Mechanisms contributing to the regional haemodynamic effects of neurotensin in conscious, unrestrained Long Evans rats

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1 The regional haemodynamic effects of i.v. bolus doses of neurotensin (10–1000 ng) were assessed in conscious, unrestrained Long Evans rats chronically instrumented with miniaturized, pulsed Doppler probes.

2 Neurotensin caused increases in blood pressure, together with dose-related tachycardias and constrictions in the renal, superior mesenteric and hindquarters vascular beds. The tachycardia elicited by the 1000 ng dose of neurotensin was preceded by a transient bradycardia.

3 In the presence of phentolamine, the pressor effect of neurotensin (1000 ng) was converted into a hypotensive effect, accompanied by reduced tachycardic and constrictor responses in the renal, superior mesenteric and hindquarters vascular beds. The tachycardia was not preceded by a bradycardia.

4 In the presence of phentolamine and propranolol, the pressor and bradycardic responses to neurotensin were unaffected, whereas the tachycardia was abolished. The renal vasoconstrictor effect was smaller, while the constrictions in the superior mesenteric and hindquarters vascular beds were not different from those in untreated rats.

5 In rats neonatally treated with capsaicin (50 mg kg⁻¹, s.c.), the pressor effects elicited by neurotensin (300 and 1000 ng) were reduced as were the constrictor responses in the renal (at the dose of 300 ng), superior mesenteric (at the dose of 300 ng) and hindquarters (at both doses) vascular beds. The bradycardia elicited by neurotensin (1000 ng) was absent, whereas the tachycardia was potentiated.

6 The results indicate that in conscious, intact rats neurotensin appears to exert cardiovascular influences through activation of sympathoadrenal mechanisms and also through non-adrenergic effects on the heart, renal, superior mesenteric and hindquarters vascular beds. The latter effects appear to involve capsaicin-sensitive nerves.

Keywords: Blood pressure; heart rate; neurotensin; regional haemodynamics; capsaicin

Introduction

Neurotensin (NT) is a tridecapeptide, first isolated from bovine hypothalamus (Carraway & Leeman, 1973), and later found in various areas of the central nervous system, the gastrointestinal tract and in circulating blood in several animal species (Uhl & Snyder, 1976; 1981; Kobayashi *et al.*, 1977; Carraway & Leeman, 1978; Carraway & Bhatnagar, 1980; Hammer *et al.*, 1980; Manberg *et al.*, 1982; Shulkes *et al.*, 1982).

NT possesses a wide range of effects on both the central and peripheral nervous systems and on peripheral tissues (Carraway & Leeman, 1973; Bissette *et al.*, 1978; Brown & Miller, 1982). NT was found to produce contraction of some isolated blood vessels, such as the portal vein (Rioux *et al.*, 1980), coronary vasculature (Quirion *et al.*, 1979) and vessels of subcutaneous adipose tissue (Rosell & Rokaes, 1981), and to have vasoconstrictor properties in the isolated, perfused hindquarters of the rat (Rioux *et al.*, 1982). The peptide also exerts potent positive inotropic and chronotropic effects on rodent isolated, spontaneously beating atria (Quirion *et al.*, 1978; Stene-Larsen & Helle, 1979). Intravenous administration of NT was found to induce hypotension in pentobarbitone anaesthetized rats, possibly through a histamine-mediated process (Quirion *et al.*, 1980), since pretreatment with compound 48/80, a well-known mast cell degranulating agent, was found not only to block the hypotensive effect of NT, but also to unmask hypertensive effects of high doses of NT (Quirion *et al.*, 1980). Using conscious rats, Sumners *et al.* (1982) reported that i.v. administration of NT elicited a fall in blood pressure which was sometimes preceded

by a slight hypertensive effect. In pentobarbitone-anaesthetized guinea-pigs, NT was found to elicit dose-dependent pressor effects, which probably resulted from an influence of the peptide on the sympathetic nervous system, and a tachycardia, which was possibly due to a direct effect of the peptide on the sino-atrial node (Kerouac *et al.*, 1981). Recently, it has been reported that both effects were significantly reduced or abolished in guinea-pigs chronically treated with the neurotoxin, capsaicin (Bachelard *et al.*, 1987). Moreover, *in vitro* studies have shown that the positive chronotropic and inotropic effects of NT were abolished in atria taken from capsaicin-treated guinea-pigs (Bachelard *et al.*, 1987). Thus, it has been suggested that capsaicin-sensitive sensory neurones participate in the cardiovascular responses to NT in guinea-pigs (Bachelard *et al.*, 1987).

The present study was undertaken to investigate the regional haemodynamic effects produced by NT injected i.v. in conscious, unrestrained, rats, chronically instrumented with intravascular catheters and miniaturized pulsed Doppler probes to measure renal, superior mesenteric and hindquarters blood flows. The contribution of adrenoceptors to the cardiovascular responses to NT was examined by peripheral administration of α - and β -adrenoceptor antagonists. Moreover, to assess the involvement of capsaicin-sensitive sensory neurones in the cardiovascular responses to NT, some experiments were carried out in rats neonatally treated with capsaicin or with the vehicle for capsaicin.

Methods

The experiments were carried out on male Long Evans rats (350–400 g) bred in the Animal Unit in Nottingham. Details of the techniques used have been published elsewhere (Gardiner

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Table 1 Baseline values for heart rate, blood pressure and regional vascular conductance in conscious, untreated ($n = 8$), vehicle-treated (Veh-treated; $n = 9$) or capsaicin-treated (Cap-treated; $n = 9$) Long Evans rats

	Heart rate (beats min ⁻¹)	Mean blood pressure (mmHg)	Doppler shift (kHz)			Vascular conductance ([kHz mmHg ⁻¹] 10 ³)		
			Renal	Mesenteric	Hindquarters	Renal	Mesenteric	Hindquarters
Untreated	379 ± 12	101 ± 2	10.1 ± 0.9	8.3 ± 0.7	5.1 ± 0.6	99 ± 8	83 ± 7	51 ± 7
Veh-treated	361 ± 11	101 ± 3	9.3 ± 1.3	9.2 ± 1.0	6.2 ± 0.7	89 ± 11	91 ± 11	61 ± 7
Cap-treated	356 ± 11	104 ± 2	10.2 ± 0.9	8.0 ± 0.3	5.3 ± 0.8	98 ± 7	78 ± 2	51 ± 10

Values are mean ± s.e.mean.

et al., 1990a, b). Briefly, animals were anaesthetized (sodium methohexitone, 60 mg kg⁻¹ i.p. supplemented as required) and had miniaturized pulsed Doppler probes (Haywood *et al.*, 1981) implanted around the left renal artery, the superior mesenteric artery and the distal abdominal aorta (below the level of the ileocaecal artery). The probe wires were tunnelled s.c. and exteriorized at the back of the neck, where they were sutured in place. Animals were given ampicillin (7 mg kg⁻¹, i.m., Penbritin, Beechams) and left to recover for at least 7 days. Thereafter, under brief anaesthesia (sodium methohexitone 40 mg kg⁻¹, i.p.), the probes wires were soldered into a microconnector (Microtech Inc., U.S.A.) and one intraarterial (distal abdominal aorta via the caudal artery) and two i.v. (right jugular vein) catheters were implanted. The catheters ran s.c. and emerged with the pulsed Doppler probe leads. The microconnector, soldered to the latter, was clamped in a harness worn by the rat and the catheters passed through a flexible spring (for protection) which was attached to the harness. Animals were returned to their home cages with free access to food and water. Experiments were not begun until the next day.

Throughout the experiments, continuous recordings were made of instantaneous heart rate, phasic and mean blood pressures and renal, mesenteric and hindquarters Doppler shift signals (Crystal Biotech VF-1 Doppler flowmeter, Holliston, MA, U.S.A.). Changes in Doppler shift signals were calculated as percentage of the baseline level. The latter have been shown to be a reliable index of volume flow (Haywood *et al.*, 1981; Wright *et al.*, 1987) and are referred to as flows in the text. The Doppler shift and corresponding mean arterial blood pressure signals were used to calculate percentage changes in regional vascular conductances (Gardiner *et al.*, 1990b).

At different times, varying between 0.5 and 30 min after peptide administration, measurements were made of heart rate, mean blood pressure and mean Doppler shift signals, to represent the full profile of the effects of the peptide, and related to the pre-drug baseline (absolute changes for the former two variables, percentages for the Doppler shifts).

Before every experiment a 30 min baseline recording period was made. Animals had free access to water and food for the duration of the experiment. All i.v. injections were given in 100 µl isotonic saline (the dead space of the catheter). NT was dissolved in isotonic saline containing 1% bovine serum albumin (BSA).

The following experiments were performed:

Cardiovascular responses to neurotensin

Animals were used on 3 consecutive days during which they received 6 randomized i.v. injections of vehicle (0.1 ml isotonic saline containing 1% bovine serum albumin (BSA)) and NT at doses varying between 10 and 1000 ng; randomized between days, but the lower doses were always given before the higher doses on any particular day. Each animal received no more than 2 i.v. injections on the same day, separated by at least 120 min. Measurements were made before, during and for 30 min following i.v. injections of vehicle or NT. Injections were given over a period of 5 s.

Effect of pretreatment with phentolamine, or phentolamine and propranolol on responses to neurotensin

The α -adrenoceptor antagonist, phentolamine, was administered i.v. as a bolus (1 mg kg⁻¹, 0.1 ml) followed by continuous infusion of 1 mg kg⁻¹ h⁻¹ (0.3 ml h⁻¹) (Winn *et al.*, 1985) to a group of 11 rats. Fifteen min after the start of the phentolamine infusion the animals received an i.v. injection of NT (1000 ng).

The next day, in the same animals, a mixture of phentolamine (1 mg kg⁻¹, i.v. bolus, 1 mg kg⁻¹ h⁻¹, infusion) and the β -adrenoceptor antagonist, propranolol, (1 mg kg⁻¹, i.v. bolus, 0.5 mg kg⁻¹ h⁻¹, infusion) (Gardiner & Bennett, 1988) was administered and 15 min after onset of the coinfusion, NT (1000 ng) was injected i.v. Measurements were made before, during, and for 30 min following i.v. injection of NT.

Table 2 Peak cardiovascular changes following intravenous administration of saline-bovine serum albumin (saline-BSA, 1%) or increasing doses of neurotensin (NT) in conscious, untreated Long Evans rats

	Δ Heart rate (beats min ⁻¹)	Δ Mean blood pressure (mmHg)	Δ Doppler shift (%)			Δ Vascular conductance (%)		
			Renal	Mesenteric	Hindquarters	Renal	Mesenteric	Hindquarters
Saline-BSA 1%	+19 ± 6†	0 ± 1	-3 ± 2	+3 ± 4	+5 ± 5	-3 ± 1	-2 ± 3	-2 ± 4
NT 10 ng	+16 ± 6	+3 ± 2	0 ± 3	-3 ± 4	+5 ± 4	-2 ± 3	-2 ± 5	0 ± 6
NT 30 ng	+16 ± 8	+8 ± 2	-3 ± 2	+8 ± 7	-8 ± 2	-9 ± 2	-4 ± 4	-11 ± 3
NT 100 ng	+25 ± 10	+11 ± 1*	0 ± 2	-14 ± 3*	-11 ± 3	-8 ± 3	-17 ± 4*	-11 ± 4
NT 300 ng	+43 ± 20*	+14 ± 2*	-6 ± 2	-41 ± 5*	-28 ± 7*	-15 ± 2*	-45 ± 4*	-35 ± 6*
NT 1000 ng	-57 ± 24*	+15 ± 2*	-24 ± 4*	-52 ± 6*	-59 ± 5*	-25 ± 4*	-55 ± 4*	-62 ± 4*
	+99 ± 12*							

Values are mean ± s.e.mean; $n = 8$, at least (some injections were given in the same animals).

* $P < 0.05$ versus control injection, analysis of variance followed by Fisher's test.

† $P < 0.05$ versus baseline, paired Student's t test for control injection of saline-BSA (1%).

With NT 1000 ng a biphasic effect was observed on heart rate; the first number indicates the initial change and below is the later change.

Cardiovascular responses to neurotensin in rats neonatally treated with capsaicin

At least 48 h after birth, Long Evans rats were anaesthetized with 1.5–2% halothane (in oxygen) and injected s.c. with either vehicle (10% ethanol, 10% Tween 80 in isotonic saline) or capsaicin (50 mg kg^{-1}) in a volume of $100 \mu\text{l}$. After weaning (day 21–30 after birth) only male rats were selected for the experiments and housed in groups of 4 or 5 with free access to food and tap water.

About 20 weeks later, two groups of rats (vehicle-treated, $n = 9$ and capsaicin-treated, $n = 9$), previously instrumented with pulsed Doppler probes and intravascular catheters (as earlier described), were used on 3 consecutive days during which they received i.v. injections of vehicle (0.1 ml isotonic saline containing 1% BSA) and NT (300 and 1000 ng), in random order. Measurements were made before, during and for 30 min following i.v. injections of vehicle or NT.

Drugs

Neurotensin (Bachem), phentolamine mesylate (Ciba), propranolol hydrochloride (Imperial Chemical Industries) and 8-methyl-N-vanillyl-6-nonenamide (capsaicin; Sigma) were used.

Statistical analysis

Values are expressed as the mean $\pm$ s.e.mean; n is the number of animals. The paired Student's t test was used to analyse the effects of control injection of saline-BSA, while comparisons between groups were made by an analysis of variance followed by Fisher's test. A P value < 0.05 was taken to indicate a significant difference.

Results

The baseline values for cardiovascular variables are given in Table 1.

Haemodynamic responses to neurotensin

Control injections caused a slight tachycardia (significant at 1 min), but had no significant effect on the other measured or calculated variables (Table 2). Table 2 shows that the lowest doses of NT tested (10 and 30 ng) did not have effects significantly different from those of control injections. However, with the 100 ng dose of NT there was a slight increase in blood pressure (significant at 0.5 min) accompanied by falls in superior mesenteric flow (significant at 1–1.5 min) and vascular conductance (significant at 1–2 min); these effects were significantly different from those following control injections. There were no changes in heart rate or renal or hindquarters flows or vascular conductances.

NT at a dose of 300 ng produced a slight increase in blood pressure (significant at 0.5 and 1.5–5 min) and a tachycardia (significant at 4–10 min). There were long-lasting falls in superior mesenteric (significant at 1–30 min) and hindquarters flows (significant at 3–30 min), but no significant change in renal flow. These responses were associated with constrictions in renal (significant at 2–5 min), superior mesenteric (significant at 1–30 min) and hindquarters (significant at 2–30 min) vascular beds (Table 2).

The highest dose of NT (1000 ng) caused a slight increase in blood pressure (significant at 2–5 min) accompanied by a transient bradycardia (significant at 1 min) followed by a tachycardia (significant at 3–30 min), and reductions in renal (significant at 4–30 min), superior mesenteric (significant at 1–

Figure 1 Cardiovascular responses to i.v. bolus injections of neurotensin (1000 ng) in conscious, unrestrained, Long Evans rats, in the absence, (●) or in the presence (○) of i.v. treatment with phentolamine (1 mg kg^{-1} , i.v. bolus, $1 \text{ mg kg}^{-1} \text{ h}^{-1}$, infusion). Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ for changes in untreated group ($n = 15$) versus changes in treated group ($n = 11$) (analysis of variance followed by Fisher's test). † $P < 0.05$ versus pre-drug baseline (Student's t test for paired data). HR = heart rate; BP = blood pressure.

30 min) and hindquarters flows (significant at 0.5–30 min) (Figure 1 and Table 2). Hence, there were constrictions in renal (significant at 2–30 min), superior mesenteric (significant at 1–30 min) and hindquarters (significant at 0.5–30 min) vascular beds (Figure 2 and Table 2).

Haemodynamic responses to neurotensin (1000 ng) in the presence of phentolamine

Fifteen min after the onset of pretreatment with phentolamine there was no change in blood pressure, but there were persistent increases in heart rate, and hindquarters flow and reductions in renal and superior mesenteric flows. There were

Figure 2 Changes in regional vascular conductances elicited by i.v. bolus injections of neurotensin (1000 ng) in conscious, unrestrained, Long Evans rats, in the absence (●) or in the presence (○) of i.v. treatment with phentolamine (1 mg kg^{-1} , i.v. bolus, $1 \text{ mg kg}^{-1} \text{ h}^{-1}$, infusion). These data were derived from those shown in Figure 1. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ for changes in untreated group ($n = 15$) versus changes in treated group ($n = 11$) (analysis of variance followed by Fisher's test). † $P < 0.05$ versus pre-drug baseline (Student's *t* test for paired data).

reductions in renal and superior mesenteric vascular conductances and an increase in hindquarters vascular conductance (all significant, $P < 0.05$) (Figures 1 and 2).

In the presence of phentolamine, NT elicited a decrease in blood pressure (significant at 0.5–1.5 min), rather than an increase as observed in untreated rats, and a tachycardia (significant at 4–10 min), which was significantly less than in untreated animals. The reduction in renal flow observed in untreated rats was absent. There was an initial increase in superior mesenteric flow (significant at 0.5 min) followed by a reduction (significant at 1–15 min); the latter was significantly smaller than that in untreated animals. There was a decrease in hindquarters flow (significant at 0.5 and 3–15 min) that was also significantly less than in untreated rats. There were initial dilatations in the renal (significant at 0.5–1.5 min) and superior mesenteric vascular beds (significant at 0.5 min) followed by constrictions in both vascular beds (renal, significant at 4–5 min; superior mesenteric, significant at 1.5–15 min) (Figure

2). Both vasodilator responses were significantly different from those seen in untreated rats, while the constrictor responses in both vascular beds were significantly smaller than in untreated animals. The constriction in the hindquarters vascular bed (significant at 3–15 min) was significantly less than in untreated rats.

Haemodynamic responses to neurotensin (1000 ng) in the presence of phentolamine and propranolol

Fifteen min after the start of pretreatment with phentolamine and propranolol there were no significant changes in blood pressure, heart rate or renal flow or vascular conductance, but there was a persistent decrease in superior mesenteric flow and an increase in hindquarters flow, together with a mesenteric vasoconstriction and hindquarters vasodilatation (all significant, $P < 0.05$) (Figures 3 and 4).

Figure 3 Cardiovascular responses to i.v. bolus injections of neurotensin (1000 ng) in conscious, unrestrained, Long Evans rats, in the absence (●) or in the presence (○) of i.v. treatment with phentolamine (1 mg kg^{-1} , i.v. bolus, $1 \text{ mg kg}^{-1} \text{ h}^{-1}$, infusion) and propranolol (1 mg kg^{-1} , i.v. bolus, $0.5 \text{ mg kg}^{-1} \text{ h}^{-1}$, infusion). Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ for changes in untreated group ($n = 15$) versus changes in treated group ($n = 11$) (analysis of variance followed by Fisher's test). † $P < 0.05$ versus pre-drug baseline (Student's t test for paired data). HR = heart rate; BP = blood pressure.

In the presence of phentolamine and propranolol, NT caused a slight decrease in blood pressure (significant at 0.5 min) followed by a modest increase (significant at 2–5 min); these changes were not significantly different from those in untreated rats (Figure 3). However, there was a persistent bradycardia (significant at 1–3 min) (Figure 3) and hence, the tachycardia observed in untreated animals was absent. There were decreases in renal (significant at 10 min) and hindquarters flows (significant at 0.5–30 min), while superior mesenteric flow initially showed an increase (significant at 0.5 min) followed by a decrease (significant at 1–30 min) (Figure 3); these effects were not different from those in untreated rats. However, the early dilatations in renal (significant at 0.5–1 min) and superior mesenteric vascular beds (significant at 0.5 min) were different from those in untreated rats (Figure 4). Furthermore, the subsequent renal vasoconstriction (significant at 3–5 min) was significantly less than in untreated animals, while the constriction in the superior mesenteric vascular bed (significant at 1–30 min) was not different (Figure 4). There was a constriction in the hindquarters vascular bed (significant at 0.5–30 min), which was not different from that in untreated rats (Figure 4).

Haemodynamic effects of neurotensin (300 and 1000 ng) in rats neonatally treated with capsaicin

The baseline values for cardiovascular variables in vehicle- and capsaicin-treated rats are given in Table 1. The resting values in vehicle- and capsaicin-treated rats were not different from those in untreated rats. Control injection (saline-BSA 1%) in vehicle-treated rats had no effect on blood pressure, but caused a slight increase in heart rate (significant at 0.5 min) and hindquarters flow (significant at 0.5 min), and vascular conductance (significant at 0.5 min) (Table 3). There was no change in renal or superior mesenteric flows or vascular conductances (Table 3). In capsaicin-treated rats, control injections had no effect on blood pressure, heart rate or renal or superior mesenteric flows, but caused an increase in hindquarters flow (significant at 0.5 min) (Table 3). However, there were no changes in renal, superior mesenteric or hindquarters vascular conductances (Table 3). The cardiovascular responses to control injections in capsaicin-treated rats were not different from those in vehicle-treated rats.

In vehicle-treated rats, NT (300 ng) caused a slight increase in blood pressure (significant at 0.5–5 min) and a tachycardia (significant at 0.5, 2 and 30 min) accompanied by decreases in superior mesenteric (significant at 1–10 min) and hindquarters flows (significant at 3–5 min), but no change in renal flow (Figure 5). Hence, there were constrictions in renal (significant at 2–4 min), superior mesenteric (significant at 1–5 min) and hindquarters vascular beds (significant at 3–5 min) (Figure 6).

Table 3 Peak cardiovascular changes following intravenous administration of saline-bovine serum albumin (saline-BSA 1%) (Control) or neurotensin (NT) in vehicle- (Veh) or capsaicin-treated (Cap) Long Evans rats

	Δ Heart rate (beats min^{-1})	Δ Mean blood pressure (mmHg)	Δ Doppler shift (%)			Δ Vascular conductance (%)		
			Renal	Mesenteric	Hindquarters	Renal	Mesenteric	Hindquarters
Control (Veh)	$+22 \pm 10^\dagger$	0 ± 1	0 ± 1	$+4 \pm 3$	$+18 \pm 6^\dagger$	$+1 \pm 2$	$+2 \pm 3$	$+23 \pm 9^\dagger$
NT 300 ng (Veh)	$+111 \pm 12^*$	$+15 \pm 2^*$	0 ± 2	$-37 \pm 8^*$	$-19 \pm 4^*$	$-13 \pm 2^*$	$-42 \pm 8^*$	$-25 \pm 4^*$
NT 1000 ng (Veh)	$-72 \pm 25^*$ $+78 \pm 16^*$	$+20 \pm 2^*$	-3 ± 4	$+33 \pm 9^*$ $-48 \pm 3^*$	$-62 \pm 4^*$	$-18 \pm 4^*$	$+31 \pm 11^*$ $-56 \pm 6^*$	$-67 \pm 3^*$
Control (Cap)	$+2 \pm 3$	-3 ± 1	$+2 \pm 1$	0 ± 2	$+17 \pm 7^\dagger$	$+3 \pm 1$	$+1 \pm 2$	$+4 \pm 4$
NT 300 ng (Cap)	$+81 \pm 16^*$	$+8 \pm 2^*$	-4 ± 2	$-20 \pm 3^*$	-7 ± 6	-4 ± 2	$-25 \pm 3^*$	$-10 \pm 7^*$
NT 1000 ng (Cap)	$+149 \pm 14^*$	$+7 \pm 2^*$	$-28 \pm 4^*$	$+43 \pm 8^*$ $-50 \pm 4^*$	$-49 \pm 3^*$	$-28 \pm 4^*$	$+42 \pm 7^*$ $-50 \pm 3^*$	$-51 \pm 3^*$

Values are mean $\pm$ s.e.mean; $n = 9$.

* $P < 0.05$ versus their respective controls, analysis of variance followed by Fisher's test.

† $P < 0.05$ versus baseline, paired Student's t test for control injections in vehicle- and capsaicin-treated rats.

With NT 1000 ng biphasic effects were observed on heart rate (vehicle-treated rats), superior mesenteric flow and vascular conductance; the first number indicates the initial change and below is the later change.

Figure 4 Changes in regional vascular conductances elicited by i.v. bolus injections of neurotensin (1000 ng) in conscious, unrestrained, Long Evans rats, in the absence (●) or in the presence (○) of i.v. treatment with phentolamine (1 mg kg^{-1} , i.v. bolus, $1 \text{ mg kg}^{-1} \text{ h}^{-1}$, infusion) and propranolol (1 mg kg^{-1} , i.v. bolus, $0.5 \text{ mg kg}^{-1} \text{ h}^{-1}$, infusion). These data were derived from those shown in Figure 3. Values are mean with s.e. mean shown by vertical lines. * $P < 0.05$ for changes in untreated group ($n = 15$) versus changes in treated group ($n = 11$) (analysis of variance followed by Fisher's test). † $P < 0.05$ versus pre-drug baseline (Student's t test for paired data).

In vehicle-treated rats, the higher dose of NT (1000 ng) produced an increase in blood pressure (significant at 1–10 min) and a bradycardia (significant at 1 min) followed by a tachycardia (significant at 3–30 min) (Figure 7). There was no significant change in renal flow, while there was an initial increase in superior mesenteric flow (significant at 0.5 min) followed by a decrease (significant at 1–30 min); there was a long-lasting decrease in hindquarters flow (significant at 0.5–30 min) (Figure 7). These changes were associated with constrictions in renal (significant at 2–10 and 30 min) and hindquarters vascular beds (significant at 0.5–30 min), while in the superior mesenteric vascular bed a dilatation (significant at 0.5 min) followed by a constriction (significant at 1–30 min) occurred (Figure 8).

In capsaicin-treated rats, the lower dose of NT (300 ng) caused a modest increase in blood pressure (significant at 0.5–1 and 3–4 min), which was significantly less, and a tachy-

cardia (significant at 0.5 min) which was not different from that in vehicle-treated rats (Figure 5). As in the vehicle-treated rats, there was no change in renal flow, but a decrease in superior mesenteric flow (significant at 1–2 min), while the slight reduction in hindquarters flow, seen in response to NT in vehicle-treated animals was absent (Figure 5). Thus, there was no change in renal vascular conductance, and this was significantly different from the response in vehicle-treated rats; moreover, the vasoconstriction in the superior mesenteric vascular bed (significant at 1–3 min) was significantly smaller than in vehicle-treated rats and the hindquarters vasoconstriction observed in the latter was absent (Figure 6).

In capsaicin-treated animals, the higher dose of NT (1000 ng) had no effect on blood pressure; this was significantly different from the response seen in vehicle-treated rats (Figure 7). Furthermore, there was no bradycardia, although the tachycardia (significant at 0.5–30 min) was greater than in

Figure 5 Cardiovascular responses to i.v. bolus injections of neurotensin in vehicle-treated (●) or capsaicin-treated (○) Long Evans rats. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ for changes in vehicle-treated group ($n = 9$) versus changes in capsaicin-treated group ($n = 9$) (analysis of variance followed by a Fisher's test). HR = heart rate; BP = blood pressure.

vehicle-treated rats (Figure 7). There was a reduction in renal flow (significant at 3–30 min) which was greater than in vehicle-treated rats (Figure 7). There was an increase in superior mesenteric flow (significant at 0.5 min) followed by a decrease (significant at 1–30 min); these effects were not different from those in vehicle-treated rats. The reduction in hindquarters flow (significant at 2–30 min) was smaller than in vehicle-treated rats (Figure 7). Hence, there were constrictions in the renal (significant at 3–30 min) and superior mesenteric vascular beds (significant at 1–30 min), but the superior mesenteric vasoconstriction was preceded by a vasodilatation (significant at 0.5 min) (Figure 8). These responses were not significantly different from those in vehicle-treated rats. However, there was a constriction in the hindquarters vascular bed (significant at 2–30 min) which was significantly smaller than that in vehicle-treated rats (Figure 8).

Discussion

The present work has shown that i.v. administration of the neuropeptide, neurotensin, can have substantial haemodynamic effects in conscious Long Evans rats. As noted in Table 2, NT caused a slight but significant pressor effect and a dose-related tachycardia which was preceded, at the higher

dose of NT (1000 ng), by a transient bradycardia. These changes were accompanied by dose-related reductions in renal, superior mesenteric and hindquarters flows and dose-related constrictions in these 3 vascular beds (Table 2).

Pressor effects of neurotensin

The finding of a pressor response to NT contrasts with previous studies carried out in pentobarbitone-anaesthetized rats, showing that NT produced hypotensive effects, probably through a histamine-mediated mechanism, and possibly involving other endogenous vasoactive substances (Quirion *et al.*, 1980). Since pentobarbitone anaesthesia reduces sympathetic nerve activity (Holmes & Scheider, 1973), it is feasible that this would inhibit any pressor effects of NT mediated through the sympathetic nervous system. However, in a study carried out in conscious Sprague-Dawley rats, hypotensive effects were reported following injection of NT (0.1 – $2.0 \mu\text{g kg}^{-1}$) (Sumners *et al.*, 1982).

Although a strain-dependent difference in sensitivity to NT could explain the discrepancy between these results and ours, the results in Table 2 show that, in spite of substantial dose-related vasoconstrictor responses in the renal, superior mesenteric and hindquarters vascular beds, the increase in blood pressure was small and not dose-related. One explanation of

Figure 6 Changes in regional vascular conductances elicited by i.v. bolus injections of neurotensin in vehicle-treated (●) or capsaicin-treated (○) Long Evans rats. These data were derived from that shown in Figure 5. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ for changes in vehicle-treated group ($n = 9$) versus changes in capsaicin-treated group ($n = 9$) (analysis of variance followed by Fisher's test).

these findings could be that NT caused increases in conductance in other vascular beds through release of endogenous vasodilator substances (Chahl, 1979; Chahl & Walker, 1981; Carraway *et al.*, 1982; Kerouac *et al.*, 1982). Such putative vasodilator effects could counterbalance the vasoconstrictor effects of NT and obscure any dose-relatedness of the pressor influence of the peptide. However, it is also feasible that NT reduced cardiac output and hence prevented any larger pressor effect resulting from the vasoconstriction.

In the presence of both propranolol and phentolamine, the pressor response to NT was similar to that seen in the unblocked state (although the haemodynamic profile was slightly different) (Figures 3 and 4). Hence, the hypotensive effect of NT in the presence of phentolamine alone (Figure 1) was probably due to an enhancement of a β -adrenoceptor-mediated vasodilator response (phentolamine causing both antagonism of prejunctional α -adrenoceptor autoinhibitory effects and suppression of postjunctional α -adrenoceptor vasoconstrictor influences). A similar observation has been made following bombesin administration in conscious Long Evans rats (Janssen *et al.*, 1991). As in that study we elected to use phentolamine and propranolol, rather than selective α_1 -, α_2 -, β_1 - and β_2 -adrenoceptor antagonists, since it was our intention simply to obtain evidence for the involvement of α - or β -adrenoceptors in the responses to neurotensin. Moreover, in our experience selective α_1 -, α_2 -, or β_2 -adrenoceptor antagonists can, themselves, produce complex haemodynamic changes in conscious rats (Gardiner & Bennett, 1988).

Heart rate effects of neurotensin

Phentolamine reduced the tachycardia and converted the pressor effect of NT into a depressor effect (Figure 1). The latter effect would have been expected to cause a greater baroreflex-mediated tachycardia, but it is possible that the

increased heart rate in the presence of phentolamine was responsible for the reduced tachycardic response to NT. On the other hand, it is also possible that cardiac α -adrenoceptors (Bennett & Kemp, 1978; Flavahan & McGrath, 1982; Tung *et al.*, 1985) were involved in the tachycardic response to NT.

Propranolol together with phentolamine had no effect on the bradycardia produced in response to NT, but abolished the tachycardia (Figure 3). Therefore, it is likely that neurally released and/or circulating catecholamines were involved in the tachycardic response to NT through activation of β - and, possibly, α -adrenoceptor-mediated mechanisms. The bradycardic response to NT might have been mediated by activation of cardiac vagal cholinergic neurones (or nerve terminals). This interpretation is supported by previous studies showing that, in guinea-pig isolated hearts, the tachycardia elicited by NT was sometimes preceded by transient bradycardia. The incidence and amplitude of the bradycardia were increased by neostigmine, but reduced by atropine, suggesting that receptors for NT exist on terminal branches of the vagus nerves, at least in guinea-pig hearts (Bachelard *et al.*, 1985). In the present work it is also feasible that NT induced a bradycardia through a vagovagal reflex (see later).

Haemodynamic effects of neurotensin in the hindquarters

In response to NT alone, the hindquarters vascular bed showed a vasoconstriction associated with a reduction in flow (Figures 1 and 2 and Table 2). In the presence of phentolamine, both responses were reduced (Figures 1 and 2). However, in the additional presence of propranolol the hindquarters vasoconstrictor response to NT, as well as the reduction in blood flow, were not different from those seen in untreated rats. Collectively, these results suggest that part of the hindquarters vasoconstrictor response to NT was due to activation of postjunctional α -adrenoceptor-mediated mechanisms and,

Figure 7 Cardiovascular responses to i.v. bolus injections of neurotensin in vehicle-treated (●) or capsaicin-treated (○) Long Evans rats. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ for changes in vehicle-treated group ($n = 9$) versus changes in capsaicin-treated group ($n = 9$) (analysis of variance followed by a Fisher's test). HR = heart rate; BP = blood pressure.

following blockade of these, and prejunctional autoinhibitory α -adrenoceptors, β -adrenoceptor-mediated hindquarters vasodilator mechanisms were augmented and contributed to the attenuation of the constrictor and pressor responses to NT seen in presence of phentolamine. Since in the additional presence of propranolol the hindquarters vasoconstrictor effect of NT was not different from that seen in untreated rats it is feasible that, in the presence of phentolamine alone, any non-adrenergic vasoconstrictor effects of NT were masked by β -adrenoceptor-mediated vasodilatation (Gardiner *et al.*, 1991).

A possible candidate for mediating non-adrenergic vasoconstrictor effects of NT is neuropeptide Y. This peptide has been shown to cause a pressor effect associated with vasoconstrictions in the renal, superior mesenteric and hindquarters vascular beds of conscious rats (Gardiner *et al.*, 1988). Moreover, neuropeptide Y has been shown to be co-localized with

noradrenaline in postganglionic sympathetic neurones (Pernow, 1988). Therefore, it is possible that stimulation of afferent nerves by NT (see later) causes reflex activation of the sympathetic nervous system, and induces the release of noradrenaline together with neuropeptide Y. However, further studies are required to substantiate this hypothesis.

Renal haemodynamic effects of neurotensin

In the renal vascular bed, phentolamine alone caused a reduction of the constrictor effect of NT, which suggests an involvement of α -adrenoceptor mechanisms in the renal vasoconstriction following NT alone. Furthermore, the finding that in the presence of phentolamine and propranolol the renal vasoconstrictor response to NT was less than in untreated rats could indicate an involvement of the renin angiotensin system in the response seen in the latter. Thus, if NT caused sympathoadrenal activation and consequent β -mediated stimulation of renin release, this would increase circulating levels of angiotensin II. Angiotensin II has been shown to cause a pressor response associated with vasoconstrictions in the renal and superior mesenteric vascular beds in conscious rats (Gardiner *et al.*, 1988). Hence, propranolol, by inhibiting renin release, would attenuate these effects.

In the presence of phentolamine alone, the renal vasoconstrictor effect of NT was preceded by a slight vasodilatation which was propranolol-resistant and absent in untreated rats. It is feasible that this was due to an indirect effect of NT mediated through nonadrenergic-noncholinergic mechanisms, possibly involving the release of an endogenous vasodilator substance, such as histamine. However, further studies are required to substantiate this possibility.

Superior mesenteric haemodynamic effects of neurotensin

In the presence of phentolamine alone, the superior mesenteric vasoconstrictor effect of NT was reduced. Therefore, the constrictor effect of NT in untreated rats was probably partially mediated through α -adrenoceptors. However, in the presence of phentolamine and propranolol the constrictor response to NT was not different from that seen in the untreated state. This might indicate that, in the presence of phentolamine alone, β -adrenoceptor-mediated vasodilatation (as discussed earlier) could have masked any nonadrenergic vasoconstrictor effects of NT.

In the presence of phentolamine alone, the superior mesenteric vasoconstrictor response to NT was preceded by a vasodilatation which was propranolol-resistant and absent in untreated animals. As discussed above, it is feasible that this was due to NT causing the release of some endogenous vasodilator substance (Chahl, 1979; Chahl & Walker, 1981; Carraway *et al.*, 1982; Kerouac *et al.*, 1982).

Haemodynamic effects of neurotensin in capsaicin-treated rats

Previous studies have demonstrated that some effects of NT are profoundly altered following treatment of animals (guinea-pig) or isolated preparations (heart or atria of guinea-pigs) with the neurotoxin, capsaicin (Rioux *et al.*, 1986; 1988; Bachelard *et al.*, 1987). In the present work we found that, in rats neonatally treated with capsaicin, the pressor response to NT (300 and 1000 ng) was reduced, as were the constrictor responses in the renal (at the dose of 300 ng), superior mesenteric (at a dose of 300 ng) and hindquarters (at both doses) vascular beds. It is likely that the reduced vasoconstrictor effects were responsible for the decrease in the pressor response to NT. Moreover, in capsaicin-treated animals the bradycardic response elicited by the higher dose of NT was absent whereas the tachycardia was potentiated. Together

Figure 8 Changes in regional vascular conductances elicited by i.v. bolus injections of neurotensin in vehicle-treated (●) or capsaicin-treated (○) Long Evans rats. These data were derived from those shown in Figure 7. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ for changes in vehicle-treated group ($n = 9$) versus changes in capsaicin-treated group ($n = 9$) (analysis of variance followed by Fisher's test).

these results suggest an involvement of capsaicin-sensitive neurones in the cardiovascular responses to NT.

Other studies have demonstrated that NT has the ability to stimulate capsaicin-sensitive primary afferents and to trigger cardiovascular reflexes following its i.p. administration or topical application to the surface of the heart in anaesthetized guinea-pigs (Rioux *et al.*, 1986; 1990). Thus, it is feasible that activation of capsaicin-sensitive neurones contributed to the pressor and vasoconstrictor effects of NT in normal animals. For example, activation of capsaicin-sensitive primary afferents by NT might lead to reflex excitation of the efferent vasomotor outflows to renal, superior mesenteric and hindquarters vascular beds.

As far as the bradycardic response to NT is concerned, a likely explanation is that this effect was due to a reflex activation of the parasympathetic nervous system in control animals. Neonatal treatment with capsaicin has been shown to induce degeneration of at least a portion of the vagal afferent cell bodies of the nodose ganglion and of the terminal projections in the nucleus of the solitary tract (Helke & Eskay, 1985) which is the central nervous system site of termination

of most vagal afferents. Thus, one would expect that if NT produced its bradycardic effect through an activation of capsaicin-sensitive afferents, neonatal treatment with capsaicin might so alter the heart rate response.

Conclusion

The present results indicate that NT exerts influences on the cardiovascular system in conscious rats through sympatho-adrenal mechanisms and possibly also through direct and/or indirect non-adrenergic effects on the heart, and on the renal, superior mesenteric and hindquarters vascular beds. The findings are consistent with an involvement of neuropeptide-containing, capsaicin-sensitive sensory neurones in part of the cardiovascular response to NT, in conscious, unrestrained rats.

This work was supported by the Canadian Heart Foundation (H.B.) and the MRC and the Wellcome Trust.

References

- BACHELARD, H., ST-PIERRE, S. & RIOUX, F. (1985). The chronotropic action of neurotensin in the guinea pig isolated heart. *Peptides*, **6**, 841–845.
- BACHELARD, H., ST-PIERRE, S. & RIOUX, F. (1987). Participation of capsaicin-sensitive neurons in the cardiovascular effects of neurotensin in guinea pigs. *Peptides*, **8**, 1079–1087.

- BENNETT, T. & KEMP, P.A. (1978). Lack of evidence for a temperature-mediated change of adrenoceptor type in the rat heart. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **301**, 217–222.
- BISSETTE, G., MANBERG, P., NEMEROFF, C.B. & PRANGE, A.J. (1978). Neurotensin, a biologically active peptide. *Life Sci.*, **23**, 2173–2183.
- BROWN, D.R. & MILLER, R.J. (1982). Neurotensin. *Br. Med. Bull.*, **38**, 239–245.
- CARRAWAY, R.E. & LEEMAN, S.E. (1973). The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalamus. *J. Biol. Chem.*, **248**, 6854–6861.
- CARRAWAY, R.E. & LEEMAN, S.E. (1978). The amino acid sequence of radioimmunoassayable neurotensin from bovine intestine. *J. Biol. Chem.*, **253**, 7996–7998.
- CARRAWAY, R.E. & BHATNAGAR, Y.M. (1980). Isolation, structure and biologic activity of chicken intestinal neurotensin. *Peptides*, **1**, 167–174.
- CARRAWAY, R.E., COCHRANE, D.E., LANSMAN, J.B., LEEMAN, S.E., PATERSON, B.M. & WELCH, H.J. (1982). Neurotensin stimulates exocytic histamine secretion from rat mast cells and elevates plasma histamine levels. *J. Physiol.*, **323**, 404–414.
- CHAHAL, L.A. (1979). The effect of putative neurotransmitters on cutaneous vascular permeability in the rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **309**, 159–163.
- CHAHAL, L.A. & WALKER, S.B. (1981). Responses of the rat cardiovascular system to substance P, neurotensin and bombesin. *Life Sci.*, **29**, 2009–2015.
- FLAVAHAN, N.A. & McGRATH, J.C. (1982). α_1 -Adrenoceptor activation can increase heart rate directly or decrease it indirectly through parasympathetic activation. *Br. J. Pharmacol.*, **77**, 319–328.
- GARDINER, S.M. & BENNETT, T. (1988). Regional hemodynamic responses to adrenoceptor antagonism in conscious rats. *Am. J. Physiol.*, **255**, H813–H824.
- GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (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. (1990a). Effects of indomethacin on the regional haemodynamic responses to low doses of endothelins and sarafotoxin. *Br. J. Pharmacol.*, **100**, 158–162.
- GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990b). Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension*, **15**, 486–492.
- GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991). Effects of N^G -nitro-L-arginine methyl ester on vasodilator responses to acetylcholine, 5'-N-ethylcarboxamidoadenosine or salbutamol in conscious rats. *Br. J. Pharmacol.*, **103**, 1725–1732.
- HAMMER, R.A., LEEMAN, S.E., CARRAWAY, R.E. & WILLIAMS, R.H. (1980). Isolation of human intestinal neurotensin. *J. Biol. Chem.*, **255**, 2476–2480.
- 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.
- HELKE, C.J. & ESKAY, R.L. (1985). Capsaicin reduces substance P immunoreactivity in the lateral nucleus of the solitary tract and nodose ganglion. *Peptides*, **6**, 121–126.
- HOLMES, J.D. & SCHEIDER, F.H. (1973). Pentobarbitone inhibition of catecholamine secretion. *Br. J. Pharmacol.*, **49**, 205–213.
- JANSSEN, P.J.J.M., GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1991). Mechanisms contributing to the differential haemodynamic effects of bombesin and cholecystokinin in conscious, Long Evans rats. *Br. J. Pharmacol.*, **102**, 123–134.
- KEROUAC, R., RIOUX, F. & ST-PIERRE, S. (1981). Mechanism of neurotensin-induced pressor effect and tachycardia in guinea pigs. *Life Sci.*, **28**, 2477–2487.
- KEROUAC, R., ST-PIERRE, S. & RIOUX, F. (1982). Influence of various drugs on the variations of blood pressure, hematocrit and plasma histamine caused by neurotensin and compound 48/80 in rats. *Neuropeptides*, **3**, 145–157.
- KOBAYASHI, R.M., BROWN, M.R. & VALE, W. (1977). Regional distribution of neurotensin and somatostatin in rat brain. *Brain Res.*, **126**, 584–588.
- MANBERG, P.J., YOUNGBLOOD, W.W., NEMEROFF, C.B., ROSSOR, M.N., IVERSEN, L.L., PRANGE, A.J. & KIZER, J.S. (1982). Regional distribution of neurotensin in human brain. *J. Neurochem.*, **38**, 1777–1780.
- PERNOW, J. (1988). Co-release and functional interactions of neuropeptide Y and noradrenalin in peripheral sympathetic vascular control. *Acta Physiol., Scand.*, **133**, (Suppl. 568), 1–56.
- QUIRION, R., RIOUX, F. & REGOLI, D. (1978). Chronotropic and inotropic effects of neurotensin on spontaneously beating auricles. *Can. J. Physiol. Pharmacol.*, **56**, 671–673.
- QUIRION, R., RIOUX, F., REGOLI, D. & ST-PIERRE, S. (1979). Neurotensin-induced coronary vessels constriction in perfused rat hearts. *Eur. J. Pharmacol.*, **55**, 221–223.
- QUIRION, R., RIOUX, F., REGOLI, D. & ST-PIERRE, S. (1980). Compound 48/80 inhibits neurotensin-induced hypotension in rats. *Life Sci.*, **27**, 1889–1895.
- RIOUX, F., BACHELARD, H., BARABE, J. & ST-PIERRE, S. (1986). The cardiovascular response to epicardial application of neurotensin in guinea pigs. *Peptides*, **7**, 1087–1094.
- RIOUX, F., BACHELARD, H., BERNOUSSI, A. & ST-PIERRE, S. (1988). Capsaicin-sensitive structures as potential target sites for neurotensin and bradykinin in guinea pig atria. *Peptides*, **9**, 883–892.
- RIOUX, F., KEROUAC, R., QUIRION, R. & ST-PIERRE, S. (1982). Neurotensin, a potent vasoconstrictor in the rat hindquarter. *Neuropeptides*, **2**, 227–232.
- RIOUX, F., LEMIEUX, M. & LEBEL, M. (1990). Further observations on the mechanism of the cardiovascular reflexes caused by exposure of the peritoneum to neurotensin. *Peptides*, **11**, 805–816.
- RIOUX, F., QUIRION, R., REGOLI, D., LEBLANC, M.A. & ST-PIERRE, S. (1980). Possible interactions between neurotensin and prostaglandins in the isolated rat portal vein. *Life Sci.*, **27**, 259–267.
- ROSELL, S. & ROKAEUS, A. (1981). Actions and possible hormonal functions of circulating neurotensin. *Clin. Physiol.*, **1**, 3–20.
- SHULKES, A., CHICK, P., WONG, H. & WALSH, J.H. (1982). A radioimmunoassay for neurotensin in human plasma. *Clin. Chim. Acta*, **125**, 49–58.
- STENE-LARSEN, G. & HELLE, K.B. (1979). Inotropic and chronotropic effects of neurotensin in the rat atrium and of physalamin in the auricles of *R. esculenta*. *Comp. Biochem. Physiol.*, **64**, 279–283.
- SUMNERS, C., PHILLIPS, M.I. & RICHARD, E.M. (1982). Central pressor action of neurotensin in conscious rats. *Hypertension*, **4**, 888–893.
- TUNG, L.H., RAND, M.L. & LOUIS, W.J. (1985). Cardiac α -adrenoceptors involving positive chronotropic responses. *J. Cardiovasc. Pharmacol.*, **6**, 121–126.
- UHL, G.R. & SNYDER, S.H. (1976). Region and subcellular distributions of brain neurotensin. *Life Sci.*, **19**, 1827–1832.
- UHL, G.R. & SNYDER, S.H. (1981). Neurotensin. In *Neurosecretion and Brain Peptides*, ed. Martin, J.B., Reichlin, S. & Bick, K.L., pp. 87–106. New York: Raven Press.
- WINN, M.J., GARDINER, S.M. & BENNETT, T. (1985). Functional involvement in the maintenance of systemic arterial blood pressure after phenoxybenzamine or phentolamine administration: studies in Long Evans and Brattleboro rats. *J. Pharmacol. Exp. Ther.*, **235**, 500–505.
- 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.

(Received July 3, 1991)

Revised September 11, 1991

Accepted September 17, 1991)