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Regional haemodynamic effects of depressor neuropeptides in conscious, unrestrained, Long Evans and Brattleboro rats

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1 The regional haemodynamic effects of i.v. bolus doses of atrial natriuretic peptide (ANP, 1 and 10 nmol), calcitonin gene-related peptide (CGRP, 0.05 and 0.5 nmol) and corticotropin-releasing factor (CRF, 1 and 5 nmol) were assessed in conscious Long Evans and Brattleboro rats chronically instrumented with miniaturized, pulsed Doppler probes.

2 The low dose of ANP was without effect on mean arterial pressure (MAP), but caused tachycardia and hindquarters vasodilatation with vasoconstriction in renal and mesenteric beds in Long Evans rats. With the high doses of ANP these effects were more pronounced and MAP fell. In Brattleboro rats there was a primary renal vasodilatation.

3 The low dose of CGRP caused a slight fall in MAP in Long Evans rats, tachycardia and a renal vasodilatation. The high dose of CGRP caused marked hypotension, tachycardia and renal, mesenteric and hindquarters vasodilatation in both strains of rat. However, only in Long Evans rats were there secondary renal and mesenteric vasoconstrictions.

4 The low dose of CRF caused falls in MAP in both strains of rat, accompanied by renal and, particularly, mesenteric vasodilatation. Administration of the high dose of CRF caused profound, prolonged hypotension, tachycardia and mesenteric vasodilatation. There was also (late onset) hindquarters vasodilatation accompanying renal vasoconstriction that followed the initial vasodilatation in this vascular bed.

5 These results indicate that appropriate doses of particular peptides may be capable of promoting flow through individual peripheral vascular beds.

Introduction

The putative roles of neuropeptides in cardiovascular regulation are almost as numerous as the neuropeptides themselves, in spite of the fact that there is little information about the cardiovascular actions of these substances *in vivo*. Moreover, notwithstanding the paucity of observations regarding their physiological roles, several peptides (and their analogues) have been proposed as likely candidates for the pharmacological manipulation of various cardiovascular disease states (e.g. MacCannell *et al.*, 1982). In the field of hypertension, for example, it is theoretically possible that depressor neuropeptides might be clinically useful. However, it is important to be aware that similar degrees of blood pressure reduction achieved with administration of appropriate doses of different hypotensive neuropeptides or their analogues could be associated with quite different regional haemodynamic profiles. Furthermore, it is likely that the latter could impinge on the overall clinical efficacy of the intervention. For example, if an agent caused reduction in systemic arterial blood pressure but this was accompanied by a potent renal vasoconstriction, such a manoeuvre would be particularly inappropriate in the presence of impaired renal function. With these sorts of considerations in mind, we have investigated the regional haemodynamic changes, in conscious, intact, normotensive rats, following administration of intravenous bolus injections of three neuropeptides. We elected to investigate α-rat atrial natriuretic peptide (ANP),

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rat calcitonin gene-related peptide (CGRP) and rat corticotropin-releasing factor (CRF) because others have found all these peptides may cause hypotension under certain conditions (see Discussion). However, we also addressed the question of whether or not a particular peptide might exert regionally selective effects on vascular resistance at a dose that had relatively little effect on systemic arterial blood pressure. Furthermore, in order to assess a possible role of vasopressin release in modifying the regional haemodynamic effects of depressor neuropeptides, we carried out comparative studies in Long Evans (i.e. normal) and Brattleboro (i.e. vasopressin-deficient) rats. Finally, we also examined the regional haemodynamic changes following a depressor dose of sodium nitroprusside (SNP) as an example of a non-neuropeptide, directly-acting vasodilator agent. Some of the results have been reported to the Physiological Society (Bennett *et al.*, 1988a,b,c).

Methods

Male, Long Evans (340–400 g, $n = 9$) and weight-matched Brattleboro rats ($n = 8$) were used. These animals were bred in Nottingham from stock originally obtained from Charing Cross Medical School. Prior to operation animals were housed 4/cage and given water and food (Labsure 41b) *ad libitum*.

Regional haemodynamic measurements were made with chronically-implanted, miniaturized pulsed Doppler probes (Haywood *et al.*, 1981). The subassembly (Crystal Biotech, Holliston, MA, U.S.A.) was built into a custom-fashioned probe suitable for implantation around the left renal or superior mesenteric artery or the abdominal aorta (below the level of the ileocaecal artery). Three probes were implanted into each animal under general anaesthesia (sodium methohexitone, Brietal, Lilly, Basingstoke; 60 mg kg^{-1}, i.p., supplemented as required). Through a mid-line laparotomy, the blood vessels were carefully separated from adjacent adipose and connective tissues, leaving paravascular nerve bundles intact. The appropriate probes were then sutured around the blood vessels and filled with ultrasonic coupling gel (Aquasonde, London). The probe wires were anchored to the body wall in positions that prevented the probes from shifting, thus occluding the vessels. They were then led through a small incision in the left flank and tunnelled subcutaneously to exit at the back of the neck. The wires were there soldered into a microconnector (Microtech Inc., Boothwyn, PA, U.S.A.) that was held in a harness worn by the rat. After operation, animals were given ampicillin trihydrate (Penbritin, Beecham, Animal Health, Brentford; 7 mg kg^{-1}, i.m.) and housed singly in their usual home cages with free access to food and water.

At least 7 days after this operation, animals that were eating, drinking and grooming normally, gaining weight and with acceptable signals from all 3 probes, were briefly re-anaesthetized (sodium methohexitone, 40 mg kg^{-1}, i.p.) and had one catheter implanted in the abdominal aorta (via the caudal artery) for recording blood pressure and 4 catheters implanted in the right jugular vein for administering substances. Catheters were led subcutaneously and exteriorised at the same site as the probe wires. Animals were left with free access to food and water until the next day before experiments were begun. Throughout the experiment, continuous recordings were made with a Gould (Cleveland, OH, U.S.A.) ES 1000 recorder, biotach amplifier and SP 400A preamplifier connected to a pulsed Doppler flowmeter (Iowa, Bioengineering Unit U.S.A.). Recordings were of heart rate (HR), phasic and mean intra-arterial blood pressure (MAP) and the Doppler shift signals from renal, mesenteric and hindquarters probes. The phasic signals were used to monitor the quality of the recording, whereas measurements were made from the electronically-derived mean signals. Although the pulsed Doppler system does not provide absolute values for volume flow there is good evidence that the Doppler shift signal is a reliable index of this variable (Haywood *et al.*, 1981; Wright *et al.*, 1987), and is referred to as flow in the text. Dividing MAP by mean Doppler shift gives an estimate of resistance from which % changes (relative to baseline) can be calculated (Haywood *et al.*, 1981).

All experiments started and finished with an assessment of the effects of administering vehicle injections (0.1 ml isotonic saline containing 1% bovine serum albumin). Thereafter ANP (1 and 10 nmol), CGRP (0.05 and 0.5 nmol) or CRF (1 and 5 nmol), dissolved in vehicle, were given in random order, although low doses were always given before high doses due to the persistence of some of the effects of the latter (see Results) and because of the likelihood that there would be unknown amounts of peptide in the catheter system after administration of the high dose. Injections of low doses of peptides were separated by at least 60 min; the intervals separating the high doses were sufficient to allow variables to return to baseline (up to 2 h). After the peptides had been administered, a bolus dose of SNP (38 nmol) was given.

Peptides Rat ANP (1–28), rat CRGP and rat CRF were obtained from Bachem (Torrance, CA, U.S.A.) with h.p.l.c. analysis showing >99% purity.

Statistical analysis Data were subject to two-way, non-parametric analysis of variance (Friedman's test). One-off, between-group comparisons were made by the Mann-Whitney U or Wilcoxon rank

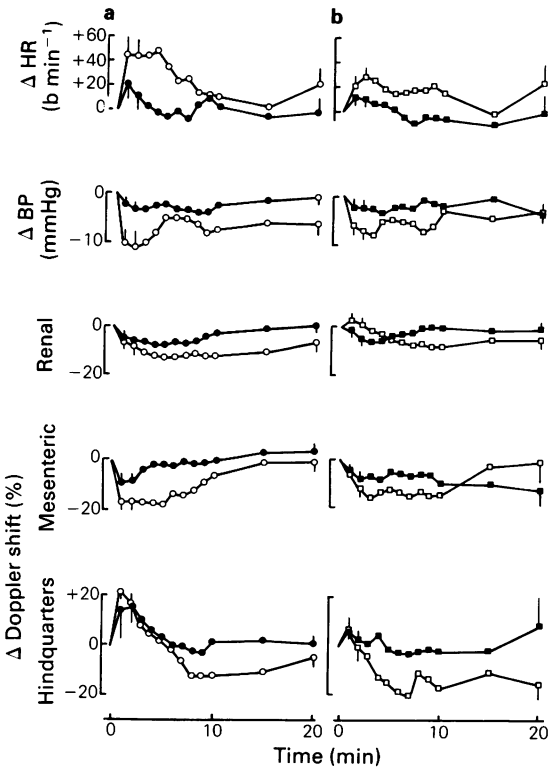

Figure 1 Group mean data for the cardiovascular responses to atrial natriuretic peptide (ANP; ●, ■ = 1 nmol; ○, □ = 10 nmol) in Long Evans (a; $n = 9$) and Brattleboro (b; $n = 8$) rats. ANP was administered at time = 0; only the s.e.means corresponding to peak effect and terminal status are given for clarity and the statistics are given in the text for the same reason.

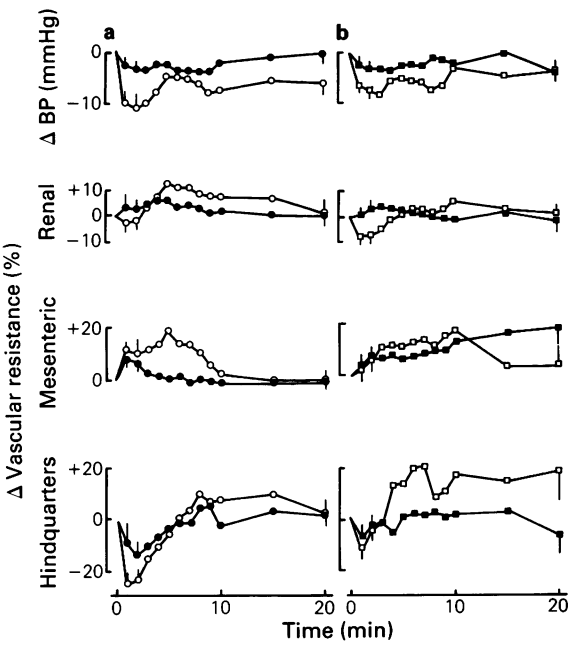

Figure 2 Changes in regional vascular resistances, in response to atrial natriuretic peptide (ANP; ●, ■ = 1 nmol; ○, □ = 10 nmol), derived from the data shown in Figure 2 (the MAP data from Figure 2 are included for reference). (a) Long Evans rats ($n = 9$); (b) Brattleboro rats ($n = 8$). ANP was administered at time = 0. Only the s.e.means corresponding to peak effect and terminal status are given for clarity and the statistics are given in the text for the same reason.

sum tests as appropriate. In the text the word significant signifies $P < 0.05$ (at least).

Results

Control injections of vehicle had no significant cardiovascular effects.

Effects of atrial natriuretic peptide (Figures 1–3)

The lower dose of ANP in Long Evans rats caused a significant tachycardia at 1 min, but this was transient, and there was no significant change in MAP (Figure 1). However, renal flow was decreased significantly between 1–10 min, and there was a reduction in mesenteric flow 1–3 min after ANP. Over the latter period, there was an increase in hindquarters flow (Figure 1). The calculated resistance changes showed significant renal vasoconstriction over 3–8 min, mesenteric vasoconstriction at 1 min only, and hindquarters vasodilatation over 1–4 min (Figure 2).

The higher dose of ANP in Long Evans rats caused significant, prolonged tachycardia (1–7 min) and hypotension (1–20 min, Figure 1), accompanied by instability of MAP (Figure 3). There were overall reductions in renal (1–20 min) and mesenteric (1–10 min) flow and an initial increase in hindquarters flow (1–3 min), followed by a decrease (7–15 min, Figure 1). Calculated vascular resistance changes showed there were significant renal and mesenteric vasoconstrictions (5–7 min and 1–8 min, respectively) and a hindquarters vasodilatation (1–4 min) (Figure 2). All these effects were more pronounced with the higher than with the lower dose of ANP, although there tended to be an early renal hyperaemia with the former before a significant vasoconstriction occurred (Figure 3).

In Brattleboro rats, the low dose of ANP caused no significant change in MAP, but there was a modest tachycardia (at 1 min only, Figure 1).

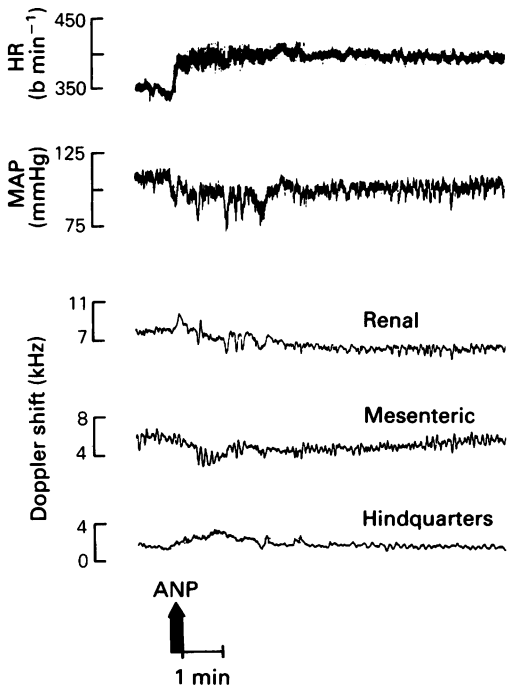

Figure 3 Cardiovascular responses to atrial natriuretic peptide (ANP 10 nmol i.v. bolus) in a conscious, Long Evans rat. ANP caused a slight fall in arterial pressure and a tachycardia. There was a transient increase, followed by a decrease in renal flow; mesenteric flow was also reduced, but hindquarters flow increased.

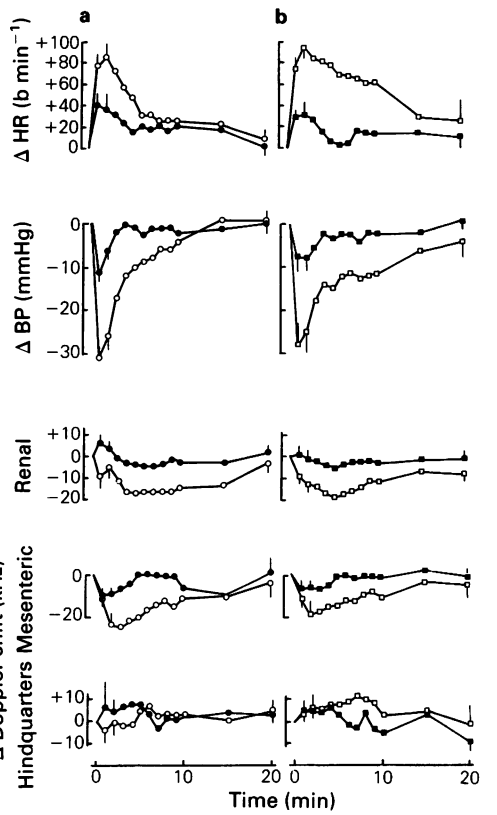

Figure 4 Group mean data for the cardiovascular responses to calcitonin gene-related peptide (CGRP; ●, ■ = 0.05 nmol; ○, □ = 0.5 nmol) in Long Evans (a; $n = 9$) and Brattleboro (b; $n = 8$) rats. CGRP was administered at time = 0; only the s.e.means corresponding to peak effect and terminal status are given for clarity and the statistics are given in the text for the same reason.

Although both renal (2–6 min) and mesenteric (2–4, 7, 9–20 min) flow was significantly reduced, there was no significant change in hindquarters flow (Figure 1). Moreover, none of the vascular beds showed any significant changes in resistance (Figure 2).

The higher dose of ANP in Brattleboro rats caused significant increases in HR (1–4 min, 7–10 min, 20 min) and decreases in MAP (1–9, 15–20 min; Figure 1). There were reductions in renal (5–20 min), mesenteric (1–10 min) and hindquarters (4–20 min) flow (Figure 1). These changes were associated with an early renal vasodilatation (1–3 min) followed by a late renal vasoconstriction (10–20 min) (Figure 2). The hindquarters vascular bed also showed a biphasic change with dilatation at 1 min and constriction later (4–7, 9–10 min, Figure 2). In the mesenteric vascular bed, there was constriction only (4–10 min, Figure 2).

Effects of calcitonin gene-related peptide (Figures 4–6)

In Long Evans rats the lower dose of CGRP caused a significant tachycardia (1–15 min) and a transient hypotension (1–2 min, Figure 4). There were no significant changes in group mean renal or hindquarters flow (but see Figure 5); mesenteric flow was reduced (1–4, 10, 15 min) (Figure 4). However, only the fall in renal vascular resistance was significant (1–2 min, Figure 6).

The higher dose of CGRP in Long Evans rats caused marked tachycardia (1–15 min) and hypotension (1–10 min) accompanied by reductions in renal (3–15 min) and mesenteric (1–7, 9 min) flows but no change in hindquarters flow (Figure 4). All 3 vascular beds showed early dilatation (renal 1–2 min; mesenteric 1 min; hindquarters 1–2 min), with vasoconstriction occurring later in renal (5–15 min) and mesenteric (4–7 and 15 min) circulations (Figure 6).

In Brattleboro rats, the lower dose of CGRP caused an increase in HR (1–3, 8–10 and 15 min), but

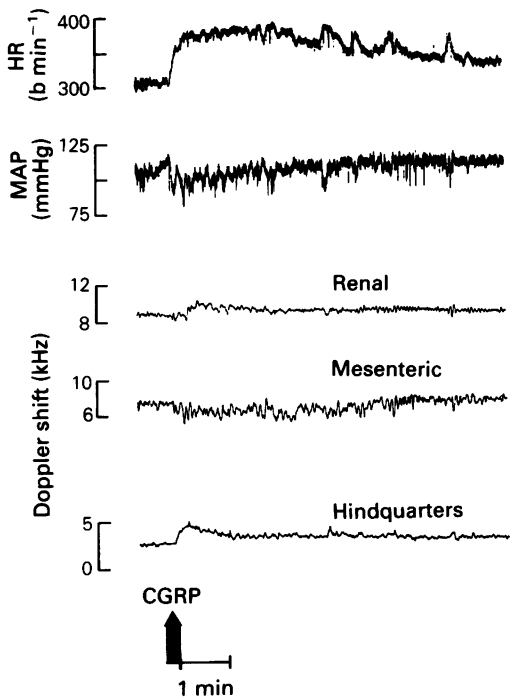

Figure 5 Cardiovascular responses to calcitonin gene-related peptide (CGRP; 0.05 nmol i.v. bolus) in a conscious, Long Evans rat. In this animal, the slight hypotension and marked tachycardia were accompanied by increases in renal and hindquarters flow, but a decrease in mesenteric flow.

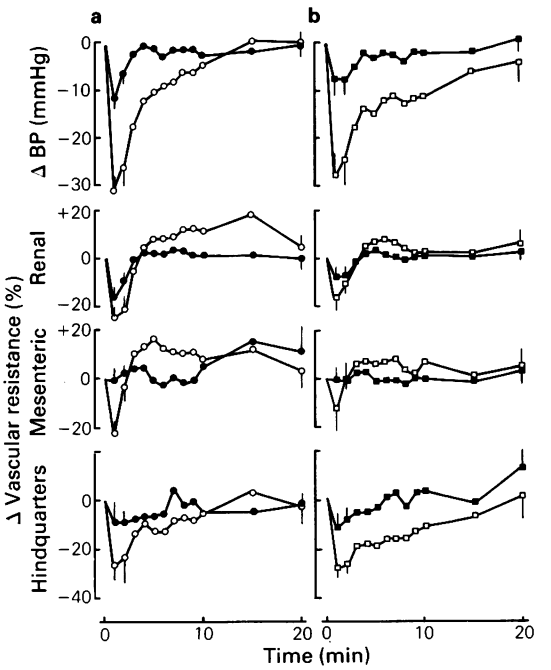

Figure 6 Changes in vascular resistances, in response to calcitonin gene-related peptide (CGRP; ●, ■ = 0.05 nmol; ○, □ = 0.5 nmol), derived from the data shown in Figure 4 (the MAP data from Figure 4 are included for reference): in (a) Long Evans rats and in (b) Brattleboro rats. Only the s.e.means corresponding to peak effect and terminal status are given for clarity and the statistics are given in the text for the same reason.

no significant changes in MAP, regional flow or vascular resistance (Figures 4 and 6). The higher dose of CGRP caused substantial tachycardia (1–15 min) and hypotension (1–10 min). Flow was reduced in the renal (1–10 min) and mesenteric (2–7 and 10 min) vascular beds, but not significantly changed in the hindquarters (Figure 4). There was early vasodilatation in the renal (1–2 min) and hindquarters (1–6 min) circulation but no significant change in mesenteric vascular resistance, and no subsequent vasoconstriction (Figure 6).

Effects of corticotropin-releasing factor (Figures 7–9)

The lower dose of CRF caused a small, but sustained, fall in MAP (1–8 min), but no change in HR in Long Evans rats (Figure 7). There was a delayed fall in renal flow (4–20 min) and an increase in mesenteric flow (1–7 and 10 min) but no change in flow through the hindquarters (Figure 7). Renal vascular resistance showed an initial fall (1–2 min) followed by a rise (9–20 min), whereas there was a mesenteric vasodilatation only (1–7 min) and no change in hindquarters vascular resistance (Figure 8).

Long Evans rats responded to the higher dose of CRF with a sustained tachycardia (1–20 min) and hypotension (1–20 min) (Figure 7). There was a progressive fall in renal flow (2–20 min), a marked increase in mesenteric flow (1–6 min) and a reduction (1–4 min) followed by an increase (15–20 min) in hindquarters flow (Figures 7 and 9). These changes were associated with a transient renal vasodilatation (1 min) followed by a sustained vasoconstriction (3–20 min), a prolonged mesenteric vasodilatation (1–15 min) and a delayed hindquarters vasodilatation (6–20 min) (Figure 8).

In Brattleboro rats, the lower dose of CRF caused a tachycardia (1–2 and 5–20 min) and hypotension (1, 7–8 and 15 min, Figure 7). There was a late fall in renal flow (5–20 min) and an early, transient increase in mesenteric flow (1 min) (Figure 7). The apparent change in hindquarters flow seen in Figure 7 was not significant. Vascular resistances fell transiently in the renal (1 min) and mesenteric (1–2 min) vascular beds,

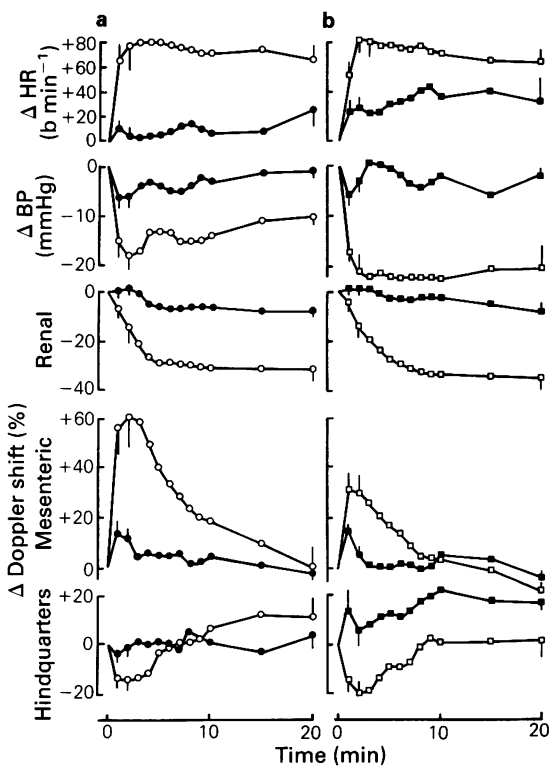

Figure 7 Group mean data for the cardiovascular responses to corticotropin-releasing factor (CRF; ●, ■ = 1 nmol; ○, □ = 5 nmol) in Long Evans (a; $n = 9$) and Brattleboro (b; $n = 8$) rats. CRF was administered at time = 0; only the s.e.means corresponding to peak effect and terminal status are given for clarity and the statistics are given in the text for the same reason.

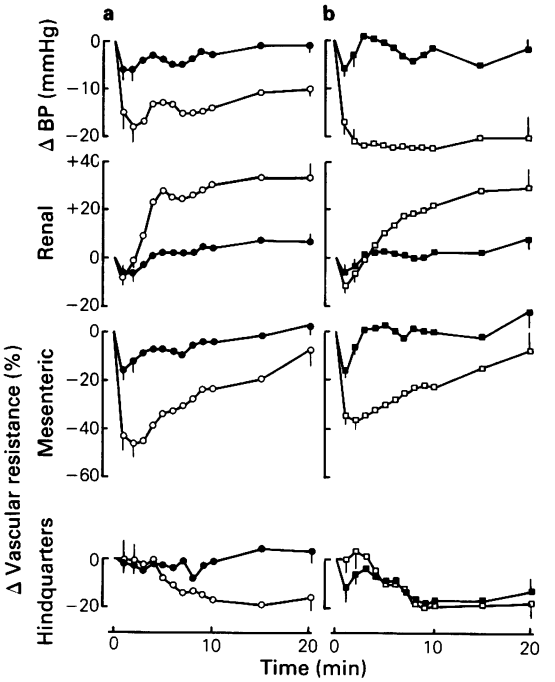

Figure 8 Changes in vascular resistances, in response to corticotropin-releasing factor (CRF; ●, ■ = 1 nmol; ○, □ = 5 nmol), derived from the data shown in Figure 7 (the MAP data from Figure 7 are included for reference): in (a) Long Evans rats and in (b) Brattleboro rats. Only the s.e.means corresponding to peak effect and terminal status are given for clarity and the statistics are given in the text for the same reason.

but the vasodilatation in the hindquarters vascular bed (Figure 8) just failed to reach significance ($P = 0.06$).

The higher dose of CRF caused sustained increases in HR (1–20 min) and decreases in MAP (1–20 min), associated with a progressive reduction in renal flow (2–20 min), an increase in mesenteric flow (1–6 min) and a decrease in hindquarters flow (1–7 min) (Figure 7). In the renal vascular bed, there was an initial dilatation (1 min) followed by a prolonged constriction (5–20 min), but there was only dilatation in the mesenteric and hindquarters vascular beds (Figure 8). In the former, this occurred rapidly (1–15 min), but in the latter it was delayed (6–20 min).

Effects of sodium nitroprusside (Figures 10–12)

The bolus dose of SNP caused significant, although transient, hypotension (0.5 min) and tachycardia (0.5–1 min) in Long Evans rats (Figure 10). Thereafter, there was an overshoot in MAP and an associated bradycardia (both significant 3–5 min). Renal flow showed a rapid, significant decrease (0.5 min) that was transient but followed by a secondary fall (3–5 min, Figure 10). Both mesenteric (particularly, Figure 11), and hindquarters flow showed a tendency to an initial increase immediately following SNP. Thereafter, there was a reduction in mesenteric (1–3 min) and hindquarters (1 min) flow (Figures 10 and 11). The changes in flow were associated with significant dilatation in all 3 vascular beds (at 0.5 min) followed by vasoconstriction in the renal (2–5 min), mesenteric (1–5 min) and hindquarters (2–3 min) beds (Figure 12).

In Brattleboro rats, SNP caused an initial fall in MAP (at 0.5 min) and increase in HR (0.5–1 min) (Figure 10). Subsequently, there was an overshoot in MAP (2–4 min) but no significant bradycardia. Renal flow showed a decrease (at 0.5 min) followed by an increase (at 1 min) with a later, secondary fall (4–5 min, Figure 10). There was a persistent (0.5 min–

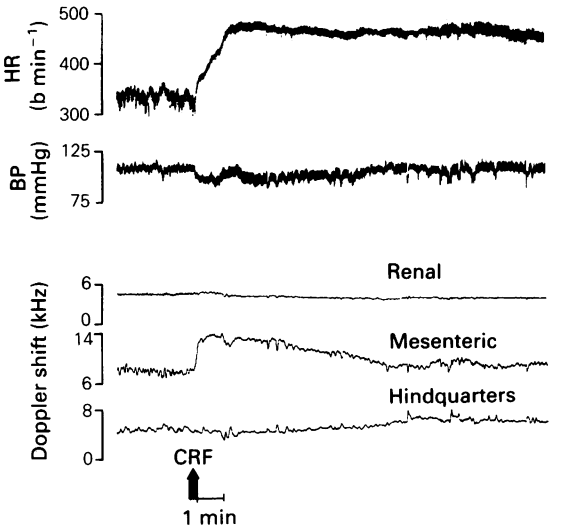

Figure 9 Cardiovascular responses to corticotropin releasing factor (CRF; 5 nmol i.v. bolus) in a conscious, Long Evans rat. In this animal, the fall in MAP was only slight, but there was a marked tachycardia. The striking increase in mesenteric flow was accompanied by an initial fall in hindquarters flow followed by a late increase. Renal flow showed a progressive reduction.

5 min) fall in mesenteric flow, but only a transient reduction (at 0.5 min) in hindquarters flow (Figure 10). The associated changes in regional resistance indicated renal vasodilatation (at 0.5 min–1 min) followed by constriction (at 4–5 min); mesenteric vasodilatation (at 0.5 min), but constriction thereafter (1–5 min); and hindquarters vasodilatation (at 0.5 min) followed by constriction (1–3 min) (Figure 12).

Discussion

The experiments we carried out were not designed, primarily, to delineate any putative physiological roles of the peptides we investigated but, rather, to define the *in vivo* profiles of effect of these substances on cardiovascular function, particularly with regard to regional haemodynamics. It is clear that the results obtained may have been influenced by various neural and hormonal mechanisms, especially in those instances where MAP fell. However, these circumstances are immediately relevant to any proposed therapeutic application of the peptides or their analogues.

The present results are particularly remarkable in three respects. Firstly, they demonstrate that, in con-

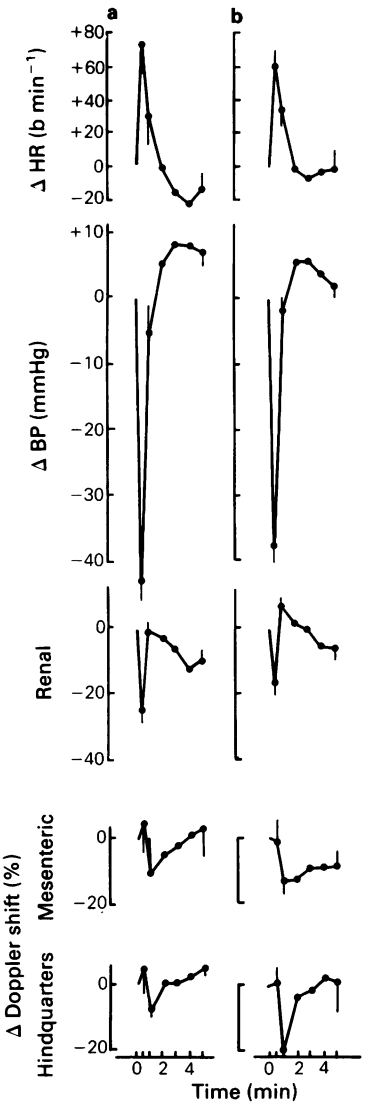

Figure 10 Group mean data for the cardiovascular responses to sodium nitroprusside (SNP; 38 nmol i.v. bolus) in Long Evans (a; $n = 9$) and Brattleboro (b; $n = 8$) rats. SNP was administered at time = 0; only the s.e.means corresponding to peak effect and terminal status are given for clarity and the statistics are given in the text for the same reason.

scious unrestrained rats, ANP, CGRP, CRF and SNP may have substantial (direct or indirect) effects on regional haemodynamics when MAP may be little different from baseline values. Secondly, they show clearly that each depressor agent may have a characteristic, differential profile of effects (direct or

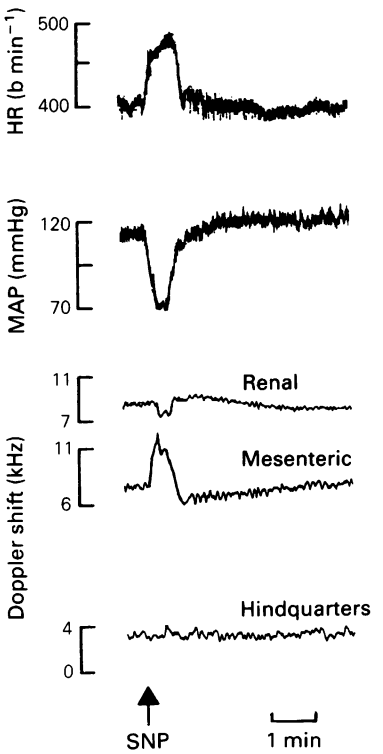

Figure 11 Cardiovascular responses to sodium nitroprusside (SNP; 38 nmol i.v. bolus) in a conscious, Long Evans rat. In this animal, the transient hypotension and tachycardia were accompanied by an initial, brief decrease in renal and an increase in mesenteric flow; thereafter flow in both these beds showed a more sustained decrease. Hindquarters flow was unchanged.

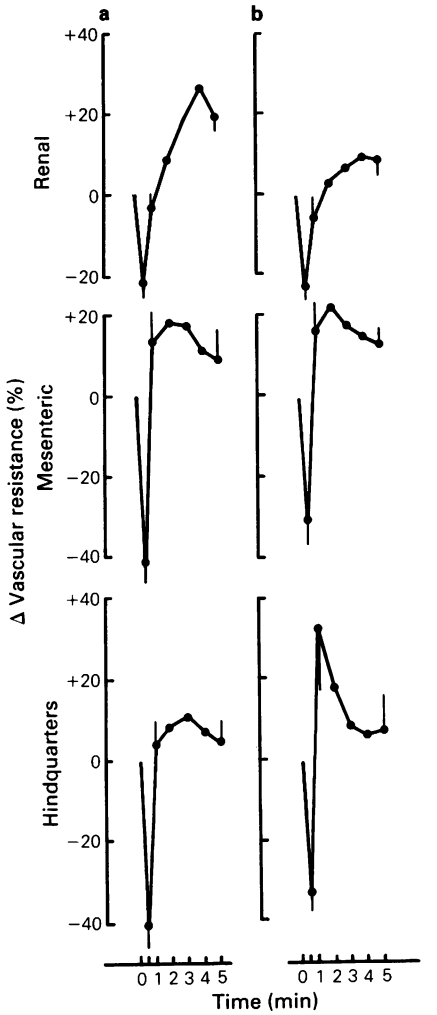

Figure 12 Changes in vascular resistance, in response to sodium nitroprusside (SNP; 38 nmol), derived from the data shown in Figure 10 (the MAP data from Figure 10 are included for reference). Only the s.e.means corresponding to peak effect and terminal status are given for clarity and the statistics are given in the text for the same reason.

indirect) on regional haemodynamics. Finally, they illustrate some interesting differences between Long Evans and Brattleboro rats that are not explained simply by the absence of the cardiovascular actions of vasopressin in the latter. We shall discuss the results by reference to each of the vasoactive agents we used.

Effects of atrial natriuretic peptide

The lower dose of ANP was without significant hypotensive effect in either strain. However, in Long Evans rats there were renal and mesenteric vasoconstrictions and hindquarters vasodilatation. Since, in none of these vascular beds, were flows constant when resistances changed, then the latter were clearly not autoregulatory. The most likely explanation of the results is that the lower dose of ANP had a potent, primary, hindquarters vasodilator effect and the resulting tendency for MAP to fall elicited secondary mesenteric and renal vasoconstrictions (Lappe *et al.*, 1985b). The renal response seemed to play a more important homeostatic role than the mesenteric response, since it was more persistent, although later in onset. While increased efferent vasomotor outflow is the most likely candidate as the mediator of these changes, it is possible that the reduction in renal flow, together with increased renal sympathetic activity, could have activated the renin-

angiotensin system. But this has to be considered against the background of a possible inhibitory effect of ANP on renin release (e.g. Burnett *et al.*, 1984; Scheuer *et al.*, 1987) and on angiotensin II-mediated vasoconstriction (Kleinert *et al.*, 1984). Evidence that angiotension II may not have been involved in the secondary increases in vascular resistances comes from the observation that this peptide has potent mesenteric vasoconstrictor effects in Long Evans rats (Bennett & Gardiner, 1986) but the change in mesenteric vascular resistance following the low dose of ANP was small. It is also unlikely that vasopressin release contributed to the haemodynamic profile seen in Long Evans rats since this would have been expected to exert preferential mesenteric constrictor effects (Bennett & Gardiner, 1986; Bennett *et al.*, 1987), but numerically there was no difference between mesenteric vascular resistance changes in Long Evans and Brattleboro rats. In the latter, the relative lack of effect of the low doses of ANP is interesting in the light of the supposed high renin status of these animals (Kinter *et al.*, 1982), but is consistent with our previous findings that the hypotensive effect of captopril was no greater in Brattleboro than in Long Evans rats (Gardiner & Bennett, 1985).

Many of the previous studies on the regional haemodynamic effects of ANP are difficult to interpret because they were carried out in anaesthetized animals (i.e. with activated renin-angiotensin systems). Those that were not (e.g. Lappe *et al.*, 1985a,b; 1986; Smits *et al.*, 1986) do not always provide information about normal animals. However, while the consensus view is that ANP may decrease cardiac output and thereby lower MAP, there has been no previous demonstration of ANP-induced hindquarters vasodilatation unaccompanied by hypotension.

The relative insensitivity of the overall haemodynamic status of Brattleboro rats to the lower dose of ANP could have been due, simply, to the lesser primary hindquarters vasodilator effect of this peptide in that strain. This proposition is supported by the findings with the higher dose of ANP. This caused a marked hindquarters vasodilatation in Long Evans rats, but only a slight, brief fall in hindquarters resistance in Brattleboro rats. Indeed, the latter showed a later, presumably indirect, hindquarters vasoconstriction, whereas the former did not. However, it is notable that the higher dose of ANP caused renal vasodilatation in Brattleboro, but not in Long Evans rats, probably indicating an interplay between different degrees of direct vasodilator and indirect vasoconstrictor influences (Lappe *et al.*, 1985b) in these different vascular beds.

There were no obvious differences in the mesenteric vascular responses in Long Evans and Brattleboro rats that could have been attributed to the absence of vasopressin in the latter, although it is possible that vasopressin release was inhibited by ANP administration (e.g. Standaert *et al.*, 1987). But it is quite clear that the similar profiles of MAP in the two strains were associated with different pictures of change in renal and in hindquarters vascular resistances. In spite of some evidence that ANP may influence baroreflex mechanisms (Thorén *et al.*, 1986), the changes in HR relative to MAP were not different, in proportional terms, to those seen with the other peptides. Furthermore, while changes in cardiac output may have contributed to the later effects of ANP (see above) it is notable that the profile of MAP at that stage, following the higher dose, reflected the regional vascular resistance changes.

In summary, it appears that bolus doses of ANP may cause particularly marked primary vasodilatation in the hindquarters of Long Evans rats and in the renal vasculature of Brattleboro rats.

Effects of calcitonin gene-related peptide

The lower dose of CGRP was slightly hypotensive in Long Evans rats, due, at least in part, to a significant renal vasdilator effect in this strain. Since renal flow tended to increase above baseline, the change in vascular resistance must have been active rather than autoregulatory. A similar tendency (although not statistically significant) was seen in the renal vascular bed in Brattleboro rats, and in the hindquarters bed in both strains, but not in the mesenteric vasculature. Thus, our results showing a relatively selective renal vasodilator effect of a low dose of CGRP are at variance with those in spontaneously hypertensive rats (Lappe *et al.*, 1987b) and in normotensive Sprague-Dawley rats (DiPette *et al.*, 1987). However, the study of DiPette *et al.* (1987) was carried out with microspheres, and this technique is not capable of detecting the subtle, transient, changes we describe here. Since there were no other significant changes in regional haemodynamics with the low dose of CGRP in Long Evans rats, it is feasible that the cardiac effects of this peptide (Satoh *et al.*, 1986; Sigrist *et al.*, 1986; Marshall *et al.*, 1986b; Lappe *et al.*, 1987a) contributed to MAP recovery such that the transient fall in the latter was insufficient to trigger mechanisms directed to other vascular beds. However, it is also possible that the lack of significant change in mesenteric and hindquarters resistance represented a balance between direct vasodilator effects of CGRP and indirect constrictor influences triggered by the hypotension. The latter proposal is consistent with the finding that the higher dose of CGRP caused marked hypotension

and initial vasodilatation in all three vascular beds in Long Evans rats. Significant falls in resistance occurred only in renal and hindquarters vascular beds in Brattleboro rats, but it is noteworthy that, in this strain, there was prolonged hindquarters vasodilatation and no late constriction in mesenteric or renal vascular beds. In Long Evans rats these latter two vascular beds did show secondary increases in resistance, and the hypotension was less prolonged and tachycardia less persistent than in Brattleboro rats.

Since the initial vasodilator effects of the higher dose of CGRP were no greater in Brattleboro than in Long Evans rats, it is not likely that the differences were due to a more persistent primary dilator effect of the peptide in the former strain. One possible explanation for our findings is that the large fall in MAP with the higher dose of CGRP triggered vasopressin release in Long Evans rats and this was contributing to the recovery in their MAP through mesenteric and renal vasoconstriction. The difference in profiles of vascular resistance change in Brattleboro rats receiving the higher dose of ANP (i.e. late vasoconstrictions in all 3 vascular beds) and the higher dose of CGRP (no secondary vasoconstrictions) could have been due to more potent generalized vasodilator actions of CGRP, offsetting any secondary vasoconstrictor mechanisms, or to CGRP inhibiting neural and/or renin-angiotensin-mediated mechanisms in Brattleboro rats.

In summary, it appears that CGRP may exert a primary renal vasodilator effect at low doses, particularly in Long Evans rats. At higher doses it does not appear to exert a selective dilator effect on the mesenteric vascular bed (Marshall *et al.*, 1986a), but its effects may be particularly marked in the hindquarters.

Effects of corticotropin-releasing factor

The initial hypotensive effect of the lower dose of CRF was associated with an active mesenteric vasodilatation. A similar phenomenon has been reported in anaesthetized dogs given CRF i.v. (MacCannell *et al.*, 1982). In those experiments intra-arterial administrations of CRF into coeliac, femoral, carotid and renal beds were without effects on flow, whereas administration of CRF into the mesenteric artery increased flow by about 80% (with a dose of 0.02 pmol). Even with doses of CRF an order of magnitude greater than we used, MacCannell *et al.* (1982) saw little change in HR, MAP or cardiac output, and the marked fall in total peripheral resistance they found was attributed to mesenteric vasodilatation alone. (They did not consider the possibility that flow through other vascular beds may have been diminished following administration of CRF). However, with the low dose of CRF, we also observed renal vasodilatation, although we cannot exclude the possibility this was autoregulatory, since there was no change in renal flow, initially. The later reduction in renal flow in Long Evans rats was associated with an increase in resistance that was probably secondary to the hypotension. The lack of a significant increase in renal resistance in Brattleboro rats may explain the more persistent hypotension in this strain, but the different profiles of HR change in the two strains indicate that cardiac contributions may not have been the same.

The higher dose of CRF caused marked, persistent hypotension in both strains of rat, accompanied by striking mesenteric vasodilatation that was prolonged, unlike the initial renal vasodilatation that gave way to secondary constrictions of large and similar magnitude in Long Evans and Brattleboro rats. In the latter strain, this phenomenon was in striking contrast to the lack of a secondary renal vasoconstriction following the higher dose of CGRP (that caused a similar initial fall in MAP). This is in line with the suggestion, made above, that CGRP might have interfered with compensatory neural and/or renin-angiotensin-mediated mechanisms, and is consistent with the finding that CRF (albeit at an extremely high dose in anaesthetized monkeys) stimulates renin release (Udelsman *et al.*, 1986a).

In both Long Evans and Brattleboro rats, there was a delayed vasodilatation in the hindquarters following the higher dose of CRF. In the former strain, this was associated with a significant hyperaemia, so it was clearly an active dilatation. Udelsman *et al.* (1986a) administered a bolus i.v. dose of 70 nmol of CRF to anaesthetized cynomolgus monkeys and observed marked hypotension, tachycardia and mesenteric and iliac vasodilatation. However, these workers did not consider the possibility that the reductions in vascular resistances in the two beds may have been mediated by different factors. From the present results, we hypothesize that the mesenteric vasodilatation was primary (direct and/or indirect, e.g. through endothelial factors; Dashwood *et al.*, 1987), and the hindquarters vasodilatation was secondary to adrenal medullary catecholamine release. Indeed, Udelsman *et al.* (1986b) have, themselves, demonstrated the presence of functional CRF receptors on adrenal medullary chromaffin tissue that are coupled to adenylate cyclase and stimulate catecholamine release. Furthermore, MacLean & Ungar (1986) have shown that CRF may be involved in catecholamine release from the adrenal gland of the dog. Elsewhere (Gardiner & Bennett, 1988a,b), we have shown that the hindquarters vascular bed in conscious Long Evans and Brattleboro rats is partic-

ularly sensitive to β_2-adrenoceptor-mediated vasodilator effects that may be markedly influenced by adrenal medullary activity. Because of the continuing active vasodilatation in mesenteric and hindquarter beds following CRF, it is feasible that any constrictor action of vasopressin would have been masked, giving rise to a similarity in haemodynamic profile in Long Evans and Brattleboro rats.

In summary, CRF exerts primary mesenteric and secondary hindquarters dilator effects. Our hypothesis would predict that the hindquarters vasodilatation and, possibly, a component of the tachycardia following administration of a high dose of CRF should be inhibited by β_2- and β_1-adrenoceptor antagonism respectively. Interestingly, Udelsman *et al.* (1986a) showed that their CRF-induced tachycardia was antagonized by propranolol, but, unfortunately, they made no measurements of vascular resistances under these conditions.

Effects of sodium nitroprusside

The vasodilator properties of the peptides considered above may be dependent or independent of the endothelium, and this may vary in different segments of the circulation (e.g. McGrath *et al.*, 1988). SNP-induced vasorelaxation is independent of endothelial function, causing direct increases in intracellular cyclic GMP levels (Murad *et al.*, 1987). The bolus dose of SNP caused initial vasodilatations in all 3 vascular beds in both strains of rat. In this respect, there was a similarity with the initial effects of the high dose of CGRP. However, unlike the picture seen with the latter, secondary vasoconstriction occurred in all 3 vascular beds in both strains of rat following SNP. The differences between the profiles in regional haemodynamics following CGRP and SNP was particularly striking in Brattleboro rats, consistent with our proposal that the CGRP might interfere with neural and/or renin-angiotensin-mediated vasoconstrictor mechanisms, possibly by a prolonged action at the endothelium and/or vascular smooth muscle. One min after SNP administration, MAP was at control levels in both strains of rat, but regional haemodynamic status was not, indicating the value of these measurements in assessing drug action. It is of interest that, in both Long Evans and Brattleboro rats, MAP overshot following the rapid hypotension caused by SNP. This may have been due to the evanescence of the latter's action, relative to those of the peptides studied, and/or because SNP does not interfere with recruitment of vasoconstrictor mechanisms.

In summary, SNP caused particularly potent mesenteric and hindquarters vasodilatation; the secondary vasoconstriction in all 3 vascular beds in Long Evans and Brattleboro rats did not differ in a way that indicated a substantial contribution from the vasoconstrictor action of vasopressin in Long Evans rats.

In conclusion, we have shown important regional differences in the haemodynamic effects of doses of peptides that have little influence on MAP or change it to similar extents. The present results raise the intriguing possibility that flow through a particular vascular bed could be increased by intravenous infusion of an appropriately selected peptide at a concentration that had insufficient effect on systemic haemodynamics to cause secondary vasoconstriction in other vascular beds.

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References

BENNETT, T., COMPTON, A.M. & GARDINER, S.M. (1987). Differential regional vascular responses to vasopressin (AVP) and neuropeptide Y (NPY) in conscious, Long Evans rats. *J. Physiol.*, **390**, 188P.

BENNETT, T., COMPTON, A.M. & GARDINER, S.M. (1988a). Changing haemodynamic profile during a sustained hypotensive response to i.v. administration of corticotropin-releasing factor in conscious rats. *J. Physiol.*, **399**, 38P.

BENNETT, T., COMPTON, A.M. & GARDINER, S.M. (1988b). Differential regional haemodynamic effects of depressor doses of rat calcitonin gene-related peptide (CGRP), rat corticotropin-releasing factor (CRF) and α-rat atrial natriuretic peptide (ANP) in conscious, Long Evans rats. *J. Physiol.*, **401**, 87P.

BENNETT, T., COMPTON, A.M. & GARDINER, S.M. (1988c). Regional haemodynamic changes following administration of sodium nitroprusside (SNP) to conscious, Long Evans and Brattleboro rats. *J. Physiol.*, **401**, 88P.

BENNETT, T. & GARDINER, S.M. (1986). Differential regional vascular effects of pressor agents in conscious Long Evans rats. *J. Physiol.*, **377**, 61P.

BURNETT, J.C., GRANGER, J.P. & OPGENORTH, T.J. (1984). Effects of synthetic atrial natriuretic factor on renal function and renin release. *Am. J. Physiol.*, **247**, F863–F866.

DASHWOOD, M.R., ANDREWS, H.E. & WEI, E.T. (1987). Binding of [I^{125}] Tyr-corticotropin-releasing factor to rabbit aorta is reduced by removal of the endothelium. *Eur. J. Pharmacol.*, **135**, 111–112.

DIPETTE, D.J., SCHWARZENBERGER, K., KERR, N. & HOLLAND, O.B. (1987). Systemic and regional hemodynamic effects of calcitonin gene-related peptide. *Hypertension*, **9** (suppl. III), III-142–III-146.

GARDINER, S.M. & BENNETT, T. (1985). Interactions between neural mechanisms, the renin-angiotensin system and vasopressin in the maintenance of blood pressure during water deprivation: studies in Long Evans and Brattleboro rats. *Clin. Sci.*, **68**, 647–657.

GARDINER, S.M. & BENNETT, T. (1988a). Involvement of β_2-adrenoceptor-mediated mechanisms in the cardiovascular responses to α_1- and α_2-adrenoceptor antagonism in conscious, unrestrained, Long Evans and Brattleboro rats. *Br. J. Pharmacol.*, **93**, 9–22.

GARDINER, S.M. & BENNETT, T. (1988b). Regional haemodynamic responses to adrenoceptor antagonism in conscious rats. *Am. J. Physiol.*, (in press).

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

KINTER, L.B., SCHRIER, D., FLAMENBAUM, W. & BEEUWKES, R. (1982). The renin angiotensin system in conscious Brattleboro strain rats. *Renal Physiol.*, **5**, 278–285.

KLEINERT, H.D., MAACK, T., ATLAS, S.D., JANUSZIEWICZ, A., SEALEY, J.E. & LARAGH, J.H. (1984). Atrial natriuretic factor inhibits angiotensin-, norepinephrine-, and potassium-induced vascular contractility. *Hypertension*, **6** (suppl. I), I-143–I-147.

LAPPE, R.W., SLIVJAK, M.J., TODT, J.A. & WENDT, R.L. (1987a). Hemodynamic effects of calcitonin gene-related peptide in conscious rats. *Regul. Peptides*, **19**, 307–312.

LAPPE, R.W., SMITS, J.F.M., TODT, J.A., DEBETS, J.J.M. & WENDT, R.L. (1985a). Failure of atriopeptin II to cause arterial vasodilation in the conscious rat. *Circ. Res.*, **56**, 606–612.

LAPPE, R.W., TODT, J.A. & WENDT, R.L. (1985b). Mechanism of action of vasoconstrictor responses to atriopeptin II in conscious SHR. *Am. J. Physiol.*, **249**, R781–R786.

LAPPE, R.W., TODT, J.A. & WENDT, R.L. (1986). Hemodynamic effects of infusion versus bolus administration of atrial natriuretic factor. *Hypertension*, **8**, 866–873.

LAPPE, R.W., TODT, J.A. & WENDT, R.L. (1987b). Regional vasodilator actions of calcitonin gene-related peptide in conscious SHR. *Peptides*, **8**, 747–749.

MACCANNELL, K.L., LEDERIS, K., HAMILTON, P.L. & RIVIER, J. (1982). Amunine (ovine CRF), urotensin I and sauvagine, three structurally related peptides, produce selective dilation of the mesenteric circulation. *Pharmacology*, **25**, 116–120.

MCGRATH, C.S., LENANSKI, C.R., CRAIG, R.K. & MARSHALL, I. (1988). Two vascular mechanisms of action for the human α-calcitonin gene-related peptide (hαCGRP). *Br. J. Pharmacol.*, **93**, 69P.

MACLEAN, M.R. & UNGAR, A. (1986). Effects of the renin-angiotensin system on the reflex response of the adrenal medulla to hypotension in the dog. *J. Physiol.*, **373**, 343–352.

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

MARSHALL, I., AL-KAZWINI, S.J., ROBERTS, P.M., SHEPPERSON, N.B., ADAMS, M. & CRAIG, R.K. (1986b). Cardiovascular effects of human and rat CGRP compared in the rat and other species. *Eur. J. Pharmacol.*, **123**, 207–216.

MURAD, F., LEITMAN, D.C., BENNETT, B.M., MOLMA, C. & WALDMAN, S.A. (1987). Regulation of guanylate cyclase by atrial natriuretic factor and the role of cyclic GMP in vasodilation. *Am. J. Med. Sci.*, **294**, 139–143.

SATOH, M., OKU, R., MAEDA, A., FUJII, N., OTAKA, A., FUNAKOSHI, S., YAJIMA, H. & TAKAGI, H. (1986). Possible mechanisms of positive inotropic action of synthetic human calcitonin gene-related peptide in isolated rat atrium. *Peptides*, **7**, 631–635.

SCHEUER, D.A., THRASHER, T.N., QUILLEN, E.W., METZLER, C.H. & RAMSAY, D.J. (1987). Atrial natriuretic peptide blocks renin response to renal hypotension. *Am. J. Physiol.*, **251**, R423–R427.

SIGRIST, S., FRANCO-CERECEDA, A., MUFF, R., HENKE, H., LUNDBERG, J.M. & FISCHER, J.A. (1986). Specific receptor and cardiovascular effects of calcitonin gene-related peptide. *Endocrinology*, **119**, 381–389.

SMITS, J.F.M., VAN ESSEN, H., STRUYKER-BOUDIER, H.A.J. & LAPPE, R.W. (1986). Lack of renal vasodilation during intrarenal infusion of synthetic atriopeptin II in conscious, intact SHR. *Life Sci.*, **38**, 81–87.

STANDAERT, D.G., CECHETTO, D.F., NEEDLEMAN, P. & SAPER, C.B. (1987). Inhibition of the firing of vasopressin neurons by atriopeptin. *Nature*, **329**, 151–153.

THORÉN, P., MARK, A.L., MORGAN, D.A., O'NEILL, T.P., NEEDLEMAN, P. & BRODY, M.J. (1986). Activation of vagal depressor reflexes by atriopeptins inhibits renal sympathetic nerve activity. *Am. J. Physiol.*, **251**, H1252–H1259.

UDELSMAN, R., GALLUCCI, W.T., BACHER, J., LORIAUX, D.L. & CHROUSOS, G.P. (1986a). Hemodynamic effects of corticotropin releasing hormone in the anesthetized cynomolgus monkey. *Peptides*, **7**, 465–471.

UDELSMAN, R., HARWOOD, J.P., MILLAN, M.A., CHROUSOS, G.P., GOLDSTEIN, D.S., ZIMLICHMAN, R., CATT, K.J. & AGUILERA, G. (1986b). Functional corticotropin releasing factor receptors in the primate peripheral sympathetic nervous system. *Nature*, **319**, 147–150.

WRIGHT, C.E., ANGUS, J.A. & KORNER, P.I. (1987). Vascular amplifier properties in renovascular hypertension in conscious rabbits. Hindquarters responses to constrictor and dilator stimuli. *Hypertension*, **9**, 122–131.

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