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Involvement of nitric oxide in the regional haemodynamic effects of perindoprilat and captopril in hypovolaemic Brattleboro rats

[1]Sheila M. Gardiner & Terence Bennett

Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH

1 Male, homozygous Brattleboro (i.e. vasopressin-deficient) rats were chronically instrumented with pulsed Doppler flow probes and intravascular catheters, and were studied 5 h after a subcutaneous injection of an hyperoncotic solution of polyethylene glycol to render them hypovolaemic, and hence dependent on the renin-angiotensin system for maintenance of haemodynamic status. Pilot experiments showed that, in this model, primed infusion of perindoprilat (0.05 mg kg^{-1} bolus, 0.05 mg kg^{-1} h^{-1} infusion) or captopril (0.2 mg kg^{-1} bolus, 0.2 mg kg^{-1} h^{-1} infusion) just abolished the pressor effect of angiotensin I (120 pmol), and had similar initial hypotensive and renal hyperaemic vasodilator effects.

2 Perindoprilat had more sustained hypotensive, and mesenteric and hindquarters vasodilator effects than captopril in the presence of saline. In the presence of N^G-nitro-L-arginine methyl ester (L-NAME 3 mg kg^{-1} h^{-1}), the renal vasodilator effects of perindoprilat were unchanged, whereas the other haemodynamic effects of perindoprilat and captopril were reduced. Hence, in the presence of L-NAME, all haemodynamic effects of perindoprilat were greater than those of captopril.

3 The renal hyperaemic vasodilator effects of acetylcholine were abolished by L-NAME and by perindoprilat, and were markedly reduced by captopril. However, since perindoprilat and captopril caused such marked renal hyperaemic vasodilatation themselves, it is feasible this change in baseline status contributed to their effects. It is unlikely this could be a full explanation of the results, because the haemodynamic effects of lemakalim were unchanged under any experimental conditions.

4 Bradykinin alone, or in the presence of saline, caused mesenteric hyperaemic vasodilatation whereas, in the presence of perindoprilat or captopril, bradykinin caused marked renal and mesenteric vasoconstrictions. However, in the additional presence of L-NAME, the mesenteric vasoconstriction was reduced, yet the hypotensive effect of bradykinin was augmented. One possible explanation of these observations is that, in the presence of L-NAME and either perindoprilat or captopril, bradykinin caused marked coronary vasoconstriction, leading to a reduction in cardiac output.

5 Neither perindoprilat nor captopril impaired the pressor, or renal, mesenteric, or hindquarters vasoconstrictor effects of L-NAME. Indeed, in their presence, the effects of L-NAME were generally enhanced, consistent with perindoprilat and captopril causing activation of nitric oxide-dependent mechanisms that were subsequently inhibited by L-NAME.

Keywords: Perindoprilat; captopril; nitric oxide; haemodynamic

Introduction

There is increasing evidence from *in vitro* studies (Kerth & Vanhoutte, 1991; Goldschmidt & Tallarida, 1991; Mombouli *et al.*, 1991; Wiemer *et al.*, 1991; Clozel, 1991; Mombouli & Vanhoutte, 1991; Illiano *et al.*, 1991; Henrion *et al.*, 1991) that various angiotensin-converting enzyme (ACE) inhibitors influence endothelial cell function. In one instance the interaction was seen with captopril, but not with enalaprilat (Goldschmidt & Tallarida, 1991), indicating that sulphydryl groups might be responsible. However, in the other studies cited above, non-sulphydryl-containing ACE inhibitors were found to exert endothelial-mediated effects, so the question is unresolved.

At the time the present study was planned there were no data regarding putative interactions between ACE inhibitors and endothelial-mediated processes *in vivo*, so one of our aims was to provide such data. However, while the experiments described here were in progress, Cachofeiro *et al.* (1992) published findings relating to the ability of the nitric oxide synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA), to attenuate the hypotensive effects of captopril or ramiprilat in spontaneously hypertensive rats. Unfortunately, Cachofeiro *et al.* (1992) carried out experiments on acutely prepared animals and provided no regional haemodynamic data.

In previous studies we had found that Brattleboro (i.e. vasopressin-deficient) rats rendered hypovolaemic by water deprivation or by subcutaneous (s.c.) injection of an hyperoncotic solution of polyethylene glycol, became exquisitely sensitive to the hypotensive and vasodilator effects of ACE inhibitors such as captopril, enalaprilat and lisinopril (Gardiner & Bennett, 1985; 1986; Gardiner *et al.*, 1988; 1989; Tomlinson *et al.*, 1990; Muller *et al.*, 1990). Therefore, we considered this model might be one in which putative interactions between ACE inhibitors and endothelial function would be particularly marked. Our major aims were, by performing experiments in conscious, chronically-instrumented Brattleboro rats rendered hypovolaemic by s.c. injection of polyethylene glycol, to determine whether or not the actions of perindoprilat and captopril were influenced by the nitric oxide (NO) synthesis inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) and to assess the influence of perindoprilat and captopril on haemodynamic responses to acetylcholine, the K^+ channel opener, lemakalim (BRL

[1] Author for correspondence.

38227), and bradykinin (i.e. vasodilators with differing degrees of 'endothelial dependence').

Methods

Male, homozygous (350–450 g) Brattleboro rats were anaesthetized (sodium methohexitone, 60 mg kg^{-1} i.p., supplemented as required) and, through a midline laparotomy, had miniaturised, pulsed Doppler probes (Haywood *et al.*, 1981) implanted around the left renal and superior mesenteric arteries and the distal abdominal aorta (to monitor hindquarters flow).

Following surgery, animals were given ampicillin (7 mg kg^{-1}, i.m. Penbritin, Beecham) and returned to individual home cages with free access to tap water and food (Biosure, GLP grade diet 41B (M)). At least 7 days later, animals were briefly anaesthetized (sodium methohexitone 40 mg kg^{-1}, i.p.) and had implanted an intra-arterial catheter in the distal abdominal aorta (via the ventral caudal artery) for blood pressure and heart rate recording, 3 catheters in the right jugular vein for drug or peptide administration, and a single s.c. catheter; they were then allowed to recover for at least 48 h before experiments were begun. The total group of 32 fully-instrumented animals was randomized into 4 subgroups of 8 (Groups 1, 2, 3 and 4).

At 07 h 00 min on the day of the experiment, animals in all groups received (through the previously implanted s.c. catheter) an injection of 5 ml of an hyperoncotic solution of polyethylene glycol (PEG; carbowax 20 M, 30% in isotonic saline) warmed to body temperature (Gardiner *et al.*, 1989). Subsequently, animals were not allowed access to drinking water, in order to achieve isosmotic hypovolaemia (Gardiner & Bennett, 1986; Gardiner *et al.*, 1989). The experimental protocol began 5 h after the injection of PEG (Gardiner *et al.*, 1989). Continuous recordings (on a Gould ES 1000 system) were made of mean and phasic arterial blood pressures, instantaneous heart rate and mean and phasic Doppler shift signals from renal, mesenteric and hindquarters probes. The latter were monitored to ensure the signals were of an acceptable quality (signal: noise $>$ 20:1). Vascular conductance changes were calculated from mean Doppler shift signals and mean arterial blood pressure (Gardiner *et al.*, 1990a,b,c).

Pilot experiments

From these experiments ($n = 10$, in total) it was found that captopril at a dose of 0.2 mg kg^{-1} bolus, 0.2 mg kg^{-1} h^{-1} infusion and perindoprilat at a dose of 0.05 mg kg^{-1} bolus, 0.05 mg kg^{-1} h^{-1} infusion just caused complete inhibition of the haemodynamic effects of angiotensin I (120 pmol), in rats treated 5 h previously with PEG. Furthermore, at these doses the initial hypotensive and renal haemodynamic effects of the ACE inhibitors were similar, and hence these doses were chosen for the full experiments.

We planned, originally, to give randomized, 3 min infusions of acetylcholine (55 nmol kg^{-1} min^{-1}) (Gardiner *et al.*, 1991a) lemakalim (35 nmol kg^{-1} min^{-1}) (Gardiner *et al.*, 1991b) and bradykinin (36 nmol kg^{-1} min^{-1}) (Gardiner *et al.*, 1992a). However, it became apparent during further pilot experiments that the dose of lemakalim caused hypotensive and tachycardic effects that were too persistent to allow a systematic protocol to be run; in addition, in the presence of the ACE inhibitors, bradykinin infusion caused irreversible cardiovascular deterioration. Eventually we determined that 3 min infusions of acetylcholine (55 nmol kg^{-1} min^{-1}) and lemakalim (8.8 nmol kg^{-1} min^{-1}) and a bolus injection of bradykinin (2.4 nmol kg^{-1}), always given in that order, evinced the most reproducible responses, so this was the protocol used in the full experiments.

Full experiments

Animals were randomized into 4 groups with similar body weights (Group 1 = 421 ± 8 g (mean ± s.e.mean); Group 2 = 403 ± 9 g; Group 3 = 418 ± 4 g; Group 4 = 412 ± 9 g). All groups were initially challenged with 3 min infusions of acetylcholine (55 nmol kg^{-1} min^{-1}) and lemakalim (8.8 nmol kg^{-1} min^{-1}) and a bolus injection of bradykinin (2.4 nmol kg^{-1}). Thereafter, animals in Groups 1 and 2 received continuous i.v. infusion of saline (0.3 ml h^{-1}) and beginning 30 min later, were re-challenged with acetylcholine, lemakalim and bradykinin (i.e. in the same order as before). Sixty min after the onset of saline infusion, animals in Group 1 were given a primed infusion of perindoprilat (0.05 mg kg^{-1} bolus, 0.05 mg kg^{-1} h^{-1} infusion) and, beginning 30 min later, were re-challenged with acetylcholine, lemakalim and bradykinin. Two h after the onset of saline infusion (i.e. 1 h after onset of perindoprilat infusion) these animals received an L-NAME infusion (3 mg kg^{-1} h^{-1}, 0.3 ml h^{-1}) for 30 min. This dose of L-NAME was chosen on the basis of previous experiments (Gardiner *et al.*, 1991a) showing that a lower dose did not abolish vasodilator responses to acetylcholine.

Animals in Group 2 were treated identically to those in Group 1 except that they received a primed infusion of captopril (0.2 mg kg^{-1} bolus, 0.2 mg kg^{-1} h^{-1} infusion) rather than perindoprilat.

Animals in Groups 3 and 4 were treated as those in Groups 1 and 2, respectively, except that they received a continuous L-NAME infusion instead of saline infusion initially (i.e. from 30 to 180 min) and saline infusion instead of L-NAME infusion at the end of the protocol (i.e. from 150 to 180 min).

Animals in Groups 1 and 2 received acetylcholine, lemakalim and bradykinin through one catheter, and saline and the ACE inhibitor, separately, through the other two catheters. L-NAME was given for the last 30 min of the experiment through the catheter which had been used to deliver the vasodilator challenges.

Identical procedures were followed for animals in Groups 3 and 4, except that L-NAME was given through a separate, unused catheter, and saline was given at the end through the catheter which had been used to deliver the vasodilator challenges.

Data analysis

All raw data were recorded on a Gould ES 1000 system in the form of hard copy of the analogue signals. Following an experiment, measurements (by hand) were made of mean arterial blood pressure, instantaneous heart rate and mean renal, mesenteric and hindquarters Doppler shift signals. These variables were averaged (by eye) over epochs of 20 s starting immediately before any intervention and, depending on the profile of response, at appropriate time points thereafter. In the case of acetylcholine and lemakalim, measurements were made for the 20 s epochs straddling the 1, 2 and 3 min time points during infusion. For bradykinin, measurements were made at the peaks or nadirs of the mesenteric and hindquarters flow changes. Following administration of perindoprilat or captopril, the values for the 20 s epochs at 5, 10 and 30 min were recorded, while for L-NAME and saline those at 30 min were noted.

For ease of presentation, group data have been rendered into the form of means ± s.e.mean, both for individual time points and for areas under or over curves (AUC and AOC, respectively). All calculations (means and s.e.mean, AUC or AOC, % changes etc) were made with a Fortran programme running on a mainframe (Vax) computer.

Data were analysed by non-parametric tests, i.e. Wilcoxon's test, Kruskal-Wallis test and Friedman's test (Theodorsson-Norheim, 1987), as appropriate. A P value < 0.05 was taken as significant.

Drugs, peptides and chemicals

Perindoprilat was supplied by Servier R & D; captopril was obtained from the Squibb Institute (U.S.A.), and lemakalim (BRL 38227) from SmithKline Beecham (UK). Acetylcholine chloride and L-NAME were obtained from Sigma (UK) and bradykinin from Bachem (UK). Polyethylene glycol (Carbowax 20 M) was obtained from BDH (UK). Perindoprilat and captopril were dissolved in isotonic saline and buffered to pH 7.4–7.6 with Na_2CO_3 (0.5%). Acetylcholine, lemakalim, L-NAME, PEG and bradykinin were dissolved in isotonic saline. In the case of bradykinin the saline contained 1% bovine serum albumin (Sigma, UK).

Results

Resting cardiovascular variables in all 4 experimental groups at the beginning of each protocol are shown in Table 1. There were no significant differences between the groups.

Effects of acetylcholine (ACh)

In all 4 groups, the first infusion of ACh caused hypotension, tachycardia, marked renal vasodilatation, slight hindquarters vasodilatation and a variable mesenteric vasoconstriction (Figures 1 and 2, Table 2). A similar picture was seen in the presence of saline, although there was a tendency for the hypotensive effect of ACh to be less (Figure 1, Table 2, Groups 1 and 2). However, during combined infusions of saline and perindoprilat there was no significant renal vasodilator response to ACh, whereas in the presence of saline and captopril, there was still a significant renal vasodilator response to ACh, albeit significantly smaller than in the presence of saline alone (Figure 1, Table 2, Groups 1 and 2). There was an increase in mesenteric vascular conductance in response to ACh in the presence of saline and either perindoprilat or captopril, which was significantly different from the mesenteric vasoconstriction seen in the presence of saline alone (Figure 1, Table 2, Groups 1 and 2).

In the presence of L-NAME, with or without captopril or perindoprilat, the renal vasodilator response to ACh was markedly attenuated, although other changes were not significantly affected (Figure 2, Table 2, Groups 3 and 4). However, in the presence of L-NAME and either ACE inhibitor, the mesenteric vascular response to ACh was significantly different from that seen in the presence of saline and either ACE inhibitor (Figures 1 and 2, Table 2).

Effects of lemakalim

In all 4 groups, the first infusion of lemakalim caused slight hypotension and a tachycardia accompanied by marked mesenteric vasodilatation, and modest and variable renal and hindquarters vasodilatations (Figures 1 and 2, Table 3).

Similar effects of lemakalim were seen in the presence of saline, or of L-NAME, and in the additional presence of perindoprilat or captopril (Figures 1 and 2, Table 3). There were no inter-group differences in the responses to lemakalim at any stage of the experimental protocols (Figures 1 and 2, Table 3).

Effects of bradykinin (BK)

In all 4 groups, the initial bolus injection of BK caused tachycardia and a tendency towards hypotension, associated with an early mesenteric vasodilatation followed by hindquarters vasodilatation; there was slight and variable renal vasodilatation (Figures 1 and 2, Table 4). A similar picture was seen in the presence of saline (Figure 1, Table 4, Groups 1 and 2). During combined infusions of saline and either captopril or perindoprilat, BK caused hypotension, marked bradycardia, renal and mesenteric vasoconstriction and hindquarters vasodilatation (Figure 1, Table 4, Groups 1 and 2). All these changes were significantly different from those seen in the presence of saline alone. There was no difference between the responses seen in the presence of captopril and those seen in the presence of perindoprilat.

The effects of BK in the presence of L-NAME differed from those in the presence of saline in respect of mean arterial blood pressure (which tended to rise, rather than fall) and renal vascular conductance (which tended to fall, rather than rise, Figures 1 and 2, Table 4).

During combined infusions of L-NAME and perindoprilat, or L-NAME and captopril, BK caused marked hypotension and bradycardia, and renal vasoconstriction and hindquarters vasodilatation (Figure 2, Table 4, Groups 3 and 4). However, there was mesenteric vasoconstriction in response to BK in the presence of L-NAME and perindoprilat, and this was significantly different from the response seen in the presence of L-NAME and captopril (Figure 2, Table 4). Moreover, the hypotensive response to BK in the presence of L-NAME and perindoprilat was significantly greater than the hypotensive response in the presence of saline and perindoprilat, consistent with the mesenteric vasoconstrictor effect of BK being greater in the latter condition (Figures 1 and 2, Table 2). The lack of mesenteric vasoconstrictor response to BK in the presence of L-NAME and captopril was associated with a tendency towards an enhanced hypotensive response, but this did not reach significance (Figures 1 and 2, Table 4), possibly because the hindquarters vasodilator effect of BK was significantly less than in the presence of saline and captopril (Table 4).

Effects of perindoprilat or captopril

Although the pilot experiments, and the results from the full experiments, indicated that the doses of perindoprilat and captopril were matched for their ability to inhibit the haemodynamic effects of angiotensin I, and for their initial

Table 1 Resting cardiovascular variables in the 4 separate experimental groups

	Group 1	*Group 2*	*Group 3*	*Group 4*
Heart rate (beats min^{-1})	328 ± 11	305 ± 10	293 ± 19	308 ± 10
Mean BP (mmHg)	114 ± 2	109 ± 2	108 ± 3	111 ± 2
Doppler shift (kHz)				
Renal	6.8 ± 1.0	7.2 ± 0.9	6.7 ± 0.8	6.3 ± 0.7
Mesenteric	5.5 ± 0.5	5.3 ± 0.5	5.3 ± 0.5	5.5 ± 0.8
Hindquarters	2.9 ± 0.3	2.6 ± 0.3	2.7 ± 0.4	2.7 ± 0.3
Vascular conductance ([kHz $mmHg^{-1}$]10^3)				
Renal	59 ± 8	66 ± 7	61 ± 5	56 ± 6
Mesenteric	48 ± 5	48 ± 4	50 ± 5	50 ± 8
Hindquarters	26 ± 3	24 ± 2	25 ± 3	24 ± 3

Values are mean ± s.e.mean, $n = 8$ (in all groups)

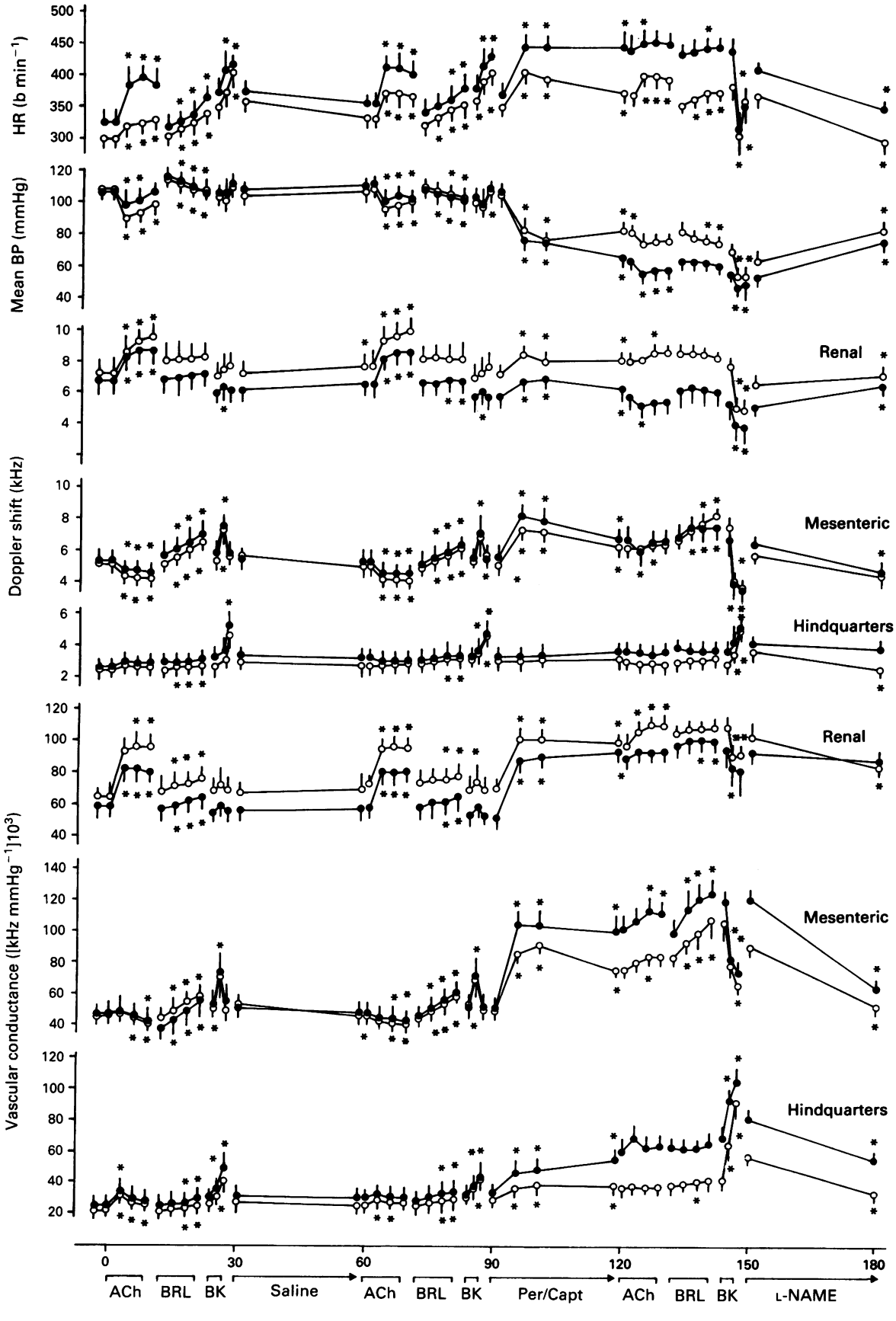

Figure 1 Cardiovascular responses to 3 min infusions of acetylcholine (ACh) or lemakalim (BRL) or bolus injection of bradykinin (BK) before and during infusion of saline, and perindoprilat (Per, ●, Group 1) or captopril (Capt, ○, Group 2) in separate groups ($n = 8$ in each) of conscious Brattleboro rats. At the end of the experiment, both groups were given a 30 min infusion of N^G-nitro-L-arginine methyl ester (L-NAME). BP = blood pressure; HR = heart rate. Values are mean and vertical bars are s.e.mean. $^*P < 0.05$ for change relative to the corresponding pre-intervention resting value. Statistics for AUC or AOC are given in the tables.

Table 2 Cardiovascular changes (AUC or AOC, arbitrary units) in response to 3 min infusions of acetylcholine under basal conditions, during infusion of saline (Groups 1 and 2) or N^G-nitro-L-arginine methyl ester (L-NAME, Groups 3 and 4), and during infusion of saline plus perindoprilat (Group 1), saline plus captopril (Group 2), L-NAME plus perindoprilat (Group 3) and L-NAME plus captopril (Group 4)

	Group 1	*Group 2*	*Group 3*	*Group 4*
ΔHeart rate	166 ± 24*	54 ± 11*[a]	83 ± 15*	105 ± 20*
ΔMean BP	−35 ± 12*	−48 ± 8*	−31 ± 6*	−50 ± 7*
ΔRenal conductance	60 ± 11*	73 ± 4*	64 ± 8*	59 ± 7*
ΔMesenteric conductance	−9 ± 3*	−9 ± 3*	−7 ± 2	−6 ± 2
ΔHindquarters conductance	14 ± 7*	16 ± 5*	14 ± 4*	20 ± 4*
	Saline		*L-NAME*	
ΔHeart rate	145 ± 17*	100 ± 14*	50 ± 10*	37 ± 14
ΔMean BP	−27 ± 6*	−28 ± 5*	−20 ± 7*	−20 ± 7*
ΔRenal conductance	57 ± 7*	63 ± 3*	8 ± 2[c]	9 ± 4*[d]
ΔMesenteric conductance	−9 ± 2*	−13 ± 4*	−6 ± 3	−9 ± 3*
ΔHindquarters conductance	6 ± 4	8 ± 2*	13 ± 4*	8 ± 2*
	Saline		*L-NAME*	
	Perindoprilat	*Captopril*	*Perindoprilat*	*Captopril*
ΔHeart rate	33 ± 7*	72 ± 13*	35 ± 13	38 ± 13
ΔMean BP	−21 ± 4*	−17 ± 6	−24 ± 9*	−28 ± 10*
ΔRenal conductance	12 ± 3	29 ± 5*[a]	11 ± 4	12 ± 3
ΔMesenteric conductance	20 ± 5*	19 ± 6*	−30 ± 8[c]	−15 ± 4*[d]
ΔHindquarters conductance	18 ± 8	8 ± 3	14 ± 4*	13 ± 4*

Values are mean ± s.e.mean, $n = 8$ (all groups).
*$P < 0.05$ for change, [a]$P < 0.05$ Group 2 vs Group 1; [c]$P < 0.05$ Group 3 vs Group 1; [d]$P < 0.05$ Group 4 vs Group 2

Table 3 Cardiovascular changes (AUC or AOC, arbitrary units) in response to 3 min infusions of lemakalim under basal conditions, during infusion of saline (Groups 1 and 2) or N^G-nitro-L-arginine methyl ester (L-NAME, Groups 3 and 4), and during infusion of saline plus perindoprilat (Group 1), saline plus captopril (Group 2), L-NAME plus perindoprilat (Group 3) and L-NAME plus captopril (Group 4)

	Group 1	*Group 2*	*Group 3*	*Group 4*
ΔHeart rate	69 ± 11*	57 ± 11*	33 ± 10	58 ± 11*
ΔMean BP	−16 ± 2*	−15 ± 2*	−11 ± 2*	−11 ± 2*
ΔRenal conductance	13 ± 3*	12 ± 3*	8 ± 3*	12 ± 2*
ΔMesenteric conductance	24 ± 4*	22 ± 3*	24 ± 3*	26 ± 5*
ΔHindquarters conductance	5 ± 1*	9 ± 1*	8 ± 2	4 ± 1
	Saline		*L-NAME*	
ΔHeart rate	51 ± 9*	61 ± 12*	20 ± 6 *	45 ± 18*
ΔMean BP	−17 ± 3*	−12 ± 2*	−18 ± 5*	−14 ± 2*
ΔRenal conductance	12 ± 2*	8 ± 1*	11 ± 3*	15 ± 3*
ΔMesenteric conductance	22 ± 3*	20 ± 3*	17 ± 3*	17 ± 3*
ΔHindquarters conductance	8 ± 2*	8 ± 2*	4 ± 1	5 ± 1
	Saline		*L-NAME*	
	Perindoprilat	*Captopril*	*Perindoprilat*	*Captopril*
ΔHeart rate	23 ± 7*	41 ± 8*	49 ± 9*	40 ± 8*
ΔMean BP	−6 ± 2	−11 ± 2*	−5 ± 2	−9 ± 4
ΔRenal conductance	10 ± 2*	7 ± 2	10 ± 4*	10 ± 4*
ΔMesenteric conductance	27 ± 4*	37 ± 6*	21 ± 4*	32 ± 8*
ΔHindquarters conductance	4 ± 2	7 ± 2	7 ± 3	5 ± 1

Values are mean ± s.e.mean, $n = 8$ (all groups).
*$P < 0.05$ for change

hypotensive and renal haemodynamic actions (Figure 1), differences between the effects of the two ACE inhibitors appeared during the 30 min following their administration. Thus, in the presence of saline, perindoprilat had significantly greater hypotensive, tachycardic and mesenteric and hindquarters vasodilator effects than captopril (Table 5).

In the presence of L-NAME, the renal vasodilator effect of perindoprilat was unchanged but the hypotension, tachycardia and mesenteric and hindquarters vasodilatation were all significantly smaller than in the absence of L-NAME (Table 5, Groups 1 and 3). In contrast, all three vascular beds showed significantly smaller vasodilatations in response to captopril during L-NAME infusion, compared to the responses seen in the absence of L-NAME (Table 5, Groups 2 and 4). Thus, the hypotensive and renal, mesenteric and hindquarters vasodilator effects of perindoprilat were all significantly greater than those of captopril during L-NAME infusion (Figure 2, Table 5, Groups 3 and 4).

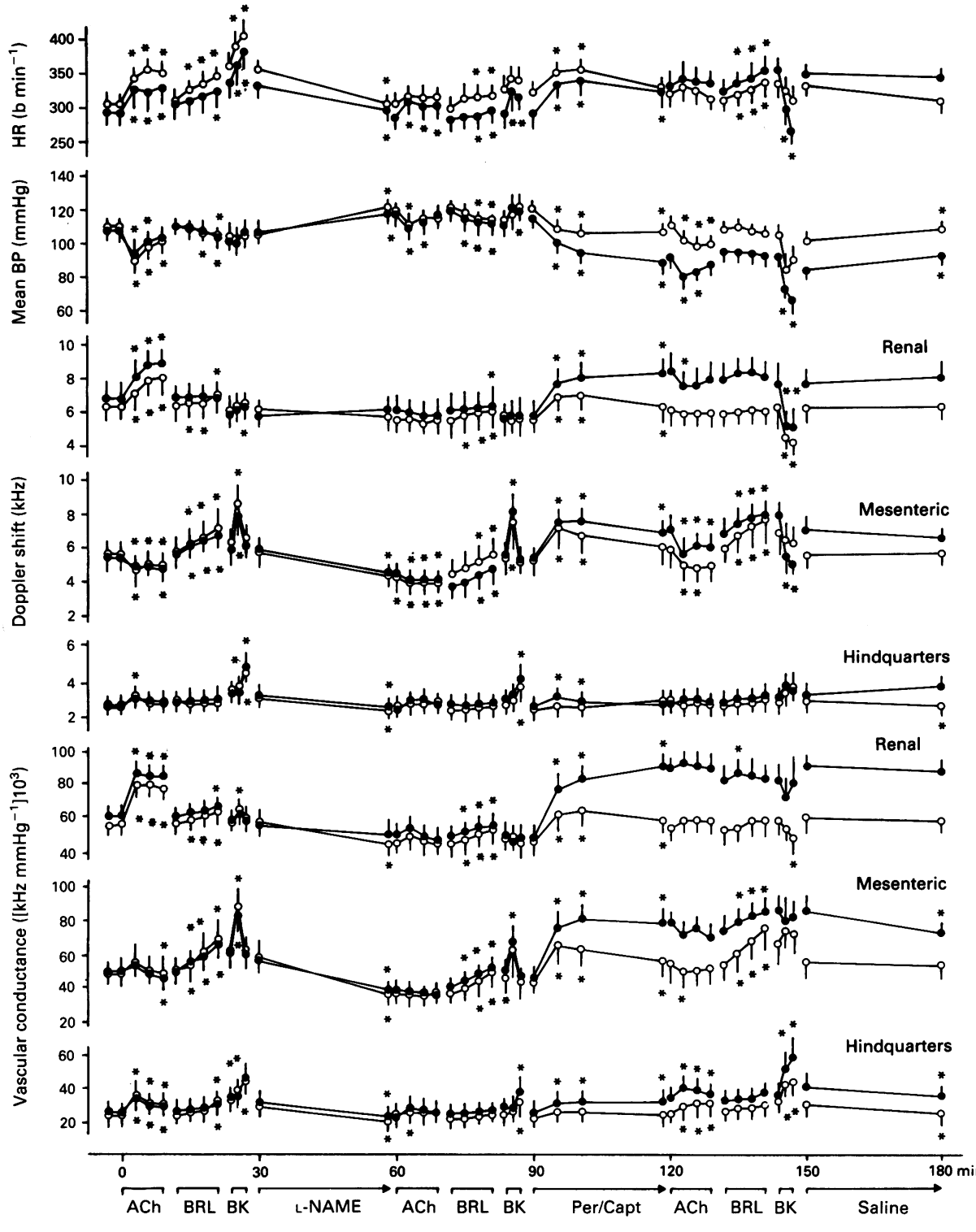

Figure 2 Cardiovascular responses to 3 min infusions of acetylcholine (ACh) or lemakalim (BRL) or bolus injection of bradykinin (BK) before and during infusion of N^G-nitro-L-arginine methyl ester (L-NAME) and perindoprilat (Per, ●, Group 3) or captopril (Capt, ○, Group 4) in separate groups ($n = 8$ in each) of conscious Brattleboro rats. At the end of the experiment, both groups were given a 30 min infusion of saline. BP = blood pressure; HR = heart rate.
Values are mean and vertical bars are s.e.mean. $^*P < 0.05$ for change relative to the corresponding pre-intervention resting value. Statistics for AUC or AOC are given in the tables.

Effects of L-NAME

L-NAME, alone, increased mean arterial blood pressure in association with bradycardia, and constrictions in renal, mesenteric and hindquarters vascular beds (Figure 2, Table 6).

In the presence of saline and perindoprilat or captopril the pattern of response to L-NAME was similar to that seen with L-NAME alone, but, with the exception of the renal vasoconstrictor response to L-NAME, all variables showed significantly greater changes in the presence of ACE inhibitors (Figures 1 and 2, Table 6).

Table 4 Cardiovascular changes (AUC or AOC, arbitrary units) in response to bolus injections of bradykinin under basal conditions, during infusion of saline (Groups 1 and 2) or N^G-nitro-L-arginine methyl ester (L-NAME, Groups 3 and 4), and during infusion of saline plus perindoprilat (Group 1), saline plus captopril (Group 2), L-NAME plus perindoprilat (Group 3) and L-NAME plus captopril (Group 4)

	Group 1	*Group 2*	*Group 3*	*Group 4*
ΔHeart rate	56 ± 6*	57 ± 15*	55 ± 14*	54 ± 5*
ΔMean BP	−15 ± 6	−13 ± 6	−9 ± 5	−11 ± 4
ΔRenal conductance	10 ± 3	8 ± 3	5 ± 2	10 ± 3*
ΔMesenteric conductance	26 ± 7*	23 ± 4*	28 ± 8*	34 ± 9*
ΔHindquarters conductance	15 ± 4*	12 ± 2*	10 ± 2*	12 ± 4*
	Saline		*L-NAME*	
ΔHeart rate	59 ± 6*	58 ± 9*	48 ± 12*	37 ± 10*
ΔMean BP	−12 ± 4	−10 ± 4	21 ± 8*[c]	14 ± 4[d]
ΔRenal conductance	8 ± 3	6 ± 1*	−13 ± 6[c]	−6 ± 2[d]
ΔMesenteric conductance	24 ± 6*	21 ± 3*	22 ± 8*	23 ± 6*
ΔHindquarters conductance	13 ± 3*	12 ± 3*	9 ± 4*	8 ± 3*
	Saline		*L-NAME*	
	Perindoprilat	*Captopril*	*Perindoprilat*	*Captopril*
ΔHeart rate	−229 ± 44*	−153 ± 39*	−138 ± 19*	−75 ± 26
ΔMean BP	−16 ± 3*	−31 ± 7*	−44 ± 6*[c]	−45 ± 12*
ΔRenal conductance	−24 ± 7*	−38 ± 9*	−32 ± 9*	−18 ± 4*
ΔMesenteric conductance	−77 ± 20*	−71 ± 13*	−24 ± 7[c]	16 ± 5[bd]
ΔHindquarters conductance	43 ± 8*	48 ± 8*	30 ± 7*	19 ± 2*[d]

Values are mean ± s.e.mean, $n = 8$ (all groups).
*$P < 0.05$ for change, [b]$P < 0.05$ Group 4 vs Group 3; [c]$P < 0.05$ Group 3 vs Group 1; [d]$P < 0.05$ Group 4 vs Group 2

Table 5 Cardiovascular changes (AUC or AOC, arbitrary units) over a 30 min period following administration of perindoprilat in the presence of saline (Group 1) or N^G-nitro-L-arginine methyl ester (L-NAME, Group 3), or captopril in the presence of saline (Group 2) or L-NAME (Group 4)

	Group 1 (Saline + perindoprilat)	*Group 2 (Saline + captopril)*	*Group 3 (L-NAME + perindoprilat)*	*Group 4 (L-NAME + captopril)*
ΔHeart rate	2089 ± 256*	1185 ± 269*[a]	1223 ± 269*[c]	652 ± 165*
ΔMean BP	−1022 ± 78*	−714 ± 67*[a]	−597 ± 59*[c]	−373 ± 64*[bd]
ΔRenal conductance	1081 ± 75*	859 ± 67*	997 ± 97*	435 ± 73*[bd]
ΔMesenteric conductance	1375 ± 145*	989 ± 65*[a]	871 ± 117*[c]	493 ± 106*[bd]
ΔHindquarters conductance	508 ± 64*	239 ± 48*[a]	212 ± 39*[c]	83 ± 17*[bd]

Values are mean ± s.e.mean, $n = 8$ (all groups).
*$P < 0.05$ for change; [a]$P < 0.05$ Group 2 vs Group 1; [b]$P < 0.05$ Group 4 vs Group 3; [c]$P < 0.05$ Group 3 vs Group 1; [d]$P < 0.05$ Group 4 vs Group 2

Table 6 Cardiovascular changes (AUC or AOC, arbitrary units) over a 30 min period following administration of N^G-nitro-L-arginine methyl ester (L-NAME) in the presence of saline and perindoprilat (Group 1), saline and captopril (Group 2) or alone (Groups 3 and 4)

	Group 1 ($n = 8$)	*Group 2 ($n = 8$)*	*Group 3 and Group 4 ($n = 16$)*
ΔHeart rate	−1706 ± 318*	−2100 ± 252*	−1284 ± 151*[b]
ΔMean BP	351 ± 51*	302 ± 63*	193 ± 23*[ab]
ΔRenal conductance	−291 ± 135*	−546 ± 99*	−222 ± 50*[b]
ΔMesenteric conductance	−1683 ± 296*	−1123 ± 303*	−535 ± 82*[ab]
ΔHindquarters conductance	−780 ± 151*	−779 ± 197*	−279 ± 33*[ab]

*$P < 0.05$ for change; [a]$P < 0.05$ Groups 3 and 4 vs Group 1; [b]$P < 0.05$ Group 3 and 4 vs Group 2

Discussion

The experimental design allowed us to study (1) the regional haemodynamic effects of perindoprilat and captopril and the influence of L-NAME thereon, (2) the effects of the ACE inhibitors, in the absence or presence of L-NAME, on the haemodynamic responses to vasodilators with differing degrees of 'endothelial dependence', and, incidentally, (3) the

effects of L-NAME in the absence and presence of ACE inhibition. The following discussion is divided into the corresponding sections.

Effects of perindoprilat or captopril

Although, during infusion of saline, both perindoprilat and captopril caused marked hypotension, tachycardia and increases in renal and mesenteric blood flow, and renal, mesenteric and hindquarters vascular conductances, all effects (except the renal vasodilatation) were greater with perindoprilat than with captopril. Hence, any interaction between captopril and NO-mediated events (Goldschmidt & Tallarida, 1991) did not confer any enhanced vasodilator ability on captopril. Indeed, it appeared that the vasodilator effects of perindoprilat were better maintained than those of captopril and this was particularly true in the presence of L-NAME (Figure 2). Under these conditions, all the haemodynamic effects of captopril were significantly less than those of perindoprilat and, proportionately, were more reduced than were those of perindoprilat, relative to the respective responses in the presence of saline. In fact, the renal vasodilator effect of perindoprilat was not significantly affected by L-NAME, although the mesenteric and hindquarters vasodilatations were. Thus, it appears that the renal vasodilator effects of perindoprilat are independent of NO, although NO may contribute to its mesenteric and hindquarters vasodilator effects, but to a lesser extent than with captopril.

The lack of an effect of L-NAME on the renal hyperaemic vasodilator action of perindoprilat is particularly striking, since it would be expected that the increase in the renal blood flow, itself, might have stimulated NO release (Hutcheson & Griffith, 1991), and Haji-ali & Zimmerman (1992) have reported that the renal hyperaemic vasodilator effects of the non-sulphydryl ACE inhibitor, lisinopril, are inhibited by N^G-nitro-L-arginine. Whatever the explanation of our results, they indicate that perindoprilat could be capable of promoting renal blood flow in the presence of impaired endothelial function, when the renal haemodynamic effects of other ACE inhibitors might be compromised.

Recently, Cachofeiro *et al.* (1992) reported that the NO synthesis inhibitor, N^G-monomethyl-L-arginine (L-NMMA), attenuated hypotensive responses to captopril, ramiprilat or the nonpeptide AT_1-receptor antagonist, losartan, in spontaneously hypertensive rats. They suggested this was not a non-specific effect, since hypotensive responses to sodium nitroprusside were not changed. However, Cachofeiro *et al.* (1992) found that the responses to sodium nitroprusside were enhanced by L-NMMA in normotensive rats, and thus the lack of change in the hypertensive animals could have represented an abnormality of the sensitization that usually occurs to nitrovasodilators following NO synthesis inhibition (Moncada *et al.*, 1991; Gardiner *et al.*, 1991a). Nevertheless, our present results, showing a diminished hypotensive response to perindoprilat or captopril in the presence of L-NAME, corroborates the finding of Cachofeiro *et al.* (1992), and extends it by demonstrating that different haemodynamic effects underlie this event in the case of the perindoprilat and captopril.

It is feasible that the haemodynamic effects of ACE inhibitors are contributed to by inhibition of degradation of endogenous BK (e.g. Wiemer *et al.*, 1991; Cachofeiro *et al.*, 1992). However, in water-deprived, Brattleboro rats, captopril is devoid of any haemodynamic effects if it is administered in the presence of losartan (Batin *et al.*, 1991a,b), indicating that ACE inhibition has no additional consequences in this circumstance. Moreover, in the present work, the complex profile of effects of exogenous BK indicates that accumulation of endogenous BK following ACE inhibition could not, alone, explain the haemodynamic effects of perindoprilat or captopril.

Effects of vasodilators

As reported elsewhere (Gardiner *et al.*, 1990c; 1991a,b; 1992a,b) we observed that ACh caused renal hyperaemia, whereas lemakalim elicited mesenteric hyperaemia and BK caused an initial mesenteric, followed by hindquarters, hyperaemia. In those vascular beds in which flow increases did not occur, any change in vascular conductance which was associated with a maintenance, or relative maintenance of flow, could have been autoregulatory. Clearly, in those instances where flow fell in association with a reduction in vascular conductance there was an active vasoconstriction that may have been direct and/or indirect (reflex or otherwise) in origin.

Acetylcholine: Although there was an indication of desensitization to ACh with repeated infusions (cf. responses to ACh alone compared to responses to ACh in the presence of saline, Table 2), L-NAME caused clear-cut, and almost total, inhibition of the renal haemodynamic effects of ACh (Figure 2, Table 2). This effect was much more dramatic than we have previously seen with acute L-NAME treatment in Long Evans rats (Gardiner *et al.*, 1990c; 1991a), although in those instances the animals were normovolaemic. However, from previous experiments on Brattleboro rats with isosmotic hypovolaemia induced by s.c. injection of PEG (Gardiner *et al.*, 1989), or hyperosmotic hypovolaemia induced by water deprivation (Gardiner *et al.*, 1988), it appears that the renal circulation is relatively well preserved and in the present work the renal vasodilator effects of ACh were not substantially different from those seen in Long Evans or Brattleboro rats under normovolaemic conditions (Gardiner *et al.*, 1991a; 1992b). Thus, the susceptibility to L-NAME of the renal haemodynamic effects of ACh in the present experiments is not likely to have been due to factors such as elevated renal vasomotor tone or impaired renal perfusion, but was probably accounted for by the infusion of a higher dose of L-NAME than in previous studies (Gardiner *et al.*, 1991a).

In the presence of saline and perindoprilat, the renal hyperaemic vasodilator effect of ACh was abolished (as in the presence of L-NAME). At first sight, it thus appears that perindoprilat has a potent inhibitory effect on NO-mediated renal haemodynamic changes. However, it should be noted that, in the experimental model employed, perindoprilat itself caused marked hypotension and hyperaemic renal vasodilatation (Figure 1, Table 5). Hence, prior to ACh infusion, systemic and renal haemodynamics were markedly different from baseline, and the lack of response to ACh could have been due to the renal haemodynamic variables being at maximal levels. However, this is not likely to be a complete explanation, since there was a renal vasodilator response to ACh in the presence of saline and captopril and this was not seen in the presence of L-NAME and captopril (Figure 2, Table 2). Thus, these findings indicate the renal vasodilator effects of ACh are NO-dependent, and they are relatively less diminished in the presence of captopril than of perindoprilat.

There are few data available relating to the effects of perindoprilat on endothelium-dependent vasorelaxations in response to ACh, and what data there are show regional heterogeneity. For example, Kerth & Vanhoutte (1991) reported that, in endothelium-intact ring preparations of the left anterior descending coronary artery of the dog, precontracted with prostaglandin $F_{2\alpha}$ and pretreated with indomethacin, the concentration-dependent relaxations evoked by ACh, BK or thrombin were enhanced by perindoprilat. However, perindoprilat was without effect on the ACh or thrombin-induced, endothelium-dependent, relaxation of rings of canine femoral arteries. Moreover, perindoprilat was devoid of any direct effect on vascular smooth muscle and did not stimulate the release of endothelium-derived relaxing factor(s). The fact that Kerth & Vanhoutte (1991) did not observe any inhibitory effects of perindoprilat on endothelium-dependent vasorelaxation might indicate that

the effects reported here were indirect rather than direct.

As indicated above, the hindquarters vasodilator effect of ACh could have been autoregulatory, consistent with its being unchanged under any experimental condition. In contrast, ACh caused a variable mesenteric vasoconstriction (possibly reflex in origin), both in the presence of saline and of L-NAME; thus, it appears that the effects of ACh in the mesenteric vasculature under these condiitons were not modulated by NO. However, when ACh was given in the presence of saline, and either of the ACE inhibitors, there was mesenteric vasodilatation. While we cannot dismiss the possibility that this vasodilatation was autoregulatory (since there was no increase in flow), it is notable that it did not occur in the presence of L-NAME and hence it is likely that NO contributed to the effect. If this were the case, then it appears that any NO-mediated vasodilator effects of ACh in the mesenteric vascular bed are not inhibited by perindoprilat in the same way as the NO-mediated effects of ACh in the kidney appear to be, at least in PEG-treated, Brattleboro rats.

Lemakalim: In our earlier experiments (Gardiner *et al.*, 1991b) we had considered the use of lemakalim as an internal reference to control for the haemodynamic effects of L-NAME itself, acknowledging problems resulting from the development of supersensitivity to nitrovasodilators following inhibition of NO synthase (Moncada *et al.*, 1991; Gardiner *et al.*, 1991a). However, we also pointed out difficulties of interpretation of responses to 'enothelium-independent' vasodilators *in vivo* (Gardiner *et al.*, 1991b). Indeed, one could argue that the haemodynamic effects of any vasoactive substance *in vivo* cannot be endothelium-independent, since, even if its primary action was not on endothelial cells, any changes in haemodynamics it caused would influence endothelial function through changes in shear forces and pulsatility (Hutcheson & Griffith, 1991). That being said, the present results indicate the absolute responses to lemakalim were not affected under any experimental condition, in spite of marked changes in baseline haemodynamics at various stages of the protocols. Unfortunately, lemakalim does not cause renal hyperaemia, and hence its effects do not provide a particularly useful comparator for those of ACh.

Bradykinin: Although BK is generally to be considered an 'endothelium-dependent' vasodilator, it has complex effects *in vivo* involving direct and indirect vasodilator and vasoconstrictor actions (Gardiner *et al.*, 1990c; 1992a; Fasciolo *et al.*, 1990; Cowan & Cohen, 1992). Initially, we had intended to administer BK by 3 min infusion but in pilot experiments we found that this intervention, in the presence of perindoprilat or captopril, caused irreversible cardiovascular deterioration. Therefore, we decided to administer BK by bolus injection at a lower dose than we have used previously (Gardiner *et al.*, 1990c; 1992a), since the PEG-treated Brattleboro rats were particularly susceptible to its hypotensive effects in the presence of ACE inhibitors. The tendency towards hypotension, and the tachycardic and mesenteric and hindquarters vasodilator effects we saw with this low dose of BK were generally similar to those observed with higher doses of BK previously (Gardiner *et al.*, 1990c; 1992a). However, the modest hindquarters vasodilator effect of BK was unaffected by L-NAME. Whilst it is feasible that this effect of BK may differ between Brattleboro and Long Evans rats (since, in the latter, L-NAME inhibits the hindquarters vasodilator effect of BK, Gardiner *et al.*, 1990c), it is also possible that any NO-mediated effects of BK in the hindquarters of PEG-treated Brattleboro rats were offset by activation of sympathetic efferent tone supported by the renin-angiotensin system (Gardiner & Bennett, 1986; Gardiner *et al.*, 1989). Furthermore, we cannot preclude the possibility that a higher dose of BK would have exerted some hindquarters vasodilator effect involving NO (see below).

A relative lack of effect of L-NAME on the mesenteric vasodilator action of BK is consistent with our previous findings (Gardiner *et al.*, 1990c; 1992a), and indicates that NO-independent mechanisms may be involved in this phenomenon (e.g. Cowan & Cohen, 1992).

In the presence of saline and perindoprilat, or saline and captopril, BK caused significant hypotension and renal and mesenteric vasoconstriction, accompanied by augmented hindquarters vasodilatation. While the latter, and the hypotensive effects of BK under these conditions, are entirely consistent with enhancement of the effects of BK, due to inhibition of its degradation by the ACE inhibitors, the explanation of the renal and mesenteric vasoconstrictions is less straightforward, particularly since these effects were associated with such clear reductions in flow. It is feasible these responses were an amalgam of the vasoconstrictor effects of BK (Fasciolo *et al.*, 1990) together with indirect actions and reflex vasoconstriction in response to the hypotension. However, additional factors must have been involved since, in the presence of L-NAME and perindoprilat, or L-NAME and captopril, the hypotensive effect of BK was much greater than in the absence of L-NAME, yet the mesenteric vasoconstriction was less (perindoprilat) or absent (captopril). At first sight this is paradoxical, since there is evidence for involvement of NO in the hypotensive and other vasodilator effects of BK (Gardiner *et al.*, 1990c; 1992a); indeed, consistent with this, the hindquarters vasodilator effect of BK was less in the presence of L-NAME and captopril than in the presence of captopril and saline (Table 4). One possibility is that, in the presence of L-NAME and perindoprilat, or L-NAME and captopril, there was a marked coronary vasoconstrictor effect of BK and this resulted in a fall in cardiac output which amplified the hypotension. It is clear there was an unusual interaction between BK and the heart in the presence of the ACE inhibitors, because profound bradycardia, rather than the usual tachycardia, was seen. However, the bradycardia itself was not responsible for the augmented hypotensive response to BK in the presence of L-NAME and the ACE inhibitors, because a similar bradycardic effect was seen in the absence of L-NAME (Table 4).

Consistent with the influence of captopril on the effects of ACh in the renal vascular bed, it appeared that the ability of captopril to enhance the hindquarters vasodilator action of BK was dependent on a substantial L-NAME-sensitive component (Table 4). In contrast, the augmentation by perindoprilat of the hindquarters vasodilator effect of BK was not significantly affected by L-NAME. It does not seem likely that the difference between perindoprilat and captopril in this regard can be explained by different degrees of BK accumulation, due to differential extents of local ACE inhibition, since the hindquarters vasodilator response to BK was the same in the presence of saline and perindoprilat as in the presence of saline and captopril.

Effects of L-NAME

Similar to its effects in animals under normal conditions (Gardiner *et al.*, 1990b), L-NAME caused hypertension and bradycardia in association with renal, mesenteric and hindquarters vasoconstrictions in PEG-treated, Brattleboro rats. Interestingly, the pressor and mesenteric and hindquarters vasoconstrictor effects of L-NAME were augmented in the presence of perindoprilat or captopril, consistent with the mesenteric and hindquarters vasodilator effects of the ACE inhibitors being dependent, to an extent, on NO (see above). Furthermore, the similar renal vasoconstrictor effect of L-NAME in the presence of saline or perindoprilat, compared to the enhanced renal vasoconstrictor effect of L-NAME in the presence of captopril (Table 6), supports the proposition that the renal vasodilator effects of the latter involve NO, whereas those of perindoprilat do not. The greater effects of L-NAME in the presence of ACE inhibition indicate that the renin-angiotensin system is not involved indispensibly in the

systemic pressor or regional haemodynamic responses to L-NAME in PEG-treated Brattleboro rats, consistent with findings in normovolaemic Long Evans rats (Gardiner *et al.*, 1990c). However, as noted earlier, in the presence of the ACE inhibitors, mean arterial blood pressure was markedly reduced and there were substantial elevations in renal, mesenteric and hindquarters vascular conductances; hence, these changes in baseline status could have affected the absolute changes in cardiovascular variables evoked by L-NAME, but this does not explain why the renal vasoconstrictor effects of L-NAME were unchanged in the presence of perindoprilat. Thus, it is more likely that all the other effects of L-NAME were enhanced in the presence of the ACE inhibitors due to the latter augmenting NO-dependent mechanisms. While it is feasible that such an interaction could occur at the level of the endothelial cells, through a direct influence on release and/or inactivation of NO, the haemodynamic response to the ACE inhibitors might have enhanced NO release through changes in the physical forces acting on the endothelial cells. However, as mentioned earlier, it is not clear why such a phenomenon should not be apparent in the renal hyperaemic vasodilator effect of perindoprilat.

In conclusion, in the PEG-treated, Brattleboro rat, perindoprilat exerts more marked and sustained hypotensive, and hyperaemic vasodilator effects in mesenteric and hindquarters vascular beds than does captopril, in spite of the effects of the latter showing more dependence on NO-mediated processes. However, both ACE inhibitors appear to inhibit ACh-induced renal hyperaemic vasodilatations (perindoprilat significantly more so than captopril), but whether or not this is a direct effect, and the extent to which endogenous BK is involved in the haemodynamic actions of perindoprilat and captopril remain to be determined.

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