to assess reflex contributions to the overall response to bradykinin under normal conditions is not without problems. Administration of mecamlamine caused clear changes in baseline cardiovascular status (Table 1) which, themselves, could have influenced responses to bradykinin. Interestingly, the mecamlamine-induced hypotension was associated with coeliac vasodilatation and mesenteric vasoconstriction. While this disparate change is consistent with the differential effects of vasopressin in these two vascular beds (Gardiner *et al.*, 1990a), and it is known that ganglion-blockade triggers vasopressin-mediated haemodynamic events in conscious rats (Gardiner & Bennett, 1985), a contribution from the 'hepatic buffer' response (Richardson & Withrington, 1981) cannot be excluded. Mecamlamine-induced hindquarters vasodilatation is in line with an important vasomotor tone in that vascular bed and its relative insensitivity to vasopressin (Gardiner *et al.*, 1990a).

Notwithstanding these incidental complications, it was clear that, in the presence of mecamlamine, the hypotensive response to bradykinin was augmented, as was its hindquarters vasodilator effect. While these findings are consistent with our suspicion that reflex mechanisms might have been offsetting the haemodynamic actions of bradykinin, it appears that additional, presently unknown, factors must have been contributing to the changes seen in the coeliac and mesenteric vascular beds, since mecamlamine did not cause significant enhancement of bradykinin-induced vasodilatation at these sites. Furthermore, the finding that, in the presence of mecamlamine, captopril had no additional effect on the response to bolus injection of bradykinin could indicate that the influence of captopril under normal circumstances was due to interference with the vasoconstrictor and prejunctional actions of AI (Zimmerman, 1981; Reid, 1992), rather than to inhibition of bradykinin degradation. While we do not know for sure that mecamlamine was without effect on ACE, it is unlikely this fully explains the results since, in the presence of mecamlamine, captopril caused additional augmentation of the hypotensive response to infusion of bradykinin. However, even in this setting, as in the presence of mecamlamine alone, it is notable that the augmentation of the hypotensive effect of bradykinin infusion was not associated with enhanced coeliac, mesenteric or hindquarters vasodilatation. As before, this indicates differential contributions from changes in cardiac output to the hypotension following bradykinin administration in different experimental circumstances. Clear, albeit indirect, evidence of this came from the finding that, in the presence of mecamlamine and captopril the enhancement of the hypotensive response to bradykinin infusion was accompanied by diminished coeliac and mesenteric vasodilator responses compared to those seen in the control experiment. Our explanation of the latter observation is that there was enhancement of the vasoconstrictor effects of bradykinin (Fasciolo *et al.*, 1990).

While this study has raised more questions than it has answered, the questions have an important bearing on our understanding of the ways in which ACE inhibition might influence integrated cardiovascular control. Apart from specific conclusions relating to the regional haemodynamic changes under our different experimental conditions, a major outcome of the present investigation is the affirmation it gives to our previous proposal that measurement of blood pressure alone may provide insufficient data if one is seeking to understand the ways in which drugs and peptides impinge on cardiovascular function *in vivo*.

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