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Regional haemodynamic effects of human and rat adrenomedullin in conscious rats

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1 Male, Long Evans rats were chronically instrumented with pulsed Doppler flow probes and intravascular catheters to permit assessment of the regional haemodynamic responses to human and rat adrenomedullin, to compare the responses to human adrenomedullin to those of human α-CGRP in the absence and presence of the $CGRP_1$-receptor antagonist, human α-CGRP [8-37], and to determine the involvement of nitric oxide (NO)-mediated mechanisms in the responses to human adrenomedullin, relative to human α-CGRP.

2 Human and rat adrenomedullin (0.3, 1, and 3 nmol kg^{-1}, i.v.) caused dose-dependent hypotension and tachycardia, accompanied by increases in renal, mesenteric and hindquarters flows and vascular conductances. At the lowest dose only, the hypotensive and mesenteric vasodilator effects of rat adrenomedullin were significantly greater than those of human adrenomedullin.

3 Human α-CGRP at a dose of 1 nmol kg^{-1} caused hypotension, tachycardia and increases in hindquarters flow and vascular conductance, but reductions in renal and mesenteric flows, and only transient vasodilatations in these vascular beds. These effects were substantially inhibited by human α-CGRP [8-37] (100 nmol kg^{-1} min^{-1}), but those of human adrenomedullin (1 nmol kg^{-1}) were not; indeed, the mesenteric haemodynamic effects of the latter peptide were enhanced by the $CGRP_1$-receptor antagonist.

4 In the presence of the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 183 nmol kg^{-1} min^{-1}), there was only a slight, but significant, inhibition of the hindquarters hyperaemic vasodilator effect of human adrenomedullin, but not that of human α-CGRP.

5 These results indicate that the marked regional vasodilator effects of human (and rat) adrenomedullin are largely independent of NO and, *in vivo*, do not involve $CGRP_1$-receptors.

Keywords: Adrenomedullin (human); rat adrenomedullin; haemodynamics; α-calcitonin gene-related peptide (α-CGRP) (human); α-CGRP [8-37] (human)

Introduction

Recently, a novel peptide, designated adrenomedullin, consisting of 52 amino acids, was isolated from human phaeochromocytoma by Kitamura *et al.* (1993). Although the levels of adrenomedullin were highest in phaeochromocytoma, the peptide was also present in normal adrenal medulla and other human tissues (Kitamura *et al.*, 1993). Subsequently, Sakata *et al.* (1993) detected adrenomedullin in various tissues from normal rats, and showed the peptide was distinct from human adrenomedullin in having 50 amino acid residues, 6 of which differed from the corresponding ones in the human peptide.

The presence of adrenomedullin in normal tissues and plasma (Ichiki *et al.*, 1994), and its synthesis and release by endothelial cells *in vitro* (Sugo *et al.*, 1994), raises the possibility that the peptide has a physiological role in cardiovascular control. This proposition is consistent with the finding that human adrenomedullin exerts a potent hypotensive effect in anaesthetized rats (Kitamura *et al.*, 1993); subsequently, this action was shown to be due to marked vasodilatation, since adrenomedullin increased cardiac index and stroke index (Ishiyama *et al.*, 1993). Interestingly, the hypotensive effect of human adrenomedullin was not accompanied by tachycardia, although Ishiyama *et al.* (1993) acknowledged this may have been due to inhibition of baroreceptor reflex function by the barbiturate anaesthetic they used. It is notable that Sakata *et al.* (1993) found rat adrenomedullin was more potent than human adrenomedullin, both in terms of eliciting hypotension in anaesthetised rats, and in stimulating cyclic AMP levels in platelets.

Recently, Kitamura *et al.* (1994) found plasma levels of adrenomedullin were elevated in patients with hypertension, and suggested the peptide might exert a beneficial arteriolar vasodilator effect in this condition. However, there are no data on the regional haemodynamic changes underlying the hypotensive effects of human or rat adrenomedullin in the absence of anaesthesia. Therefore, the first objective of this work was to determine the dose-relatedness of the regional haemodynamic actions of rat and human adrenomedullin in conscious rats.

Although human, and rat, adrenomedullin show only slight homology with human α-calcitonin gene-related peptide (α-CGRP) (Kitamura *et al.*, 1993), it has been reported that, in the isolated mesenteric vascular bed of the rat, human adrenomedullin caused vasodilatation which was blocked by the $CGRP_1$-receptor antagonist, human α-CGRP [8-37] (Nuki *et al.*, 1993). Since endogenous CGRP is localized to perivascular nerve fibres in the mesenteric vascular bed (Kawasaki *et al.*, 1988; Han *et al.*, 1990), the findings of Nuki *et al.* (1993) could be explained either by human adrenomedullin interacting with $CGRP_1$- receptors, and/or by adrenomedullin releasing endogenous CGRP. However, although exogenous CGRP is a potent vasodilator of the isolated mesenteric vascular bed of the rat (Marshall *et al.*, 1986), systemic administration of human or rat CGRP causes mesenteric vasoconstriction, probably as a reflex response to the systemic hypotension (Gardiner *et al.*, 1988; 1989a,b). Therefore, our second objective was to compare regional haemodynamic responses to human adrenomedullin and human α-CGRP in conscious rats, and to determine the influence of human α-CGRP [8-37] upon them.

There is some evidence that components of the regional

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haemodynamic response to infusion of human α-CGRP involve nitric oxide (NO)-mediated mechanisms (Gardiner *et al.*, 1991), but the possible involvement of NO in the haemodynamic effects of human adrenomedullin has not been examined. Therefore, our third objective was to assess the influence of the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), on responses to human adrenomedullin. However, since L-NAME causes substantial cardiovascular changes (Gardiner *et al.*, 1990a) which could influence responses to human adrenomedullin, independent of an action involving inhibition of NO synthase, we also assessed the effects of human adrenomedullin in the presence of a co-infusion of angiotensin II and vasopressin that, within experimental constraints, simulated the pressor and regional haemodynamic effects of L-NAME. Some of the results have been presented to the British Pharmacological Society (Gardiner *et al.*, 1994).

Methods

Male, Long Evans rats (350–450 g), bred in the Animal Unit in Nottingham, were used for all experiments. Under sodium methohexitone anaesthesia (Brietal, Lilly; 40–60 mg kg^{-1}, i.p., supplemented as required) pulsed Doppler flow probes and intravascular catheters were implanted to monitor renal, mesenteric and hindquarters flow, to measure blood pressure, and to administer substances into the right jugular vein, as described in detail previously (Gardiner *et al.*, 1993). Doppler probes were implanted at least 7 days before intravascular catheters, and the latter were implanted no less than 24 h before experiments were begun in conscious, unrestrained animals. The following protocols were run:

Dose-responses to human or rat adrenomedullin

Separate groups ($n = 8$ in each) of rats were given 3 increasing i.v. doses (0.3, 1.0, 3.0 nmol kg^{-1}) of human or rat adrenomedullin (Groups 1 and 2, respectively), with doses separated by at least 2 h.

Effect of human α-CGRP [8-37] on responses to human α-CGRP or human adrenomedullin

Rats ($n = 8$; Group 3) were randomised to receive human adrenomedullin (1 nmol kg^{-1}) or human α-CGRP (1 nmol kg^{-1}) followed 2 h later by the other peptide. Two hours thereafter, an infusion of human α-CGRP [8-37] (100 nmol kg^{-1} min^{-1}; Gardiner *et al.*, 1990b) was begun and 15 min later, human adrenomedullin or human α-CGRP was again administered (whichever was the peptide given first earlier). After a further 2 h, the infusion of human α-CGRP [8-37] was begun again, and, 15 min later, human adrenomedullin or human α-CGRP was again administered (whichever peptide was given second earlier).

In a separate group of rats ($n = 8$; Group 4) the protocol above was followed, with the exception that an infusion of isotonic saline (154 mmol l^{-1} NaCl) was given instead of human α-CGRP [8-37]. This allowed us to assess the reproducibility of responses to human adrenomedullin and human α-CGRP in the absence of the $CGRP_1$-receptor antagonist.

Effect of L-NAME or vasopressin plus angiotensin II on responses to human α-CGRP or human adrenomedullin

Animals were randomized to receive human adrenomedullin (1 nmol kg^{-1}) or human α-CGRP (1 nmol kg^{-1}) followed 2 h later by the other peptide. A further 2 h later, in one group ($n = 8$; Group 5) of animals, an infusion of L-NAME (183 nmol kg^{-1} min^{-1}; Gardiner & Bennett, 1992) was begun, and 90 min later human adrenomedullin or human α-CGRP was administered (whichever peptide was given first earlier). The infusion of L-NAME was continued and 2 h later, human adrenomedullin or human α-CGRP was again administered (whichever peptide was given second earlier).

In a separate group of rats ($n = 9$; Group 6) the protocol above was followed with the exception that, instead of L-NAME, a co-infusion of vasopressin (3 pmol kg^{-1} min^{-1}) and angiotensin II (33 pmol kg^{-1} min^{-1}) was given, to simulate, as far as possible, the pressor and regional haemodynamic effects of L-NAME. Pilot experiments showed that the co-infusion was more effective in this respect than either peptide alone.

Data analysis

Continuous recordings were made of phasic and mean arterial blood pressure and instantaneous heart rate, together with phasic and mean renal, mesenteric and hindquarters Doppler shift signals. Percentage changes in mean Doppler shift signals were taken as indices of flow changes, and changes in vascular conductance were calculated by dividing mean arterial blood pressure into mean Doppler shift and expressing the change as a %. Within-group analysis was by Friedman's test applied to changes relative to baseline or to integrated responses (areas under or over curves); between group comparisons were made by the Mann-Whitney U test or the Kruskal-Wallis test, as appropriate. A value for $P < 0.05$ was taken as significant.

Drugs and peptides

Human and rat adrenomedullin were obtained from the Peptide Institute through their UK agents (Scientific Research Associates). Human α-CGRP, angiotensin II and arginine vasopressin were obtained from Bachem (UK), and human α-CGRP [8-37] was a gift from Dr R. Foulkes (Celltech Ltd.). L-NAME hydrochloride was obtained from Sigma. All peptides were dissolved in sterile saline (154 mmol l^{-1} NaCl) containing 1% bovine serum albumin (Sigma); L-NAME was dissolved in sterile saline. Bolus injections were given in 100 μl; infusions were at 400 μl h^{-1}. Administration of saline alone in these volumes has no consistent effects.

Results

Resting values for cardiovascular variables in the 6 groups of rats studied are shown in Table 1. There were some slight differences in hindquarters Doppler shift values only.

Dose-responses to human or rat adrenomedullin

Human adrenomedullin caused hypotension and tachycardia, the magnitudes and durations of which were dose-dependent (Figure 1, Table 2). There were significant increases in renal, mesenteric and hindquarters flow with all 3 doses of human adrenomedullin, and these were associated with vasodilatations (Figure 1) which were dose-dependent when expressed as the integrated response (Table 2).

Rat adrenomedullin also caused dose-dependent hypotension, tachycardia and dilatation in the renal, mesenteric and hindquarters vascular beds (Table 2). However, only at the lowest dose did rat adrenomedullin have a greater hypotensive effect than human adrenomedullin; this difference was accompanied by a greater mesenteric vasodilatation only (Table 2).

Effect of human α-CGRP [8-37] on responses to human α-CGRP or human adrenomedullin

Bolus injections of human α-CGRP caused hypotension and tachycardia, together with reductions in renal and mesenteric flow and an increase in hindquarters flow; there were only

transient increases in renal and mesenteric vascular conductance, but a marked hindquarters vasodilatation (Figure 2). Repeated injection of human α-CGRP in the presence of saline evoked responses similar to those above, with the exception that the hindquarters vasodilator response was enhanced (Figure 2). In contrast, in the presence of human α-CGRP [8-37], the hypotensive and tachycardic effects of human α-CGRP were substantially inhibited, as were the reductions in renal and mesenteric flow; indeed, under these circumstances, human α-CGRP evoked a transient increase in renal flow (Figure 2). However, the initial rise in renal vascular conductance was significantly attenuated, and the subsequent renal and mesenteric vasoconstrictions were abolished in the presence of human α-CGRP [8-37], as were the increases in hindquarters flow and vascular conductance (Figure 2).

In the absence of human α-CGRP [8-37], the haemodynamic responses to human adrenomedullin were very reproducible (Figure 3). Interestingly, in the presence of human α-CGRP [8-37], the increases in mesenteric flow and vascular conductance evoked by human adrenomedullin were significantly enhanced; no effect of the latter peptide was inhibited by the $CGRP_1$-receptor antagonist.

Effect of L-NAME or vasopressin plus angiotensin II on responses to human α-CGRP or human adrenomedullin

Prior to infusion of L-NAME or vasopressin plus angiotensin II, responses to bolus injection of human α-CGRP were as described above (Figure 4).

The pressor effects of L-NAME and vasopressin plus angiotensin II were well-matched (Figure 4). However, infusion of vasopressin plus angiotensin II caused significantly greater bradycardia and reductions in renal and mesenteric flow and mesenteric vascular conductance than did L-NAME (Figure 4). In the presence of L-NAME, the integrated tachycardic and renal hyperaemic effects of human α-CGRP were significantly less than in the presence of

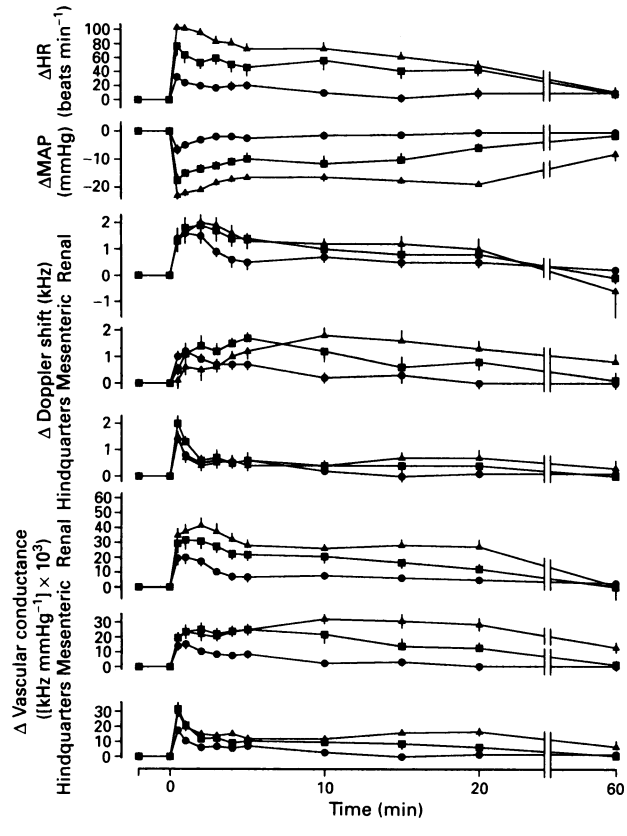

Figure 1 Cardiovascular changes following i.v. bolus injection of human adrenomedullin (●, 0.3 nmol kg^{-1}; ■, 1 nmol kg^{-1}; ▲, 3 nmol kg^{-1}), in the same conscious Long Evans rats ($n = 8$; Group 1). Values are mean ± s.e.mean; for clarity statistics for the integrated responses are shown in Table 2. HR = heart rate; MAP = mean arterial blood pressure.

Table 1 Resting cardiovascular variables in the 6 groups of conscious, Long Evans rats studied (see Methods)

	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 5*	*Group 6*
Heart rate (beats min^{-1})	338 ± 7	334 ± 6	316 ± 4	319 ± 7	312 ± 4	325 ± 1
Mean blood pressure (mmHg)	105 ± 2	103 ± 2	101 ± 2	103 ± 2	105 ± 1	104 ± 2
Renal Doppler shift (kHz)	7.1 ± 1.2	7.2 ± 0.4	6.8 ± 0.4	6.3 ± 0.5	6.5 ± 0.1	7.0 ± 0.5
Mesenteric Doppler shift (kHz)	6.8 ± 0.5	7.5 ± 0.7	6.1 ± 0.4	7.6 ± 0.7	7.5 ± 1.0	6.8 ± 0.7
Hindquarters Doppler shift (kHz)	4.5 ± 0.3	4.2 ± 0.2	3.6 ± 0.3	3.9 ± 0.5	3.1 ± 0.3[1,2]	3.5 ± 0.2[1]
Renal vascular conductance ([kHz $mmHg^{-1}$]10^3)	67 ± 11	70 ± 4	68 ± 5	62 ± 6	62 ± 2	68 ± 5
Mesenteric vascular conductance ([kHz $mmHg^{-1}$]10^3)	65 ± 5	73 ± 7	60 ± 3	75 ± 9	75 ± 9	66 ± 7
Hindquarters vascular conductance ([kHz $mmHg^{-1}$]10^3)	43 ± 3	41 ± 3	36 ± 3	38 ± 4	29 ± 3	34 ± 2

Values are mean ± s.e.mean.
Superscript numbers $P < 0.05$ versus corresponding groups (Kruskal-Wallis).

Table 2 Integrated (area under or over curve 0–60 min) cardiovascular changes during infusion of human or rat adrenomedullin in 2 separate groups of conscious, Long Evans rats

	Adrenomedullin 0.3 nmol kg^{-1}		*Adrenomedullin* 1.0 nmol kg^{-1}		*Adrenomedullin* 3.0 nmol kg^{-1}	
	Human a	*Rat* b	*Human* c	*Rat* d	*Human* e	*Rat* f
Heart rate (% min)	245 ± 98	378 ± 104	619 ± 82[a]	880 ± 162[b]	758 ± 108[a]	1567 ± 157[b,d]
Mean blood pressure (% min)	−101 ± 35	−284 ± 39[a]	−332 ± 68[a]	−461 ± 97[b]	−790 ± 60[a,c]	−801 ± 67[b,d]
Renal conductance (% min)	675 ± 240	788 ± 152	904 ± 182[a]	1378 ± 252[b]	1814 ± 324[a,c]	2136 ± 245[b,d]
Mesenteric conductance (% min)	362 ± 120	868 ± 143[a]	1175 ± 274[a]	1775 ± 224[b]	1990 ± 308[a,c]	2359 ± 226[b,d]
Hindquarters conductance (% min)	463 ± 111	835 ± 224	1119 ± 281[a]	1343 ± 272	2003 ± 356[a,c]	2034 ± 415[b]

Values are mean ± s.e.mean.
Superscript letters $P < 0.05$ versus corresponding column (Wilcoxon or Mann-Whitney U test, as appropriate).

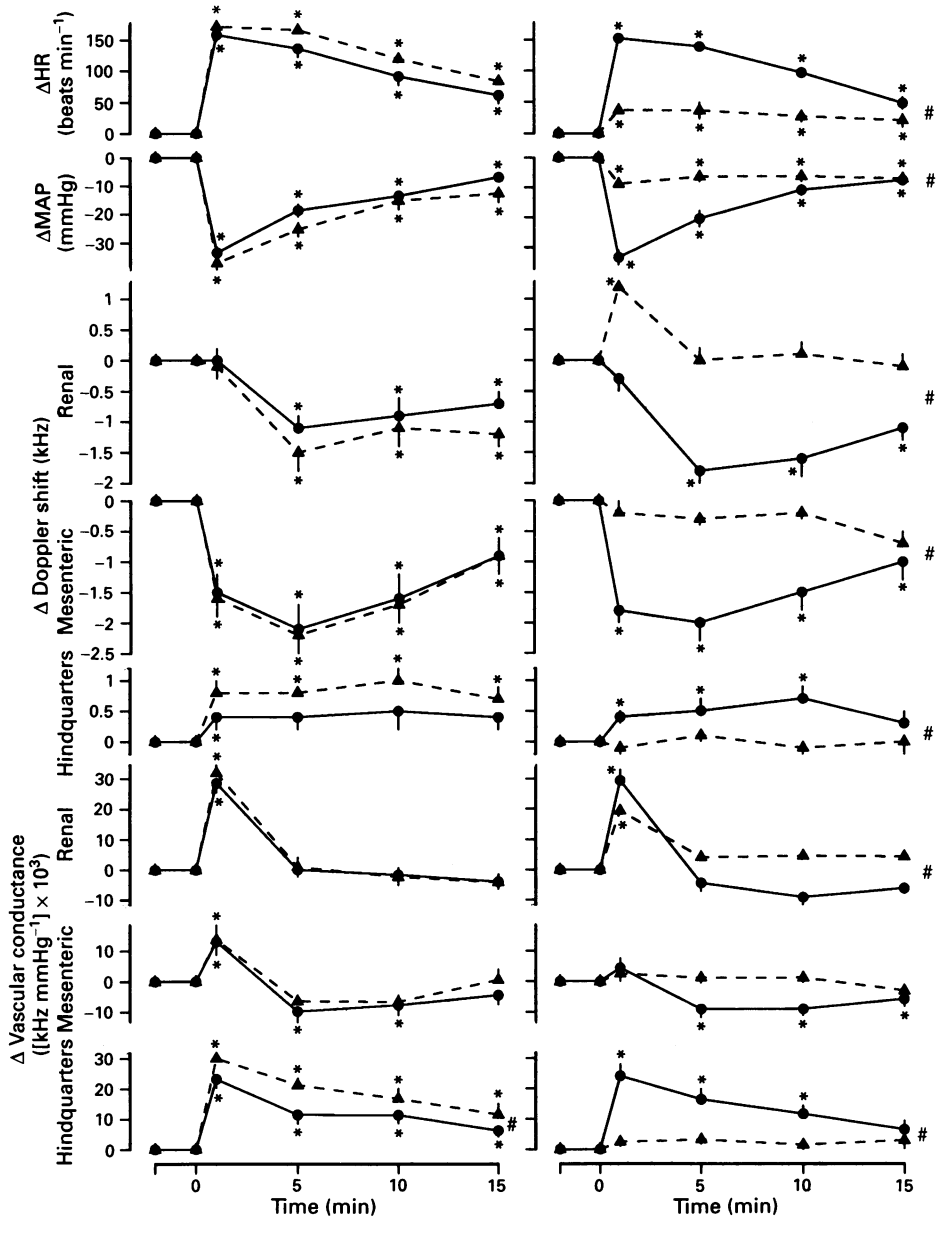

Figure 2 Cardiovascular changes following i.v. bolus injection of human α-calcitonin gene-related peptide (αCGRP) (1 nmol kg^{-1}). Left-hand panels, responses in the absence (●) and during infusion of saline (▲) in the same conscious, Long Evans rats ($n = 8$; Group 3). Right-hand panels, responses in the absence (●) and during infusion of human α-CGRP [8-37] (▲, 100 nmol kg^{-1} min^{-1}) in the same conscious, Long Evans rats ($n = 8$; Group 4). Values are mean ± s.e.mean; $^{*}P < 0.05$ versus baseline (Friedman's test), $^{\#}P < 0.05$ for the difference between the integrated responses in the absence and presence of saline (left-hand panels) or human α-CGRP [8-37] (right-hand panels) (Wilcoxon's test).

vasopressin and angiotensin II; however, the renal, mesenteric and hindquarters vasodilator actions of human α-CGRP were not significantly different in the two conditions (Figure 4).

Prior to infusion of L-NAME or vasopressin plus angiotensin II, responses to human adrenomedullin were as described above (Figure 5).

As in the experiment above, the pressor effects of L-NAME and vasopressin plus angiotensin II were the same, although the bradycardia and reductions in mesenteric and hindquarters flows and vascular conductances in the presence of vasopressin plus angiotensin II were greater than those in the presence of L-NAME (Figure 5).

The integrated tachycardic and hindquarters hyperaemic vasodilator responses to human adrenomedullin were slightly, but significantly, less in the presence of L-NAME than in the presence of vasopressin plus angiotensin II (Figure 5).

Discussion

The present work in conscious rats shows that human adrenomedullin has clear, dose-dependent hypotensive and tachycardic effects. While the changes in mean arterial blood pressure are consistent with previous reports (Kitamura *et al.*, 1993; Ishiyama *et al.*, 1993; Hao *et al.*, 1994), the marked increases in heart rate contrast with the absence of tachycardic responses to human adrenomedullin in anaesthetized rats (Ishiyama *et al.*, 1993) and cats (Hao *et al.*, 1994). This difference is consistent with the heart rate effects of adrenomedullin in our conscious rats being reflex responses to the hypotension, and the absence of tachycardia in the experiments of Ishiyama *et al.* (1993) and Hao *et al.* (1994) being due to inhibition of cardiac baroreflexes by anaesthesia, rather than adrenomedullin having a unique profile of action (Hao *et al.*, 1994).

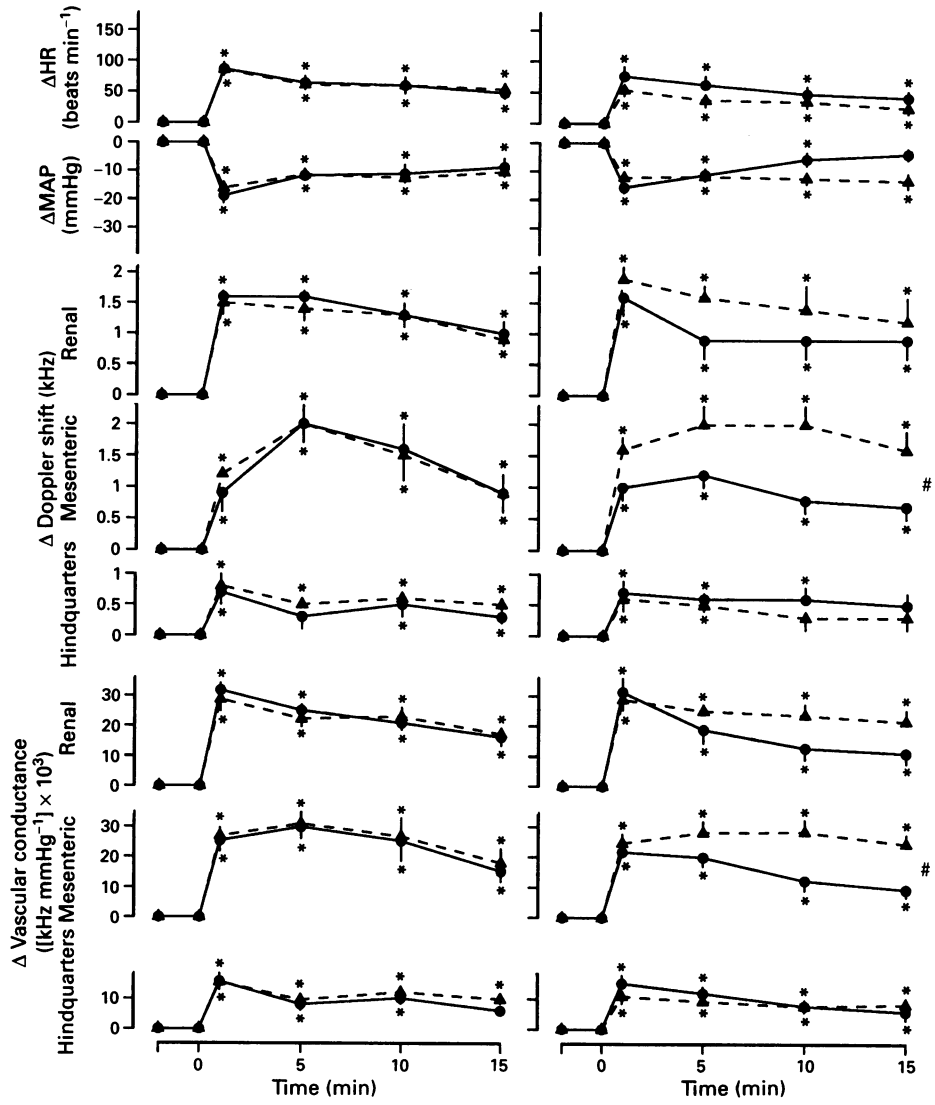

Figure 3 Cardiovascular changes following i.v. bolus injection of human adrenomedullin (1 nmol kg^{-1}). Left-hand panels, responses in the absence (●) and during infusion of saline (▲) in the same conscious, Long Evans rats ($n = 8$; Group 3). Right-hand panels, responses in the absence (●) and during infusion of human α-calcitonin gene-related peptide (α-CGRP) [8-37] (▲, 100 nmol kg^{-1} min^{-1}) in the same conscious, Long Evans rats ($n = 8$; Group 4). Values are mean ± s.e.mean; $^*P < 0.05$ versus baseline (Friedman's test), $^*P < 0.05$ for the difference between the corresponding integrated responses in the absence and presence of human α-CGRP [8-37] (Wilcoxon's test).

It has been reported that rat adrenomedullin has a more potent hypotensive action than human adrenomedullin (Sakata *et al.*, 1993; Lin *et al.*, 1994) in anaesthetized rats. However, such a difference was only apparent at the lowest dose (0.1 nmol kg^{-1}) used in the present study in conscious rats, and that difference was accompanied only by a greater mesenteric vasodilatation.

Although there is some evidence that $CGRP_1$-receptors may be involved in the mesenteric vasodilator effects of human adrenomedullin *in vitro* (Nuki *et al.*, 1993), we could find no evidence for their involvement *in vivo*. Using equimolar doses (1 nmol kg^{-1}) of human α-CGRP and human adrenomedullin, the hypotensive and tachycardic actions of the former were about twice those of the latter. Human α-CGRP caused reductions in renal and mesenteric flows, and only transient increases in renal and mesenteric vascular conductances. In contrast, human adrenomedullin caused marked and persistent increases in renal and mesenteric flows and vascular conductances. Interestingly, however, both human α-CGRP and human adrenomedullin caused increases in hindquarters flows and vascular conductances. Elsewhere, (Gardiner *et al.*, 1988; 1989a) we have suggested that the relative lack of renal and mesenteric vasodilator responses to human α-CGRP was due to activation of counter-regulatory vasoconstrictor mechanisms, such as the renin-angiotensin system (Kurtz *et al.*, 1988). However, since the hypotensive effect of the highest dose of human adrenomedullin (3 nmol kg^{-1}) was similar to that of human α-CGRP at 1 nmol kg^{-1}, it appears that any activation of endogenous vasoconstrictor mechanisms was not able to mask the vasodilator actions of human adrenomedullin. These differences, alone, argue against the latter peptide interacting, directly or indirectly, with $CGRP_1$-receptors, a proposal which is supported by the finding that human α-CGRP [8-37] caused clear inhibition of the haemodynamic effects of human α-CGRP, but not those of human adrenomedullin. It is notable that the inhibition of the hypotensive action of human α-CGRP by human α-CGRP [8-37] was accompanied by clear suppression of its renal and mesenteric vasoconstrictor effects, consistent with these being secondary to the usual hypotension (see above).

Rather than inhibiting the haemodynamic effects of human

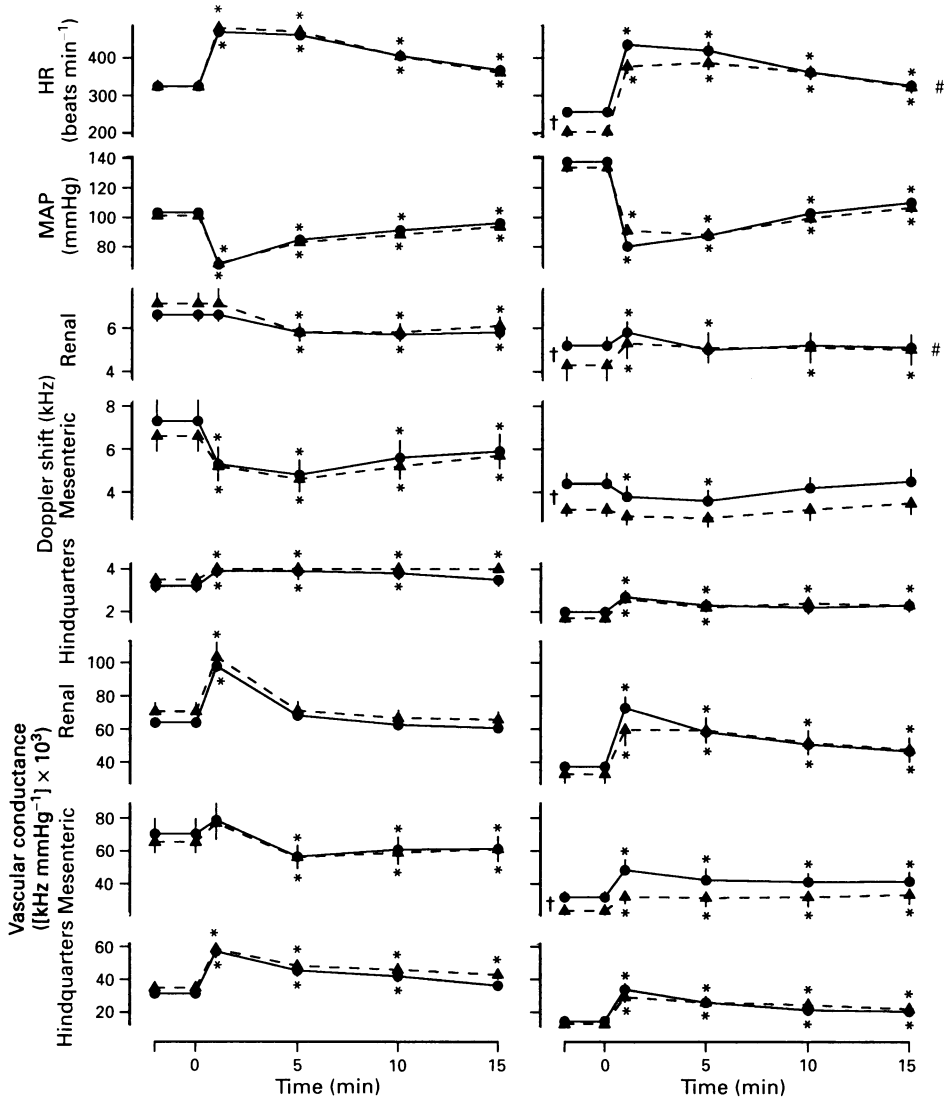

Figure 4 Cardiovascular changes following i.v. bolus injection of human α-calcitonin gene-related peptide (α-CGRP) (1 nmol kg^{-1}) before (left-hand panels, ●) and during infusion of N^G-nitro-L-arginine methyl ester (L-NAME) (right-hand panels, ●) in the same conscious, Long Evans rats ($n = 8$; Group 5), or before (left-hand panels, ▲) and during infusion of vasopressin plus angiotensin II (right-hand panels, ▲) in a separate group of animals ($n = 8$; Group 6). Values are mean ± s.e.mean; $^*P < 0.05$ versus baseline (Friedman's test); †$P < 0.05$ for difference between resting values (Mann-Whitney U test); $^\#P < 0.05$ for difference between integrated responses (Mann-Whitney U test) in the presence of L-NAME or vasopressin plus angiotensin II.

adrenomedullin, the $CGRP_1$-receptor antagonist enhanced its mesenteric haemodynamic action, and tended to do the same in the renal vascular bed. One possibility is that antagonism of the action of endogenous CGRP by human α-CGRP [8-37] sensitizes the mesenteric (and renal) vascular beds to human adrenomedullin. Although speculative, this suggestion is consistent with the finding that human adrenomedullin stimulates cyclic AMP formation in rat vascular smooth muscle cells, and this action is not inhibited by human α-CGRP [8-37] (Ishizaka *et al.*, 1994).

A difference between human α-CGRP and human adrenomedullin was also apparent when the possibility of the involvement of NO was investigated by comparing responses to each peptide in the presence of L-NAME or vasopressin plus angiotensin II to simulate, as far as possible, the haemodynamic effects of the former. Thus, the hindquarters hyperaemic vasodilator effect of human adrenomedullin was only slightly, but significantly, less in the presence of L-NAME than in the presence of vasopressin plus angiotensin II, indicating a small contribution from NO to this response. Such a difference was not apparent for human α-CGRP, although a slow infusion, rather than bolus injection, of the latter peptide appears to activate NO-mediated hindquarters vasodilatation (Gardiner *et al.*, 1991). While it is clear that any involvement of NO in the responses to human adrenomedullin was very marginal, this is an important observation, since it would be reasonable to assume otherwise, considering the marked and widespread vasodilator profile of human adrenomedullin.

Overall, then, human and rat adrenomedullin are potent vasodilator peptides that, unlike human α-CGRP, cause marked increases in renal and mesenteric flows and vascular conductances, as well as hindquarters hyperaemic vasodilatation. While all the haemodynamic effects of human α-CGRP are inhibited by human α-CGRP [8-37], none of the effects of human adrenomedullin are, at least in conscious rats. Contrary to the findings of Nuki *et al.* (1993) these results indicate that the actions of adrenomedullin do not involve $CGRP_1$-receptors, consistent with the recent findings of Ishizaka *et al.* (1994).

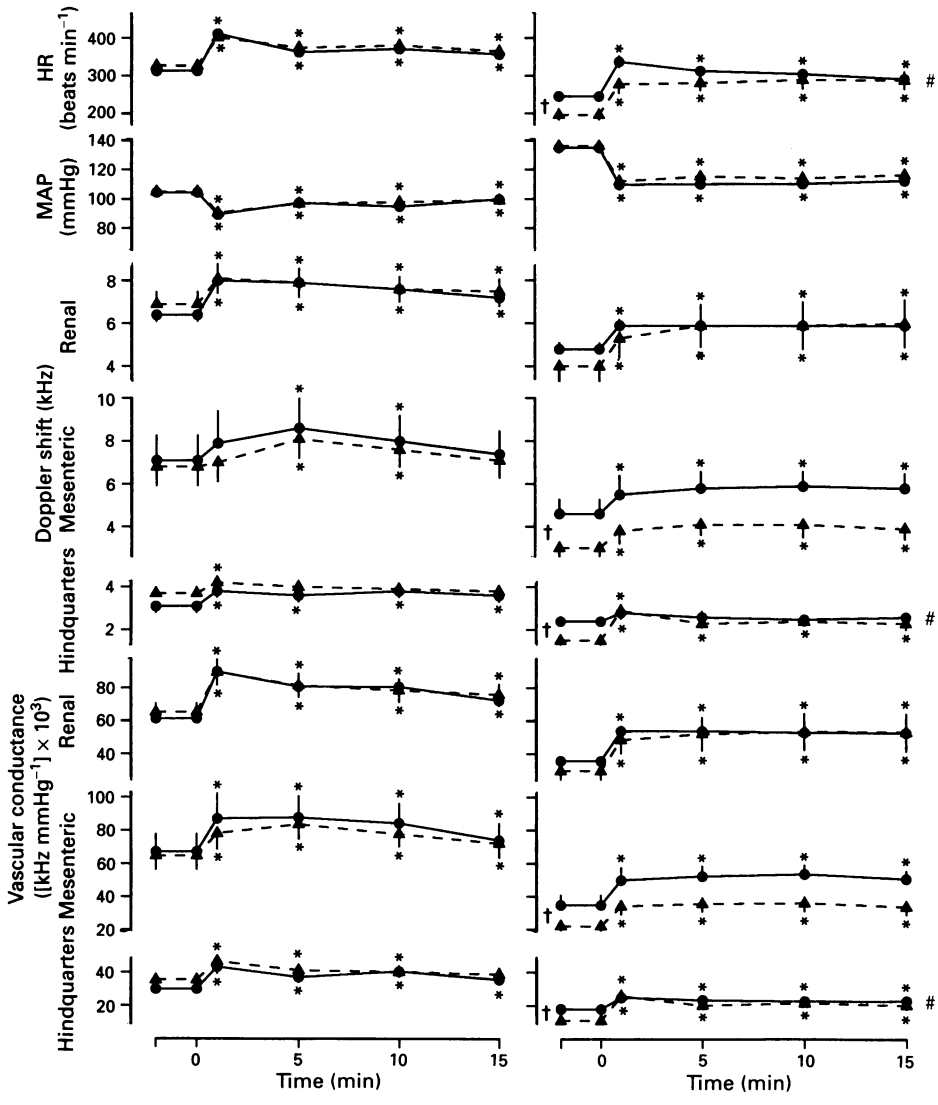

Figure 5 Cardiovascular changes following i.v. bolus injection of human adrenomedullin (1 nmol kg^{-1}) before (left-hand panels, ●) and during infusion of N^G-nitro-L-arginine methyl ester (L-NAME) (right-hand panels, ●) in the same conscious, Long Evans rats ($n = 8$; Group 5), or before (left-hand panels, ▲) and during infusion of vasopressin plus angiotensin II (right-hand panels, ▲) in a separate group of animals ($n = 8$; Group 6). Values are mean ± s.e.mean; $^*P < 0.05$ versus baseline (Friedman's test); $\dagger P < 0.05$ for difference between resting values (Mann-Whitney U test); $^\#P < 0.05$ for difference between corresponding integrated responses (Mann-Whitney U test) in the presence of L-NAME or vasopressin plus angiotensin II.

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