Br. J. Pharmacol. (1991), **103**, 1256–1262

Haemodynamic effects of human α-calcitonin gene-related peptide following administration of endothelin-1 or N^G-nitro-L-arginine methyl ester in conscious rats

[1]S.M. Gardiner, A.M. Compton, P.A. Kemp, T. Bennett, *R. Foulkes & *B. Hughes

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and *Department of Pharmacology, Celltech Ltd., Slough

1 We investigated the peripheral haemodynamic effects of human α-calcitonin gene-related peptide (CGRP) following administration of endothelin-1 or N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide production, in conscious, chronically-instrumented, Long Evans rats.

2 Infusion of endothelin-1 (3 nmol $kg^{-1} h^{-1}$) caused hypertension, bradycardia and renal, mesenteric and hindquarters vasoconstrictions. Co-infusion of human α-CGRP (1.5 nmol $kg^{-1} h^{-1}$) reduced the hypertension and abolished the hindquarters vasoconstriction caused by endothelin-1 but the renal and mesenteric vasoconstrictor actions of endothelin-1 were not affected.

3 Infusion of human α-CGRP (15 nmol $kg^{-1} h^{-1}$) in the presence of endothelin-1 caused hypotension and hyperaemic vasodilatation in the hindquarters; the mesenteric vasoconstrictor effects of endothelin-1 were diminished, but there was only a transient reversal of the renal vasoconstrictor effects of endothelin-1.

4 Pretreatment with the non-peptide angiotensin II receptor antagonist, DuP 753 (10 mg kg^{-1}), caused slight hypotension associated with renal, mesenteric and hindquarters vasodilatations, but DuP 753 did not affect responses to endothelin-1 infusion. However, under these conditions co-infusion of human α-CGRP (15 nmol $kg^{-1} h^{-1}$) caused a sustained reversal of the renal vasoconstrictor effects of endothelin-1.

5 These results indicate that the failure of human α-CGRP to cause sustained reversal of the renal vasoconstrictor effects of endothelin-1 in the absence of DuP 753 was due to activation of the renin-angiotensin system (possibly as a consequence of the hypotension).

6 In the second experiment, L-NAME (10 mg kg^{-1}) caused renal, mesenteric and hindquarters vasoconstrictions similar to those seen in the presence of endothelin-1. However, the renal vasoconstrictor effects of L-NAME were reversed completely by human α-CGRP (15 nmol $kg^{-1} h^{-1}$), even though the latter caused hypotension comparable to that seen in the presence of endothelin-1. These results are consistent with a lack of functional activation of the renin-angiotensin system by human α-CGRP in the presence of L-NAME.

7 The vasoconstrictor effects of L-NAME on the hindquarters were completely reversed by infusion of human α-CGRP, but hindquarters flow and vascular conductance did not rise above baseline levels. Hence these results indicate the hindquarters hyperaemic vasodilator effects of human α-CGRP seen in the presence of endothelin-1 were contributed to by nitric oxide-mediated mechanisms.

Keywords: Human α-CGRP; endothelin-1; N^G-nitro-L-arginine methyl ester (L-NAME); peripheral blood flow

Introduction

In a recent study (Gardiner *et al.*, 1990d) it was found that infusion of endothelin-1 caused marked constriction of the internal carotid vascular bed in conscious rats, and that this effect was antagonized by co-infusion of nimodipine or human α-calcitonin gene-related peptide (CGRP). In the case of human α-CGRP it was suggested that the antagonism of the vasoconstrictor effects of endothelin-1 might show regional variations, since the internal carotid vasoconstriction was reversed at a time when systemic arterial blood pressure was still elevated, indicating persistent vasoconstriction in other vascular beds. Thus, the first aim of the present study was to investigate the functional antagonism between the vasoconstrictor effects of endothelin-1 and the influence of human α-CGRP in the renal, mesenteric and hindquarters vascular beds.

The results indicated that the hindquarters vasoconstrictor response to endothelin-1 was readily reversed by co-infusion of human α-CGRP, whereas the renal vasoconstrictor effects of endothelin-1 were resistant to reversal by human α-CGRP (see Results). Previously we (Bennett *et al.*, 1989; Gardiner *et al.*, 1990a) had found that the renal vasodilator effects of rat α-CGRP were enhanced by the angiotensin converting enzyme inhibitor, captopril, and suggested that activation of the renin-angiotensin system either directly (Kurtz *et al.*, 1988) and/or indirectly might oppose the vasodilator action of the rat α-CGRP. However, we could not exclude the possibility that captopril inhibited the catabolism of the peptide and thereby enhanced its effects.

Therefore, the second aim of the present study was to determine if inhibition of the cardiovascular actions of angiotensin II influenced the ability of human α-CGRP to oppose the renal vasoconstrictor effects of endothelin-1. In order to avoid the putative problems with the use of captopril in this protocol (see above), and the complications arising from the partial agonistic effects of the peptide analogues of angiotensin II (e.g. Tomlinson *et al.*, 1990), we used the non-peptide angiotensin II receptor antagonist, DuP 753 (Wong *et al.*, 1990; Batin *et al.*, 1991), which is extremely potent but totally lacking in agonistic effects.

In vivo, endothelin-1 is produced by endothelial cells and its release is inhibited by nitric oxide (Boulanger & Lüscher, 1990), the major endothelium-derived relaxing factor (see Moncada & Higgs, 1990). In conscious rats, administration of N^G-nitro-L-arginine methyl ester (L-NAME; Moore *et al.*, 1990) causes hypertension associated with marked regional vasoconstrictions (Gardiner *et al.*, 1990f). L-NAME inhibits production of nitric oxide from L-arginine (Ishii *et al.*, 1990; Mülsch & Busse, 1990), hence its regional haemodynamic effects could be due to loss of nitric oxide-mediated vasodilator tone and/or disinhibition of endothelin-1 release. If the

[1] Author for correspondence.

latter were the case the haemodynamic profile of L-NAME and endothelin-1 might be similar. The question of whether or not human α-CGRP opposes the vasoconstrictor effects of L-NAME and endothelin-1 in a comparable fashion depends on the extent to which the vasodilator action of human α-CGRP is dependent on release of nitric oxide from endothelial cells; this is contentious (Gray & Marshall, 1990) and could vary in different vascular beds. Therefore, the third aim of the present work was to compare the regional haemodynamic profiles of endothelin-1 and L-NAME and to assess the degree of reversal of these effects by human α-CGRP.

Methods

All experiments were carried out on male, Long Evans rats (350–450 g). Under sodium methohexitone anaesthesia (60 mg kg^{-1}, i.p., supplemented as required) miniaturized, pulsed Doppler probes (Haywood *et al.*, 1981) were implanted to monitor renal, superior mesenteric and hindquarters blood flows in conscious animals. All the techniques have been described in detail elsewhere (Gardiner *et al.*, 1989a,b; 1990d,f).

Continuous recordings of the Doppler shift signals were made by use of a Crystal Biotech VF-1 mainframe (Crystal Biotech, Holliston, USA) modified to operate with a pulse repetition frequency of 125 kHz and fitted with modified HVPD-20 modules, to avoid problems with signal aliasing (Gardiner *et al.*, 1990c). Mean arterial blood pressure and mean Doppler shift signals were used to calculate percentage changes in regional vascular conductances (Gardiner *et al.*, 1990c). The following protocols were run:

Effects of endothelin-1

Conscious, Long Evans rats (n = 8) received an i.v. infusion of endothelin-1 (3 nmol kg^{-1} h^{-1} at 0.3 ml h^{-1}) over a period of 20 min.

Effects of human α-CGRP during infusion of endothelin-1

Ten min after the onset of endothelin-1 infusion (as above), conscious Long Evans rats (n = 8) were given a concurrent infusion of human α-CGRP (1.5 nmol kg^{-1} h^{-1} at 0.3 ml h^{-1}) and the two peptides were given together for the following 10 min.

The protocol as above was also performed but with a 10 fold higher dose of human α-CGRP (i.e. 15 nmol kg^{-1} h^{-1} at 0.3 ml h^{-1}).

The same animals (Group 1 in Table 1) were used in protocols 1 and 2; the protocols were randomized and spread over 2 days. At least 90 min separated each experimental run and all variables were back to baseline before the next intervention was begun.

Effects of human α-CGRP during infusion of endothelin-1 in the presence of DuP 753

In a separate group (Group 2 in Table 1) of conscious, Long Evans rats (n = 9) human α-CGRP (15 nmol kg^{-1} h^{-1}; 0.3 ml h^{-1}) was administered during the last 10 min of a 20 min infusion of endothelin-1 (as above) in animals that had received DuP 753 (10 mg kg^{-1} bolus in 100 μl; Wong *et al.*, 1990; Batin *et al.*, 1991), 10 min before the onset of the endothelin infusion.

Effects of human α-CGRP after administration of L-NAME

Elsewhere (Gardiner *et al.*, 1990f) we have shown that an i.v. bolus dose of L-NAME (10 mg kg^{-1}) causes haemodynamic effects that are established within 10 min of administration and are maintained for the following 60 min at least. Therefore, an additional group (Group 3 in Table 1) (n = 8) of Long Evans rats was given an i.v. bolus injection of L-NAME (10 mg kg^{-1} in 100 μl) and 10 min later an infusion of human α-CGRP (15 nmol kg^{-1}, h^{-1}, 0.3 ml h^{-1}) was begun and continued for the following 10 min.

The resting, baseline values for all cardiovascular variables in the 3 separate groups of animals used in the present study are shown in Table 1. There were no significant differences between the groups (Kruskal-Wallis test).

Drugs and peptides

Human α-CGRP (Celltech Ltd) and endothelin-1 (Peptide Institute) were dissolved in isotonic saline containing 1% bovine serum albumin (Sigma). 2-n-butyl-4-chloro-5-hydroxymethyl-1-[2-(1H-tetrazole-5-yl)bi-phenyl-4-yl-methyl]imidazole, potassium salt (DuP 753, Du Pont de Nemours, USA; gift from Dr R. Smith) and L-NAME hydrochloride (Sigma) were dissolved in isotonic saline.

Statistics

Within-group comparisons were carried out with Friedman's test (Theodorsson-Norheim, 1987) and between-group comparisons with Wilcoxon's rank sums test or the Kruskal-Wallis test, as appropriate.

Results

Effects of endothelin-1

Infusion of endothelin-1 at 3 nmol kg^{-1} h^{-1} for 20 min caused a progressive rise in mean arterial blood pressure and a bradycardia (Figure 1), accompanied by reductions in blood flows and conductances in the renal, mesenteric and hindquarters vascular beds (Figures 1 and 2). The profiles of change were similar to those described previously (Gardiner *et al.*, 1990b).

Table 1 Resting, baseline cardiovascular variables in the three separate groups of Long Evans rats studied

Group	*1* (n = 8)	*2* (n = 9)	*3* (n = 8)
Heart rate (beats min^{-1})	325 ± 15	318 ± 16	319 ± 10
Mean arterial blood pressure (mmHg)	104 ± 3	105 ± 1	102 ± 4
Doppler shift (kHz)			
Renal	8.5 ± 1.4	11.0 ± 0.9	11.4 ± 1.5
Mesenteric	6.3 ± 0.9	5.3 ± 0.4	7.9 ± 0.5
Hindquarters	4.0 ± 0.5	4.4 ± 0.3	4.0 ± 0.4
Vascular conductance ([kHz mmHg^{-1}]10^3)			
Renal	83 ± 14	104 ± 8	111 ± 14
Mesenteric	61 ± 9	51 ± 4	77 ± 5
Hindquarters	39 ± 5	42 ± 2	40 ± 5

Values are mean ± s.e.mean.

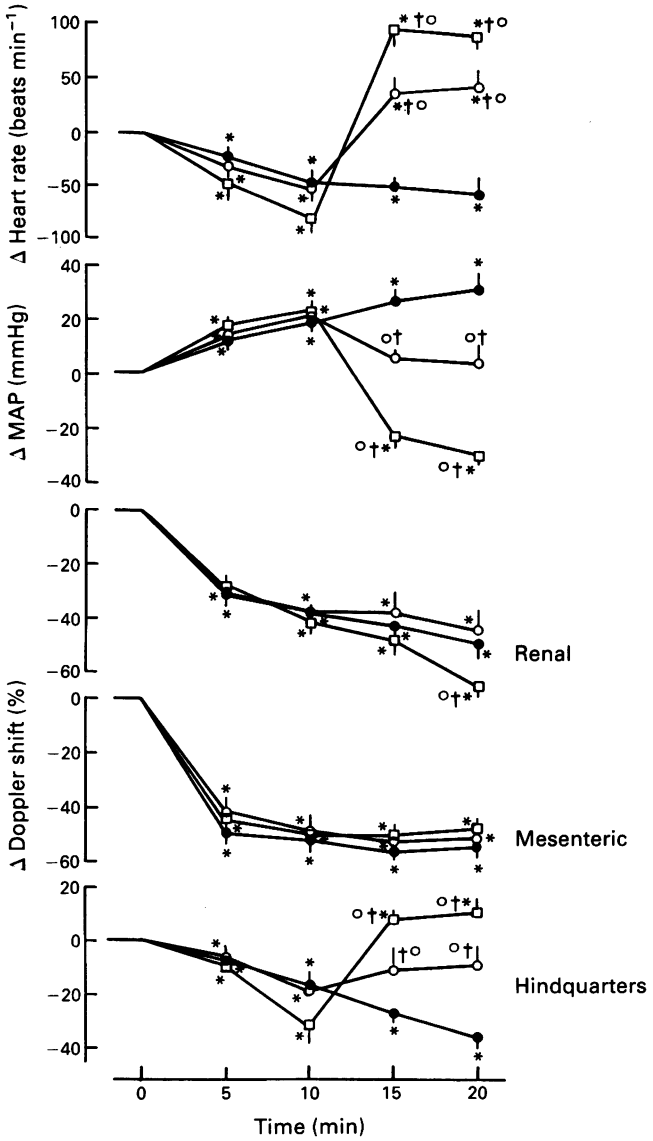

Figure 1 Changes in heart rate, mean arterial blood pressure (MAP) and regional blood flows (Doppler shift) in the same Long Evans rats ($n = 8$) during infusion of endothelin-1 alone (3 nmol kg^{-1} h^{-1}) for 20 min (●), during infusion of endothelin-1 alone for 10 min followed by co-infusion of endothelin-1 and human α-calcitonin gene-related peptide (α-CGRP) (1.5 nmol kg^{-1} h^{-1}) for the subsequent 10 min (○) or during infusion of endothelin-1 and human α-CGRP (15 nmol kg^{-1} h^{-1}) for the subsequent 10 min (□). Values are mean and bars show s.e.means. * $P < 0.05$ versus baseline. † $P < 0.05$ versus 10 min value, ° $P < 0.05$ versus corresponding value in the presence of endothelin-1 alone.

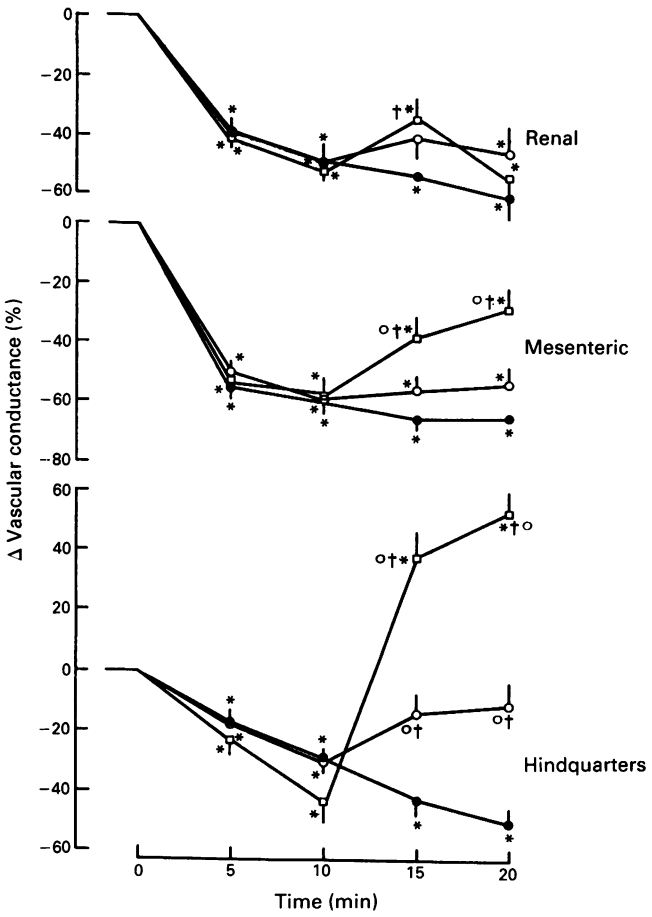

Figure 2 Changes in regional vascular conductances in the same Long Evans rats ($n = 8$) as in Figure 1 during infusion of endothelin-1 alone (3 nmol kg^{-1} h^{-1}) for 20 min (●), during infusion of endothelin-1 alone for 10 min followed by co-infusion of endothelin-1 and human α-calcitonin gene-related peptide (α-CGRP) (1.5 nmol kg^{-1} h^{-1}) for the subsequent 10 min (○), or during infusion of endothelin-1 alone for 10 min followed by co-infusion of endothelin-1 and human α-CGRP (15 nmol kg^{-1} h^{-1}) for the subsequent 10 min (□). Values are mean and bars show s.e.means. * $P < 0.05$ versus baseline, † $P < 0.05$ versus 10 min values, ° $P < 0.05$ versus corresponding value in the presence of endothelin-1 alone.

Effects of human α-CGRP during infusion of endothelin-1

While endothelin-1 was being infused alone during the first 10 min the cardiovascular effects were indistinguishable from those described above. Co-infusion of human α-CGRP at 1.5 nmol kg^{-1} h^{-1} caused a significant ($P < 0.05$) reduction in the endothelin-1-induced hypertension, accompanied by a pronounced tachycardia (Figure 1). Although the reduction in hindquarters blood flow caused by endothelin-1 was abolished by human α-CGRP, the latter did not significantly affect renal or mesenteric blood flows (Figure 1). Thus, the hindquarters vasoconstrictor effect of endothelin-1 was reversed by human α-CGRP at a time when the renal and mesenteric vasoconstrictor effects were not significantly changed (Figure 2).

Infusion of endothelin-1 alone in the third protocol caused effects very similar to the responses evoked by the peptide in the first protocol (Figures 1 and 2). Co-infusion of human α-CGRP at 15 nmol kg^{-1} h^{-1} caused hypotension and tachycardia (Figure 1). The reduction in renal blood flow was enhanced, whereas the reduction in hindquarters blood flow was converted to a significant increase above baseline during infusion of endothelin-1 and human α-CGRP; mesenteric blood flow showed no significant change (Figure 1). Hence there was a significant increase in hindquarters vascular conductance above baseline levels; mesenteric vasoconstriction was diminished, but there was only a transient rise in renal vascular conductance, which always remains below baseline (Figure 2).

Effects of human α-CGRP during infusion of endothelin-1 in the presence of DuP 753

Injection of DuP 753 (10 mg kg^{-1}) caused a slight, but sustained fall in mean arterial blood pressure (maximum at 10 min, -9 ± 2 mmHg, $P < 0.05$), accompanied by a tachycardia (40 ± 9 beats min^{-1}, $P < 0.05$). There were increases in flow in renal ($9 \pm 2\%$, $P < 0.05$), mesenteric ($23 \pm 5\%$, $P < 0.05$) and hindquarters ($14 \pm 4\%$, $P < 0.05$) vascular beds, together with rises in vascular conductance (renal, $19 \pm 2\%$; mesenteric, $35 \pm 6\%$; hindquarters, $23 \pm 5\%$, all $P < 0.05$). In the presence of DuP 753 the haemodynamic effects of endothelin-1 infusion were not different from those seen with endothelin-1 alone. However, the subsequent administration of human α-CGRP elicited a sustained renal vasodilatation in

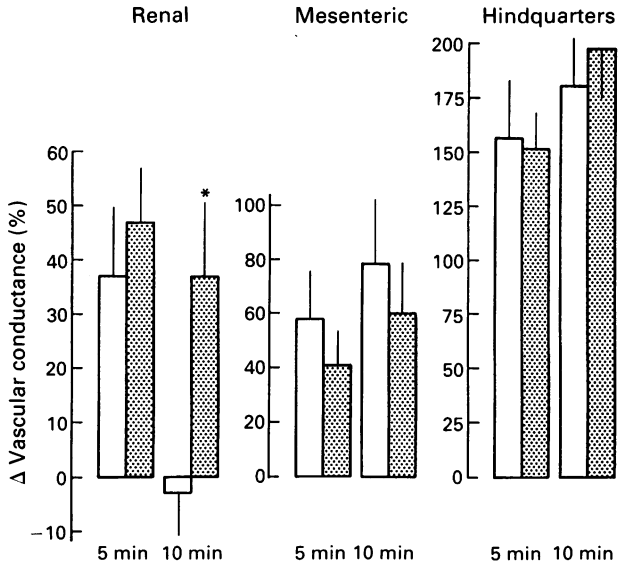

Figure 3 Changes in regional vascular conductances 5 and 10 min after infusion of human α-calcitonin gene-related peptide (α-CGRP) (15 nmol kg^{-1} h^{-1}) in the presence of an endothelin-1 infusion (3 nmol kg^{-1} h^{-1} beginning 10 min before human α-CGRP) (open columns, data from Figure 2) or in the presence of an endothelin-1 infusion beginning 10 min before human α-CGRP and 10 min after administration of DuP 753 (10 mg kg^{-1}) (stippled columns) in a separate group of animals ($n = 9$). Values are mean and bars show s.e.means. The changes are expressed relative to the value before human α-CGRP was administered. * $P < 0.05$ versus value in the presence of endothelin-1 alone.

contrast to the transient effect seen in the absence of DuP 753 (Figure 3). The mesenteric and hindquarters vasodilator responses to human α-CGRP in the presence of endothelin-1 were unaffected by DuP 753 (Figure 3).

Effects of human α-CGRP following administration of L-NAME

Injection of L-NAME caused hypertension and bradycardia (Figure 4) accompanied by reductions in renal, mesenteric and hindquarters blood flows and conductances (Figures 4 and 5). The changes in vascular conductances following administration of L-NAME were very similar to those seen during infusion of endothelin-1 (Figure 5), although the changes in mean arterial blood pressure, heart rate and renal blood flow were different (Figure 4). In the presence of L-NAME, human α-CGRP caused tachycardia and hypotension similar to those seen in the presence of endothelin-1 (Figure 4). However, there were differences in the changes in renal and hindquarters blood flows indicating that human α-CGRP caused more marked renal vasodilatation and less marked hindquarters vasodilatation in the presence of L-NAME than in the presence of endothelin-1 (Figures 4 and 5). The mesenteric vasodilator response to human α-CGRP was similar under the two conditions (Figure 5).

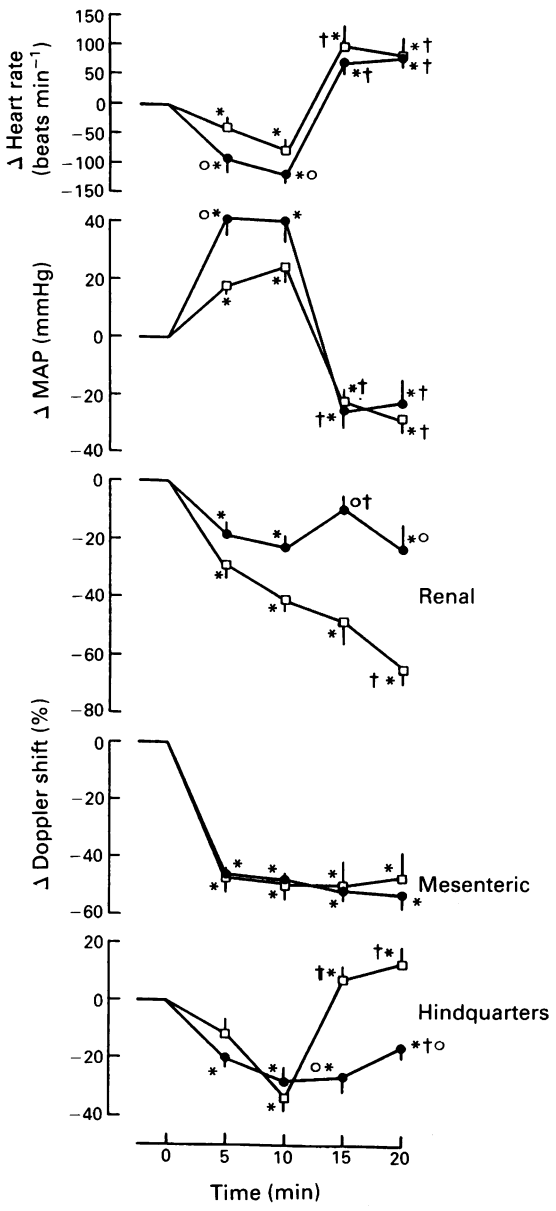

Figure 4 Changes in heart rate, mean arterial blood pressure (MAP) or regional blood flows (Doppler shift) following infusion of endothelin-1 alone (3 nmol kg^{-1} h^{-1}) for 10 min and co-infusion of endothelin-1 and human α-calcitonin gene-related peptide (α-CGRP) (15 nmol kg^{-1} h^{-1}) for the subsequent 10 min (□), (data from Figure 1): (●) indicates changes in cardiovascular variables after injection of N^{G}-nitro-L-arginine methyl ester (10 mg kg^{-1} bolus) followed 10 min later by infusion of human α-CGRP (15 nmol kg^{-1} h^{-1}) for the subsequent 10 min in a separate group of Long Evans rats ($n = 8$). Values are mean and bars show s.e.means. * $P < 0.05$ versus baseline, † $P < 0.05$ versus 10 min value. ° $P < 0.05$ versus corresponding value in the presence of endothelin-1.

Discussion

The present work produced two main findings: (1) the ability of human α-CGRP to oppose the vasoconstrictor effects of endothelin-1 varies in different vascular beds; (2) the haemodynamic changes following administration of endothelin-1 or L-NAME and the ability of human α-CGRP to reverse these changes are not identical.

In a previous study (Gardiner *et al.*, 1990d) we suggested that the functional antagonism between the vasoconstrictor effects of endothelin-1 and the vasodilator effects of human α-CGRP might vary in different vascular beds. This was demonstrated to be so in the present work since the hindquarters vasoconstrictor effects of endothelin-1 were abolished by infusion of human α-CGRP at a rate that had no effect on renal or mesenteric vascular conductances. Furthermore, in the presence of endothelin-1, infusion of human α-CGRP at a higher rate, sufficient to cause hypotension, tachycardia and marked elevations in hindquarters blood flow and vascular conductance above baseline levels, caused only a modest reduction in the mesenteric vasoconstriction and a transient reversal in renal vasoconstriction. In the latter vascular bed the ability of human α-CGRP to reverse endothelin-1-induced vasoconstriction was augmented in the presence of DuP 753, a non-peptide, angiotensin II antagonist (Wong *et al.*, 1990). These results are consistent with activation of the renin-

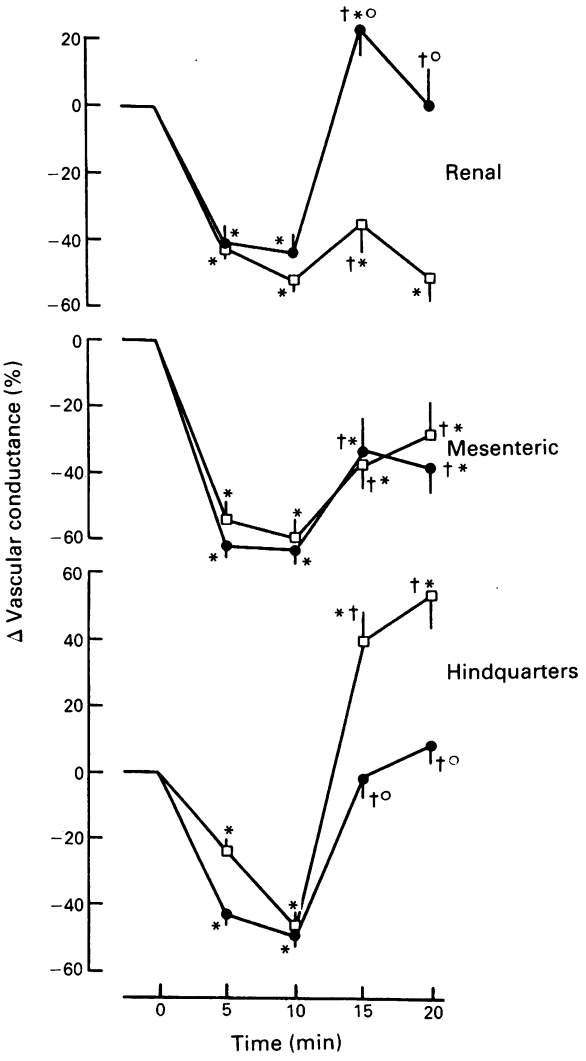

Figure 5 Changes in regional vascular conductances following infusion of endothelin-1 (3 nmol kg^{-1} h^{-1}) for 10 min and co-infusion of endothelin-1 and human α-calcitonin gene-related peptide (α-CGRP) (15 nmol kg^{-1} h^{-1}) for the subsequent 10 min (□), (data from Figure 2); (●) indicates changes in cardiovascular variables after injection of N^G-nitro-L-arginine methyl ester (10 mg kg^{-1} bolus) followed 10 min later by infusion of human α-CGRP (15 nmol kg^{-1} h^{-1}) for the subsequent 10 min in a separate group of Long Evans rats ($n = 8$). Values are mean and bars show s.e.means. * $P < 0.05$ versus baseline; † $P < 0.05$ versus 10 min value; ° $P < 0.05$ versus corresponding value in the presence of endothelin-1.

angiotensin system in the presence of human α-CGRP offsetting its renal vasodilator effects. Such findings corroborate previous observations showing that captopril causes marked renal vasodilatation in the presence of rat α-CGRP (Bennett *et al.*, 1989; Gardiner *et al.*, 1990a), and indicate that those effects were due to antagonism of angiotensin II-mediated vasoconstriction rather than to some other action of captopril augmenting the effects of rat α-CGRP.

It is possible that activation of the renin-angiotensin system by human α-CGRP was a direct (Kurtz *et al.*, 1988), and/or an indirect effect, consequent upon the fall in mean arterial blood pressure. Whatever the explanation, it is notable that this occurred in the presence of endothelin-1 which has been shown to inhibit renin release *in vitro* (Rakugi *et al.*, 1988; Takagi *et al.*, 1988, 1989; Matsumura *et al.*, 1989b). However, *in vivo*, any inhibitory effects of endothelin-1 on renin release may be obscured by stimulatory effects secondary to changes in renal function (e.g. Goetz *et al.*, 1988; Miller *et al.*, 1989; Otsuka *et al.*, 1989; Matsumura *et al.*, 1989a; Tsuchiya *et al.*, 1990). Moreover, it is feasible that the stimulatory effect of human α-CGRP on renin release is greater than endothelin's inhibitory effect. However, the relative potencies of endothelin-1 to inhibit, and human α-CGRP to stimulate, renin release have not been assessed.

It is notable that in the presence of endothelin-1, human α-CGRP acted to cause mesenteric vasodilatation, albeit to a modest extent. In previous studies in conscious rats, administration of human α-CGRP alone was found to cause a fall in mesenteric vascular conductance, and we argued that this might have been due to reflex vasoconstrictor effects (Gardiner *et al.*, 1989a). The present results are consistent with *in vitro* studies showing mesenteric vasodilator responses to human α-CGRP, since those experiments were carried out in preconstricted preparations (Marshall *et al.*, 1986). The lack of a marked mesenteric vasodilator response to human α-CGRP in the present work *in vivo*, even when the mesenteric vasculature was preconstricted with endothelin-1, could have been due to activation of baroreflex mechanisms consequent upon the hypotension caused by human α-CGRP. In this context it is interesting that the mesenteric vasodilator effects (unlike the renal effects) of human α-CGRP were not augmented in the presence of DuP 753. A similar picture was seen in the hindquarters, consistent with a relative lack of vasoconstrictor effect of angiotensin II in that vascular bed (Gardiner *et al.*, 1988). However, angiotensin II can exert substantial mesenteric vasoconstrictor effects (Gardiner *et al.*, 1988), as was clear from the marked vasodilator response to DuP 753. Hence, the apparent lack of involvement of the renin-angiotensin system in opposing the mesenteric vasodilator effects of human α-CGRP in the presence of endothelin-1 was probably due to the hypotension eliciting baroreflex-mediated vasoconstrictor effects that had a more extensive influence in this vascular bed than in the renal circulation.

As reported elsewhere, administration of L-NAME caused hypertension associated with marked renal, mesenteric and hindquarters vasoconstrictions, consistent with a substantial involvement of nitric oxide-mediated mechanisms in the maintenance of resting regional vascular conductances (Gardiner *et al.*, 1990e,f). In the present work we compared the haemodynamic effects of endothelin-1 and L-NAME since there is a possibility that disinhibition of endothelin-1 release contributes to the vasoconstrictor effects seen following suppression of nitric oxide production (Boulanger & Lüscher, 1990) with L-NAME. Although we were able to match very closely the renal, the mesenteric and the hindquarters vasoconstrictor effects of endothelin-1 and L-NAME, the changes in mean arterial blood pressure, heart rate and renal blood flow were different following administration of the two substances. At the doses used, L-NAME produced a greater rise in mean arterial blood pressure than did endothelin-1 even though their vasoconstrictor effects were the same. Hence it is likely that endothelin-1 produced a more marked decrease in cardiac output than did L-NAME. However, it does not follow that release of endogenous endothelin-1 would cause the same haemodynamic effects as administering exogenous endothelin-1. Therefore, direct assessment of the possible contribution of endogenous endothelin-1 to the haemodynamic actions of L-NAME will have to await the availability of a selective antagonist of the cardiovascular actions of endothelin-1.

In the presence of L-NAME, human α-CGRP caused a complete reversal of the renal vasoconstriction in spite of mean arterial blood pressure falling to a level similar to that seen in the presence of endothelin-1 (under which circumstances there was activation of the renin-angiotensin system sufficient to oppose the renal vasodilator effects of human α-CGRP; see above). These results indicate that in the presence of L-NAME, human α-CGRP may have caused little stimulation of the renin-angiotensin system, but there are no studies available regarding the effects of L-NAME on renin release. The fact that the initial overshoot in renal vascular conductance following administration of human α-CGRP in

the presence of L-NAME was transient (see Figure 5) might indicate activation of the renin-angiotensin system at that stage.

The occurrence of renal and mesenteric vasodilator responses to human α-CGRP in the presence of L-NAME that were greater (renal) or similar (mesenteric) to those in the presence of endothelin-1 might indicate that endothelium-derived nitric oxide was not involved in these responses, consistent with other findings (Grace *et al.*, 1987). However, *in vivo*, even classical, endothelium-dependent vasodilators can elicit substantial responses in the presence of L-NAME (Gardiner *et al.*, 1990g).

The picture in the hindquarters was more clear-cut in as much as the overshoot in blood flow and vascular conductance seen following administration of human α-CGRP in the presence of endothelin-1 was absent when human α-CGRP was given after L-NAME, although the hindquarters vasoconstrictor effect of the latter was still reversed by human α-CGRP. These findings indicate that about 50% of the hindquarters vasodilator response to human α-CGRP in the present experiments could have been mediated by nitric oxide-mediated mechanisms sensitive to L-NAME. Differential involvement of such processes in different vascular beds could account for the regional differences in sensitivity to the vasodilator effects of human α-CGRP and the variations in the ability of this peptide to reverse endothelin-1-induced regional vasoconstrictions (see above). In this connection it is of interest that, although the carotid vascular bed is more sensitive than the hindquarters to the vasodilator effects of human α-CGRP under normal conditions (Gardiner *et al.*, 1989a), in the presence of endothelin-1 the carotid vasodilator effects of human α-CGRP (Gardiner *et al.*, 1990d) were less than those in the hindquarters (this study). The augmented hindquarters vasodilator effect of human α-CGRP was abolished in the presence of L-NAME, whereas the latter has no effect on the carotid vasodilator effects of human α-CGRP (unpublished observations). Collectively these results indicate a substantial nitric oxide-mediated vasodilator effect of human α-CGRP localized to the hindquarters vascular bed.

References

BATIN, P., GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1991). Differential regional haemodynamic effects of the non-peptide angiotensin II antagonist, DuP 753, in water-replete and water-deprived Brattleboro rats. *Life Sci.*, **48**, 733–739.

BENNETT, T., COMPTON, A.M. & GARDINER, S.M. (1989). Captopril enhances the renal vasodilator effects of rat α-calcitonin gene-related peptide (CGRP) in conscious Long Evans rats. *J. Physiol.*, **413**, 28P.

BOULANGER, C. & LÜSCHER, T.F. (1990). Release of endothelin from the porcine aorta. Inhibition by endothelium-derived nitric oxide. *J. Clin. Invest.*, **85**, 587–590.

GARDINER, S.M., BENNETT, T. & COMPTON, A.M. (1988). Regional haemodynamic effects of neuropeptide Y, vasopressin and angiotensin II in conscious, unrestrained, Long Evans and Brattleboro rats. *J. Auton. Nerv. Syst.*, **24**, 15–27.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989a). Regional haemodynamic effects of human α- and β-calcitonin gene-related peptide in conscious Wistar rats. *Br. J. Pharmacol.*, **98**, 1225–1232.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1989b). Regional haemodynamic effects of calcitonin gene-related peptide. *Am. J. Physiol.*, **256**, R332–R338.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1990a). Renal vasodilator response to captopril in the presence of calcitonin gene-related peptide. In *Diuretics III, Chemistry, Pharmacology, and Clinical Applications* ed. Puschett, J.B. & Greenberg, A. pp. 279–281. New York: Elsevier.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1990b). Regional haemodynamic effects of endothelin-1 and endothelin-3 in conscious Long Evans and Brattleboro rats. *Br. J. Pharmacol.*, **99**, 107–112.

GARDINER, S.M., COMPTON, A.M., BENNETT, T. & HARTLEY, C.J. (1990c). Can the pulsed Doppler technique be used to measure changes in cardiac output in conscious rats? *Am. J. Physiol.*, **259**, H448–H456.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., KEMP, P.A. & NEY, U. (1990d). Synergistic internal carotid vasodilator effects of human α-calcitonin gene-related peptide and nimodipine in conscious rats. *Br. J. Pharmacol.*, **99**, 830–834.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990e). Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension*, **15**, 486–492.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990f). Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats. *Br. J. Pharmacol.*, **101**, 625–631.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990g). Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats: effects of N^G-nitro-L-arginine methyl ester. *Br. J. Pharmacol.*, **101**, 632–639.

GOETZ, K.L., WANG, B.C., MADWED, J.B., ZHU, J.L. & LEADLEY, R.J. (1988). Cardiovascular, renal, and endocrine responses to intravenous endothelin in conscious dogs. *Am. J. Physiol.*, **255**, R1064–R1068.

GRACE, G.C., DUSTING, G.J., KEMP, B.E. & MARTIN, T.J. (1987). Endothelium and the vasodilator action of rat calcitonin gene-related peptide (CGRP). *Br. J. Pharmacol.*, **91**, 729–733.

GRAY, D.W. & MARSHALL, I. (1990). Calcitonin gene-related peptide (CGRP) endothelium-dependent relaxation in rat aorta is inhibited by L-NMMA. *Br. J. Pharmacol.*, **99**, Proc. Suppl., 104P.

HAYWOOD, J.R., SHAFFER, R.A., FASTENOW, C., FINK, G.D. & BRODY, M.J. (1981). Regional blood flow measurement with pulsed Doppler flow meter in conscious rat. *Am. J. Physiol.*, **241**, H273–H278.

ISHII, K., CHANG, B., KERWIN, J.F., HUANG, Z.-J. & MURAD, F. (1990). N^{ω}-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. *Eur. J. Pharmacol.*, **176**, 219–223.

KURTZ, A., MUFF, R., BORN, W., LUNDBERG, J.M., MILLBERG, B.-I., GNADINGER, M.P., UEHLINGER, D.E., WEIDMANN, P., HOKFELT, T. & FISCHER, J.A. (1988). Calcitonin gene-related peptide is a stimulator of renin secretion. *J. Clin. Invest.*, **82**, 538–543.

MARSHALL, I., AL-KAZWINI, S.J., HOLMAN, J.J. & CRAIG, R.K. (1986). Human and rat α-CGRP but not calcitonin cause mesenteric vasodilatation in rats. *Eur. J. Pharmacol.*, **123**, 217–222.

MATSUMURA, Y., HISAKI, K., OHYAMA, T., HAYASHI, K. & ORIMOTO, S. (1989a). Effects of endothelin on renal function and renin secretion in anaesthetized rats. *Eur. J. Pharmacol.*, **166**, 577–580.

MATSUMURA, Y., NAKASE, K., IKEGAWA, R., HAYASHI, K., OHYAMA, T. & MORIMOTO, S. (1989b). The endothelium-derived vasoconstrictor peptide endothelin inhibits renin release *in vitro*. *Life Sci.*, **44**, 149–157.

MILLER, W.L., REDFIELD, M.M. & BURNETT, J.C. (1989). Integrated cardiac, renal, and endocrine actions of endothelin. *J. Clin. Invest.*, **83**, 317–320.

MONCADA, S. & HIGGS, E.A. (eds) (1990). *Nitric Oxide from L-Arginine: a Bioregulatory System.* Amsterdam: Excerpta Medica.

MOORE, P.K., AL-SWAYEH, O.A., CHONG, N.W.S., EVANS, R.A. & GIBSON, A. (1990). L-N^G-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation *in vitro*. *Br. J. Pharmacol.*, **99**, 408–412.

MÜLSCH, A. & BUSSE, R. (1990). N^G-nitro-L-arginine (N^5-[imino-(nitroamino)methyl]-L-ornithine) impairs endothelium-dependent dilations by inhibiting cytosolic nitric oxide synthesis from L-arginine. *Naunyn Schmiedebergs Arch. Pharmacol.*, **341**, 143–147.

OTSUKA, A., MIKAMI, H., KATAHIRA, K., TSUNETOSHI, T., MINAMITANI, K. & OGIHARA, T. (1989). Changes in plasma renin activity and aldosterone concentration in response to endothelin injection in dogs. *Acta Endocrinol.*, **121**, 361–364.

RAKUGI, H., NAKAMARU, M., SAITO, H., HIGAKI, J. & OGIHARA, T. (1988). Endothelin inhibits renin release from isolated rat glomeruli. *Biochem. Biophys. Res. Commun.*, **155**, 1244–1247.

TAKAGI, M., MATSUOKA, H., ATARASHI, K. & YAGI, S. (1988). Endothelin: a new inhibitor of renin release. *Biochem. Biophys. Res. Commun.*, **157**, 1164–1168.

TAKAGI, M., TSUKADA, H., MATSUOKA, H. & YAGI, S. (1989). Inhibitory effect of endothelin on renin release *in vitro*. *Am. J. Physiol.*, **257**, E833–E838.

THEODORRSON-NORHEIM, E. (1987). Friedman & Quade tests: BASIC computer program to perform non-parametric two-way analysis of variance and multiple comparisons on ranks of several related samples. *Comput. Biol. Med.*, **17**, 85–99.

TOMLINSON, K.C., GARDINER, S.M. & BENNETT, T. (1990). Hypotensive effects of angiotensin II analogues and angiotensin converting enzyme inhibitors in water-deprived Brattleboro rats. *J. Cardiovasc. Pharmacol.*, **15**, 562–568.

TSUCHIYA, K., NARUSE, M., SANAKA, T., NARUSE, K., KATO, Y., ZENG, Z.P., NITTA, K., SHIZUME, K., DEMURA, H. & SUGINO, N. (1990). Effects of endothelin on renal hemodynamics and excretory functions in anaesthetized dogs. *Life Sci.*, **46**, 59–65.

WONG, P.C., PRICE, W.A., CHIA, A.T., DUNCIA, J.V., CARINI, D.J., WEXTER, R.R. JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1990). Nonpeptide angiotensin II receptor antagonists. VII Characterization of functional antagonism displayed by DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.*, **252**, 719–725.

(Received October 16, 1990
Revised January 16, 1991
Accepted January 22, 1991)