Br. J. Pharmacol. (1991), **102**, 123–134

Mechanisms contributing to the differential haemodynamic effects of bombesin and cholecystokinin in conscious, Long Evans rats

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1 Long Evans rats were chronically instrumented with intravascular catheters and pulsed Doppler probes to assess changes in renal, mesenteric and hindquarters blood flows and vascular conductances in response to bombesin (2.5 $\mu g\,kg^{-1}$, i.v.) and cholecystokinin (CCK) (0.5 and 5.0 $\mu g\,kg^{-1}$, i.v.).

2 Bombesin caused an increase in heart rate and blood pressure, together with a transient renal vasoconstriction and prolonged mesenteric vasodilatation; there was an early hindquarters vasodilatation followed by vasoconstriction.

3 In the presence of phentolamine, bombesin caused a fall in blood pressure due to enhanced hindquarters vasodilatation; these effects were reversed by propranolol and hence were possibly due to circulating adrenaline acting on vasodilator β_2-adrenoceptors.

4 During concurrent administration of phentolamine, propranolol and atropine, bombesin caused prolonged tachycardia and a rise in blood pressure. The renal vasoconstrictor and mesenteric vasodilator effects of bombesin were not reduced under these conditions and thus probably were direct and/or indirect non-adrenergic, non-cholinergic (NANC) effects.

5 CCK caused dose-dependent increases in blood pressure accompanied by renal, mesenteric and hindquarters vasoconstrictions followed, after the higher dose, by vasodilatations. The lower dose of CCK increased heart rate but there was a bradycardia followed by a tachycardia after the higher dose.

6 Experiments with antagonists as described above indicated the pressor effect of CCK was mediated largely through α-adrenoceptors, as were the mesenteric and hindquarters vasoconstrictor effects; CCK exerted NANC negative chronotropic effects.

7 All the effects of CCK were markedly inhibited by L364,718. This observation, and the finding that L364,718 had no effect on the responses to bombesin, together with the dissimilarities in the regional haemodynamic effects of exogenous CCK and bombesin, indicate that the cardiovascular actions of the latter were not dependent on the release of endogenous CCK.

Introduction

Bombesin is a tetradecapeptide originally isolated from the skin of the frog *Bombina bombina* in 1971 (see Erspamer & Melchiorri, 1973). Gastrin-releasing peptide, a 27 amino acid peptide, is the mammalian homologue (Sunday *et al.*, 1988) and produces similar effects to bombesin after administration in mammals. Bombesin causes a reduction in food and water intake after central (Willis *et al.*, 1984) or peripheral administration (Gibbs *et al.*, 1981), which may be due to satiety-like effects, although it is difficult to exclude the possibility of nausea or malaise contributing to these actions. Bombesin also releases several gut peptides such as gastrin and cholecystokinin (CCK) (Ghatei *et al.*, 1982; Walsh, 1989).

CCK was originally described as a gut hormone causing gallbladder contraction. Subsequently the peptide was identified in neurones by use of anti-gastrin antibodies (CCK and gastrin share the same carboxyterminus), as described by Dockray (1988). Central or peripheral administration of CCK reduces food intake (Lukaszewski & Praissman, 1988; Griesbacher *et al.*, 1989; Rehfeld, 1989). Administration of CCK enhances the release of oxytocin, but not of vasopressin *in vivo* (Verbalis *et al.*, 1986; Carter & Lightman, 1987), although *in vitro* studies indicate that CCK can release both peptides (Bondy *et al.*, 1989).

Bombesin and CCK have been shown to exert cardiovascular actions. Intravenous or subcutaneous injection of bombesin in anaesthetized or conscious rats causes an increase in blood pressure and a tachycardia, although the latter may be slight and the pressor effect may not show clear dose-dependence (Melchiorri *et al.*, 1971; Erspamer *et al.*, 1972; Fisher *et al.*, 1985). Erspamer *et al.* (1972) obtained similar results in pithed rats, although Bayorh & Feuerstein (1985) observed dose-dependent pressor and tachycardic responses to bombesin under these conditions.

CCK, given i.v. in anaesthetized rats, causes dose-dependent bradycardia and complex blood pressure changes (Marker & Roberts, 1988). The ability of bombesin and CCK to overcome severe haemorrhagic shock in rats has been reported (Guarini *et al.*, 1987; 1988a,b; 1989), and recently it was claimed that the effects of bombesin were mediated by the release of endogenous CCK (Guarini *et al.*, 1989).

Since little information is available regarding the regional haemodynamic effects of bombesin or CCK administered i.v. in conscious rats, and because of the suggested relationship between these neuropeptides (Guarini *et al.*, 1989), the present study was designed to answer the following questions:

(1) Are the regional haemodynamic responses to i.v. injections of bombesin or CCK similar in conscious, unrestrained Long Evans rats?

(2) Are the regional haemodynamic responses to CCK influenced by L364,718, a selective antagonist of peripheral (A-type) CCK receptors?

(3) Given the possibility that bombesin releases endogenous CCK, are the regional haemodynamic responses to bombesin influenced by L364,718 at a dose that inhibits the effects of exogenous CCK?

(4) Since it is possible that CCK releases vasopressin, are the regional haemodynamic responses to CCK influenced by a vasopressin V_1-receptor antagonist?

(5) To what extent do the cardiovascular responses to bombesin or CCK involve adrenoceptors or muscarinic receptors?

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Methods

Male, Long Evans rats (350–400 g) were used and throughout the experiments the rats were housed in cages with a solid floor, covered with sawdust. They had free access to water and food and the room temperature was kept between 18 and 21°C.

Animals were anaesthetized with sodium methohexitone (Lilly Ltd., 60 mg kg^{-1}, i.p., supplemented when required) prior to surgery. A midline abdominal incision was made and the connective tissue was carefully separated from the left renal and the superior mesenteric arteries and from the abdominal aorta below the ileocaecal artery. Appropriate sized pulsed Doppler probes (Haywood *et al.*, 1981; made in Nottingham but using DBF-120A-XS sub-assemblies from Crystal Biotech, Holliston, U.S.A.) were sutured around the vessels (with Ethicon 6/0 silk suture) and the leads of the probes were sutured to the abdominal wall (with Ethicon 3/0) in order to prevent possible changes in the position of the probes. The probe wires were tunnelled subcutaneously to exit at the back of the neck, where they were sutured in place. After replacement of the viscera and irrigating the abdominal cavity with sterile saline, the abdominal incision was closed with surgical silk (Ethicon 3/0). After surgery the rats were given an intramuscular injection of ampicillin (Penbritin, Beecham Ltd., 7 mg kg^{-1}) and were allowed to recover for at least 7 days.

After this period the rats were briefly re-anaesthetized with sodium methohexitone (40 mg kg^{-1}, i.p., supplemented when required), and prior to catheterization the leads of the implanted probes were soldered to a 6-way micro-connector (Microtech Inc., Boothwyn, U.S.A.) and the signals were checked on an oscilloscope (Telequipment DM64). All 3 phasic pulsed Doppler signals had to be of a good quality (signal: noise, 20 : 1); if this criterion could not be met the animal was rejected from the study. Between 2 and 4 catheters (Portex Ltd., i.d. 0.28 mm, o.d. 0.62 mm) were implanted in the right jugular vein and an intra-arterial catheter was implanted in the distal aorta, via the caudal artery, for measurement of blood pressure and heart rate (Gardiner & Bennett 1985; 1988).

The arterial catheter consisted of a 7 cm length of polyethylene catheter with a small diameter (Portex Ltd., i.d. 0.28 mm, o.d. 0.62 mm) fused to a more rigid, nylon catheter with a larger diameter (Portex Ltd., i.d. 0.58 mm, o.d. 1.02 mm). The catheters were tunnelled subcutaneously to the back of the neck where they emerged at the same point as the Doppler probe wires. The micro-connector, soldered to the latter, was clamped in a custom-made harness worn by the rat and the catheters ran through a flexible spring attached to the harness. The rats were allowed to recover for a day before experiments started (Gardiner & Bennett, 1988; Gardiner *et al.*, 1988a, b; 1990b).

At the end of every day the arterial catheter was filled with a strong saline/heparin solution (Monoparin, CP Pharmaceuticals Ltd., 450 units ml^{-1}, 1 ml) in order to prevent clots forming in the catheter. If a catheter became blocked a plasmin solution (porcine plasmin, Sigma Chemicals Ltd.; 1 unit ml^{-1}) was flushed down it in order to dissolve the blood clot.

The probes were connected to a pulsed Doppler flowmeter (Crystal Biotech, Holliston, U.S.A.), constructed to the original design by Hartley & Cole (1974) and Hartley *et al.* (1978), but operating with a pulse repetition frequency of 125 kHz (Gardiner *et al.*, 1990a). The mean Doppler signal represents the average velocity of the erythrocytes. The relationship between mean Doppler shift (kHz) and volume flow (ml min^{-1}) measured with an electromagnetic flowmeter, is linear (Haywood *et al.*, 1981) and hence the percentage change in mean Doppler shift relative to baseline was taken as an index of change in flow.

During the experiments 9 different variables were recorded (heart rate, phasic and mean blood pressure, and phasic and mean Doppler shift signals from renal, mesenteric and hindquarters probes). The purpose of recording phasic Doppler shift signals was to ensure that they were of an acceptable quality during the experiments. The pulsed Doppler flowmeter gives an electronic zero that corresponds to zero volume flow. Zero lines for all 3 regional flow signals were recorded continuously.

At selected time points heart rate, mean blood pressure and mean Doppler shifts were measured and related to the predrug baseline (absolute changes for the former two variables, percentages for the Doppler shifts). In addition, the Doppler shift was divided by mean blood pressure in order to obtain the vascular conductance changes (% relative to baseline; Gardiner *et al.*, 1990b). Before every experiment baseline measurements were made over a period of 30 min.

Experimental protocols

Three separate groups of rats were studied ($n = 8$ in each).

Group 1 Animals were given bombesin (2.5 $\mu g\,kg^{-1}$, i.v. bolus) on two occasions separated by at least 1.5 h.

The next day animals were given the vehicle for L364,718 (2% dimethylsulphoxide (DMSO) and 2% Tween 80 in isotonic saline; i.v. 0.1 ml bolus and 0.3 ml h^{-1} infusion), followed, after 10 min, by i.v. bombesin (2.5 $\mu g\,kg^{-1}$, i.v. bolus). Then, after at least 1.5 h, these animals were given L364,718 (Guarini *et al.*, 1989; 50 $\mu g\,kg^{-1}$, i.v. bolus, 150 $\mu g\,kg^{-1}\,h^{-1}$, infusion) followed, after 10 min, by bombesin (2.5 $\mu g\,kg^{-1}$, i.v. bolus).

Group 2 Animals were given 2 doses of CCK (0.5 and 5.0 $\mu g\,kg^{-1}$, i.v. bolus) separated by at least 1.5 h. Subsequently, (after at least 1.5 h) administration of the vehicle for L364,718 (see above) was started, and 10 min later CCK (5.0 $\mu g\,kg^{-1}$, i.v. bolus) was given.

The next day these animals were given L364,718 (50 $\mu g\,kg^{-1}$, i.v. bolus, 150 $\mu g\,kg^{-1}\,h^{-1}$, infusion) followed 10 min later by CCK (0.5 $\mu g\,kg^{-1}$, i.v. bolus). At least 1.5 h after this time the animals were given L364,718 (50 $\mu g\,kg^{-1}$, i.v. bolus, 150 $\mu g\,kg^{-1}\,h^{-1}$, infusion) followed 10 min later by CCK (5.0 $\mu g\,kg^{-1}$, i.v. bolus).

Group 3 Animals were given a vasopressin V_1-receptor antagonist (10 $\mu g\,kg^{-1}$, i.v. bolus) and after 10 min CCK (5.0 $\mu g\,kg^{-1}$, i.v. bolus) was administered. At least 1.5 h later phentolamine (1 mg kg^{-1}, i.v. bolus, 1 mg $kg^{-1}\,h^{-1}$, infusion) administration was started, followed, after 30 min, by bombesin (2.5 $\mu g\,kg^{-1}$, i.v. bolus) and, at least 45 min after bombesin administration, CCK (5.0 $\mu g\,kg^{-1}$, i.v. bolus) was given.

The next day phentolamine (1 mg kg^{-1}, i.v. bolus, 1 mg $kg^{-1}\,h^{-1}$, infusion) and propranolol (1 mg kg^{-1}, i.v. bolus, 0.5 mg $kg^{-1}\,h^{-1}$, infusion) administrations were started and 30 min after onset of the infusions, bombesin (2.5 $\mu g\,kg^{-1}$, i.v. bolus) was given. At least 45 min after bombesin administration CCK (5.0 $\mu g\,kg^{-1}$, i.v. bolus) was injected. Following a further 45 min during which the infusions of phentolamine and propranolol were continued, atropine (1 mg kg^{-1}, i.v. bolus, 1 mg $kg^{-1}\,h^{-1}$, infusion) administration was started, followed 30 min later by bombesin (2.5 $\mu g\,kg^{-1}$, i.v. bolus) and, after at least a further 45 min, by CCK (5.0 $\mu g\,kg^{-1}$, i.v. bolus).

Rats in group 1 were exposed to bombesin (2.5 $\mu g\,kg^{-1}$) on 4 occasions over 2 days and there were no systematic differences in the responses. Hence in those experiments where the responses to bombesin were affected by pretreatment (see Results), these differences must have been due to the pretreatment rather than the repeated exposure to bombesin.

Rats in group 2 were exposed to CCK (5.0 $\mu g\,kg^{-1}$) on 2 occasions during one day; no systematic differences in responses were observed. So, the different responses to CCK, observed in animals in groups 2 and 3 following administration of different antagonists (see Results), are likely to have

Table 1 Peak cardiovascular changes following increasing i.v. doses of bombesin in conscious, Long Evans rats

	Dose of bombesin		
	0.25 μg kg⁻¹	*2.5 μg kg⁻¹*	*25 μg kg⁻¹*
Heart rate (beast min⁻¹)	24 ± 10*	94 ± 10*	83 ± 11*
Mean blood pressure (mmHg)	3 ± 2	18 ± 3*	20 ± 2*
Flow (%)			
Renal	−13 ± 7	−76 ± 5*	−77 ± 4*
Mesenteric	23 ± 11	43 ± 3*	47 ± 4*
Hindquarters	26 ± 10*	55 ± 16*	51 ± 9*
Conductance (%)			
Renal	−15 ± 8	−77 ± 5*	−78 ± 3*
Mesenteric	21 ± 12	23 ± 4*	24 ± 4*
Hindquarters	23 ± 10*	57 ± 13*	45 ± 14*

Values are mean ± s.e.mean; $n = 8$.
* $P < 0.05$ versus baseline (Friedman's test).

been caused by those pretreatments rather than the repeated exposure to CCK.

Peptides and drugs

The following peptides and drugs were used during the experiments: bombesin (Bachem Ltd); CCK (26–33), sulphated (Bachem Ltd.); L364,718 (3S(−)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide), (Merck, Sharp and Dohme Ltd.); vasopressin V_1-receptor antagonist, $d(CH_2)_5$[Tyr(Et)]DAVP (Prof. Manning, Medical College of Ohio); phentolamine mesylate (Ciba Geigy Ltd.); propranolol hydrochloride (Imperial Chemical Industries Ltd.); atropine methyl nitrate (Sigma Chemicals Ltd.).

Bombesin and CCK (26–33) were dissolved in 1% BSA in isotonic saline. L364,718 was dissolved in 2% DMSO and 2% Tween 80 in isotonic saline. The vasopressin V_1-receptor antagonist was dissolved in 0.5 ml glacial acetic acid, diluted to the working concentration with isotonic saline; phentolamine, propranolol and atropine were dissolved in saline.

The dose of bombesin was determined in pilot experiments that showed a ten fold lower dose had little effect, whereas a ten fold higher dose had no greater effect than the dose employed (Table 1). The range of the CCK concentrations was based on the studies by Marker & Roberts (1988), while the dose of L364,718 was that used by Guarini *et al.* (1989). The concentrations of the vasopressin antagonist, phentolamine, propranolol and atropine used were based on published findings (Gardiner & Bennett, 1985; Winn *et al.*, 1985; Gardiner & Bennett, 1988; Fisher *et al.*, 1985, respectively).

Statistical analysis

Two different statistical tests were applied to the data. For intra-group comparisons Friedman's test (Theodorsson-Norheim, 1987) was used; for paired comparisons Wilcoxon's rank sums test was used, and for unpaired, inter-group comparisons the Mann-Whitney U-test was applied.

A P value < 0.05 was taken to indicate a significant difference. In the results, time values given in parentheses indicate points at which values were significantly different from baseline or significantly different from values obtained in rats not pretreated with antagonists. Values at time points other than those mentioned were not significantly different.

Results

Cardiovascular effects of bombesin

Haemodynamic responses to bombesin (2.5 μg kg⁻¹) (Figures 1 and 2) The cardiovascular effects of bombesin in a single rat are shown in Figure 1, and Figure 2 shows the group mean results.

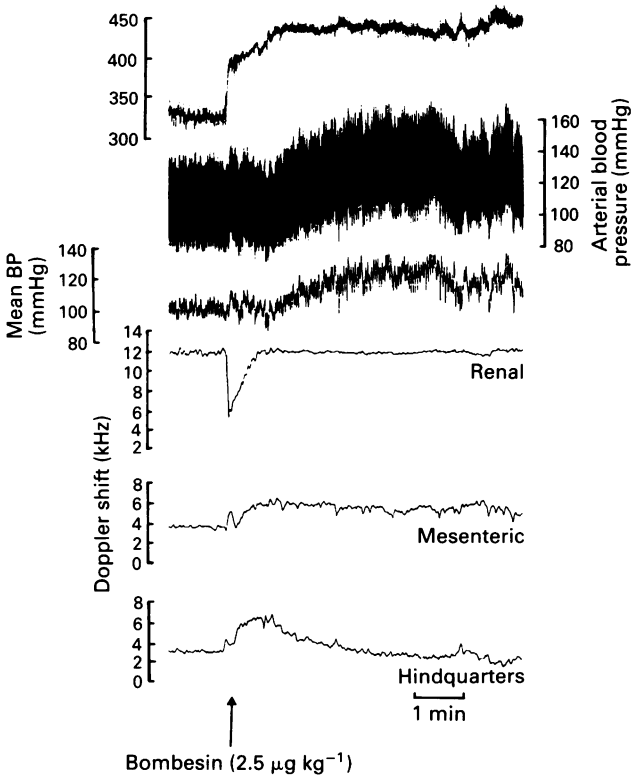

Figure 1 Haemodynamic responses to bombesin (2.5 μg kg⁻¹, bolus, i.v.) in a conscious, unrestrained, Long Evans rat.

A marked tachycardia (significant at 1–5 min, $P < 0.05$) and a small increase in blood pressure (significant at 2–5 min, $P < 0.05$) occurred after administration of bombesin. Renal flow decreased (significant at 0.5 min, $P < 0.05$), while mesenteric (significant at 0.5–30 min, $P < 0.05$) and hindquarters (significant at 0.5–2 min, $P < 0.05$) flows increased. There was a profound, but very short-lasting (significant at 0.5 min, $P < 0.05$) renal vasoconstriction. However, the mesenteric vascular bed showed a prolonged (significant at 1–30 min, $P < 0.05$) vasodilatation, while the hindquarters vascular bed dilated initially (significant at 0.5–1 min, $P < 0.05$) and constricted thereafter (significant at 5 min, $P < 0.05$).

Haemodynamic responses to bombesin (2.5 μg kg⁻¹) in the presence of vehicle or L364,718 Pretreatment with vehicle or with the peripheral CCK receptor antagonist, L364,718, did not change baseline levels or the haemodynamic responses to bombesin (Table 2).

Table 2 Peak cardiovascular changes following administration of vehicle or L364,718, or bombesin in the presence of L364,718

	Vehicle	*L364,718*	*Bombesin in the presence of L364,718*
Heart rate (beats min⁻¹)	+5 ± 4	+19 ± 12	+108 ± 13*
Mean blood pressure (mmHg)	+1 ± 1	+5 ± 3	+16 ± 3*
Flow (%)			
Renal	+2 ± 1	+1 ± 2	−58 ± 4*
Mesenteric	+1 ± 3	−6 ± 5	+44 ± 6*
Hindquarters	+1 ± 4	−4 ± 5	+74 ± 15*
Conductance (%)			
Renal	+1 ± 1	−3 ± 3	−62 ± 4*
Mesenteric	+1 ± 3	−10 ± 5	+32 ± 7*
Hindquarters	+1 ± 4	−8 ± 5	+60 ± 16*

Values are mean ± s.e.mean; $n = 8$.
* $P < 0.05$ versus baseline; compare with Figure 2.

Haemodynamic responses to bombesin (2.5 μg kg^{-1}) in the presence of phentolamine (Figure 2) Thirty min after onset of phentolamine administration the fall in blood pressure was not significant, but there was a persistent tachycardia, a reduction in renal flow, an increase in hindquarters flow, a constriction of the renal and dilatation of the hindquarters vascular beds (all significant, $P < 0.05$).

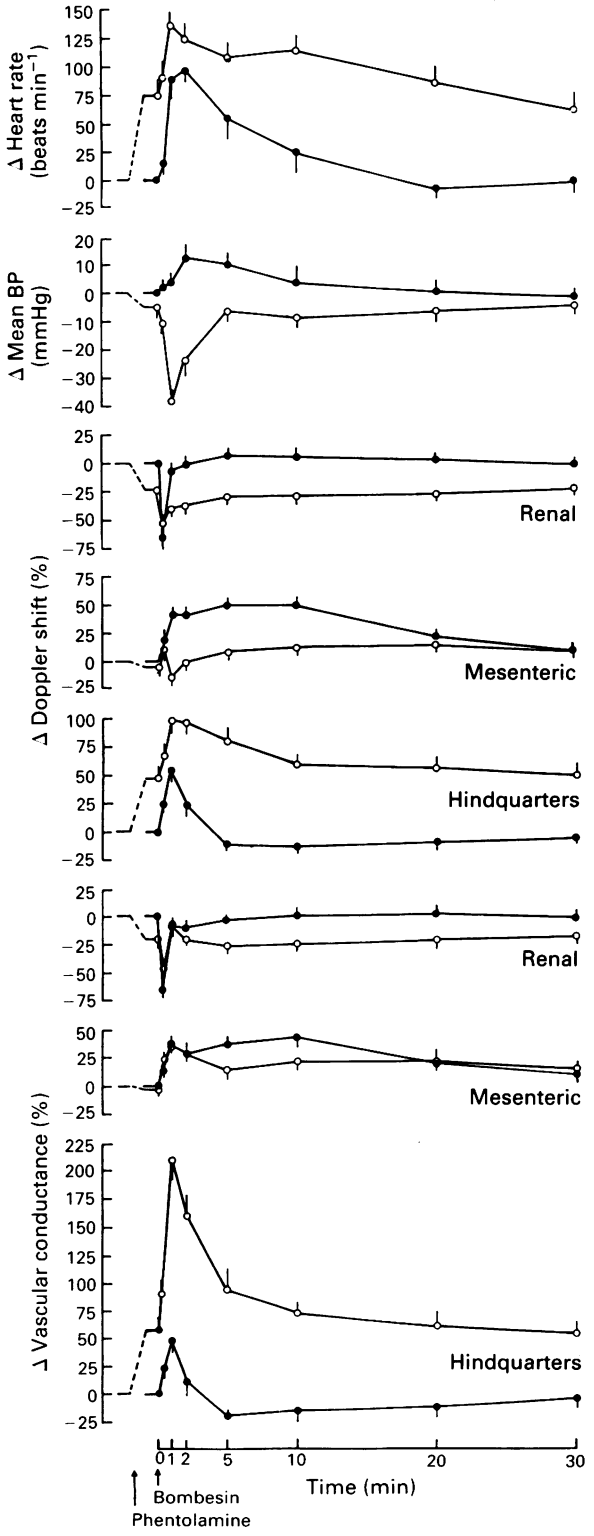

Figure 2 Haemodynamic responses to bombesin (2.5 μg kg^{-1}, bolus, i.v.) in conscious, unrestrained, Long Evans rats, in the absence, (●), or in the presence of phentolamine, (○). The dotted line represents the response to phentolamine. Values are mean ($n = 8$) with s.e.mean shown by vertical lines. All statistics are given in the text.

In the presence of phentolamine, bombesin caused a tachycardia (significant at 0.5–5 min, $P < 0.05$), which was less than in untreated animals (significant at 0.5–5 min, $P < 0.05$) even though the blood pressure decreased (significant at 0.5–2 min, $P < 0.05$), i.e. an effect opposite (significant at 0.5–5 min, $P < 0.05$) to that seen in untreated rats. There was a renal vasoconstriction (significant at 0.5 min, $P < 0.05$), followed by vasodilatation (significant at 1 min, $P < 0.05$); the vasoconstriction was less than that in untreated rats (significant at 0.5 min, $P < 0.05$). The mesenteric vascular bed showed a long-lasting vasodilatation (significant at 0.5–30 min, $P < 0.05$) which was not different from that in untreated rats. However, there was a hindquarters vasodilatation (significant at 0.5–2 min, $P < 0.05$) which was greater (significant at 1–5 min, $P < 0.05$) than the response observed in untreated rats, and there was no subsequent vasoconstriction.

Haemodynamic responses to bombesin (2.5 μg kg^{-1}) in the presence of phentolamine and propranolol (Figure 3) Thirty min after the onset of phentolamine and propranolol adminis-

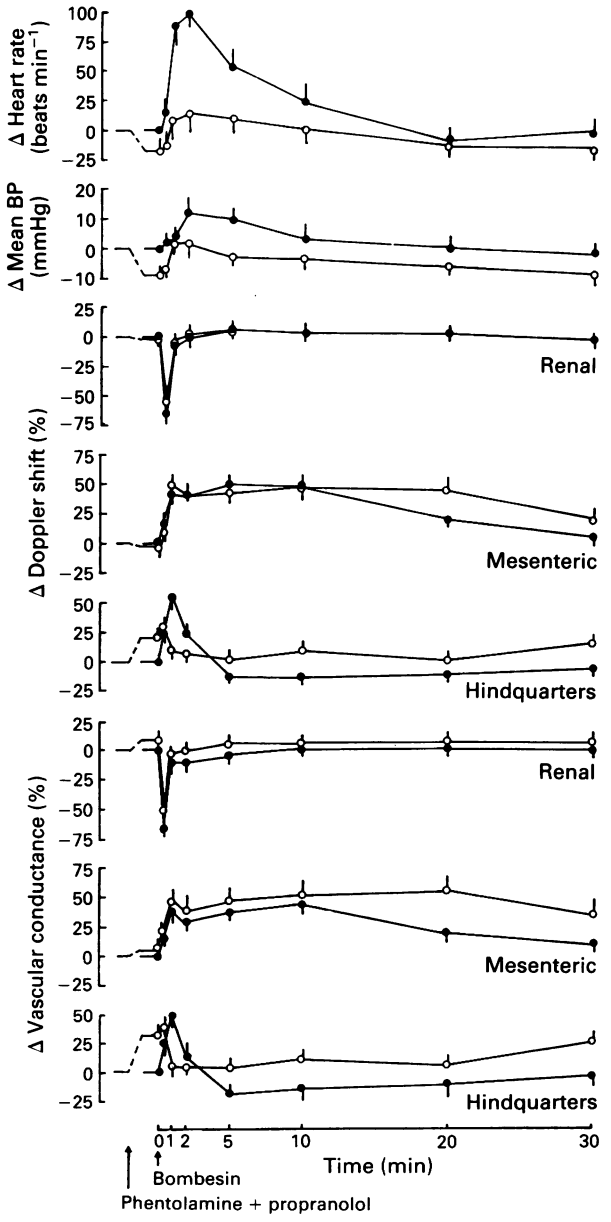

Figure 3 Haemodynamic responses to bombesin (2.5 μg kg^{-1}, bolus, i.v.) in conscious, unrestrained, Long Evans rats, in the absence, (●), or in the presence of phentolamine and propranolol, (○). The dotted line represents the response to infusions of phentolamine and propranolol. Values are mean ($n = 8$) with s.e.mean shown by vertical lines. All statistics are given in the text.

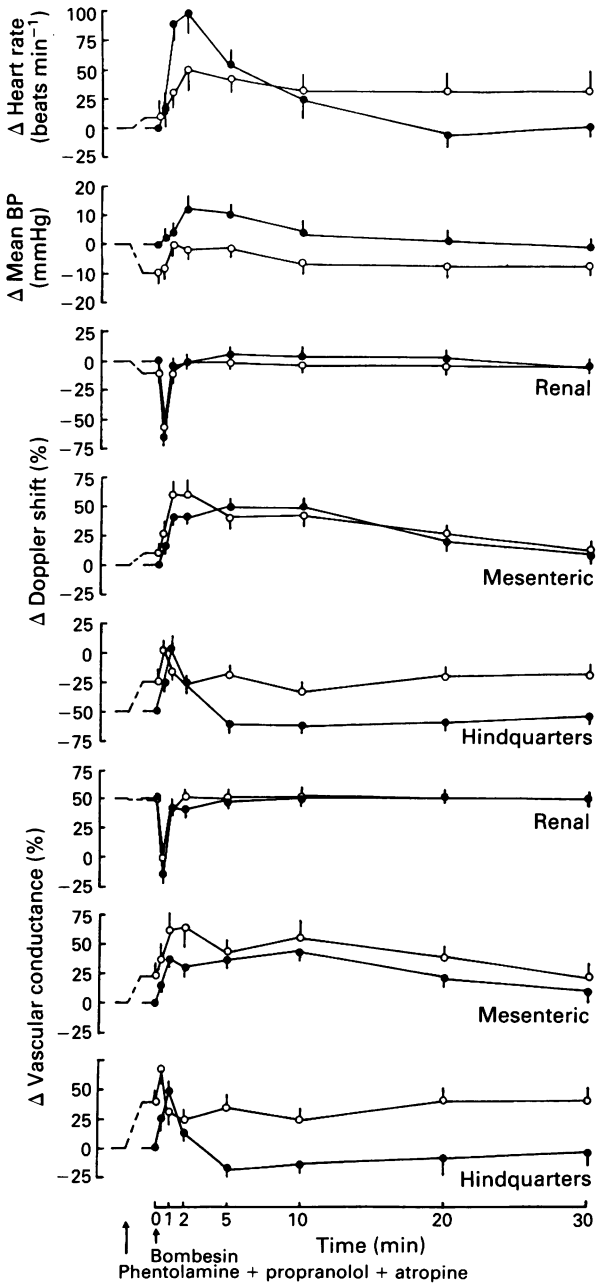

Figure 4 Haemodynamic responses to bombesin (2.5 $\mu g\,kg^{-1}$, bolus, i.v.) in conscious, unrestrained, Long Evans rats, in the absence, (●), or in the presence of phentolamine, propranolol and atropine, (○). The dotted line represents the response to phentolamine, propranolol and atropine administration. Values are mean ($n = 8$) with s.e.mean shown by vertical lines. All statistics are given in the text.

tration there was a persistent bradycardia, a decrease in blood pressure, an increase in hindquarters flow, and renal, mesenteric and hindquarters vasodilatations (all significant, $P < 0.05$).

In the presence of phentolamine and propranolol, bombesin caused a tachycardia (significant at 1–10 min, $P < 0.05$) which was less (significant at 1–2 min, $P < 0.05$), and a pressor response (significant at 1–10 min, $P < 0.05$) which was greater (significant at 1 min, $P < 0.05$), than in rats receiving bombesin alone. The renal vasoconstriction (significant at 0.5 min, $P < 0.05$) was similar to that in untreated animals, but the mesenteric vasodilatation (significant at 0.5–30 min, $P < 0.05$) was larger (significant at 20 min, $P < 0.05$). The transient hindquarters vasodilatation seen in untreated rats was absent, but a prolonged hindquarters vasoconstriction (significant at 1–20 min, $P < 0.05$) occurred.

Haemodynamic responses to bombesin (2.5 $\mu g\,kg^{-1}$) in the presence of phentolamine, propranolol and atropine (Figure 4) Thirty min after the onset of atropine administration, in the presence of phentolamine and propranolol, blood pressure was decreased ($P < 0.05$), but heart rate was not different from baseline. There was a decrease in renal and an increase in hindquarters flow, together with mesenteric and hindquarters vasodilatations (all significant, $P < 0.05$).

In the presence of phentolamine, propranolol and atropine, bombesin caused a sustained tachycardia (significant at 2–30 min, $P < 0.05$), but the maximum tachycardic response was smaller (significant at 1–2 min, $P < 0.05$) than in untreated rats. However, subsequently the tachycardia was greater (significant at 20 min, $P < 0.05$) than in untreated rats. The pressor response (significant at 1–10 min, $P < 0.05$) was slightly greater (significant at 1 min, $P < 0.05$), the renal vasoconstriction (significant at 0.5 min, $P < 0.05$) smaller, and the mesenteric vasodilatation (significant at 1–2 and 10 min, $P < 0.05$) not different, from those in untreated rats. The hindquarters showed a vasodilatation (significant at 0.5 min, $P < 0.05$) followed by a vasoconstriction (significant at 2 and 10 min, $P < 0.05$). The change in hindquarters vascular conductance was less (significant at 1 min, $P < 0.05$) than in untreated rats.

Cardiovascular effects of cholecystokinin

Haemodynamic responses to CCK (0.5 $\mu g\,kg^{-1}$) in the absence or the presence of L364,718 (Figure 5) After administration of CCK there was a tachycardia (significant at 1–10 min, $P < 0.05$) and a short-lived rise in blood pressure (significant at 0.25–0.5 min, $P < 0.05$). Renal (significant at 0.25–1 min, $P < 0.05$) and hindquarters (significant at 1–2 min, $P < 0.05$) flows were increased slightly, whereas mesenteric flow initially showed a decrease (significant at 0.25–1 min, $P < 0.05$) followed by a small increase (significant at 5–10 min, $P < 0.05$). Vasoconstrictions occurred in the renal (significant at 0.5 min, $P < 0.05$), mesenteric (significant at 0.25–1 min, $P < 0.05$) and hindquarters (significant at 0.5 min, $P < 0.05$) vascular beds. Subsequently there were mesenteric (significant at 5 min, $P < 0.05$) and hindquarters (significant at 1–2 min, $P < 0.05$) vasodilatations.

Pretreatment with the CCK receptor antagonist did not affect baseline values, but in its presence CCK (0.5 $\mu g\,kg^{-1}$) had no significant effects ($n = 8$, data not shown).

Haemodynamic responses to CCK (5.0 $\mu g\,kg^{-1}$) alone or in the presence of vehicle or L364,718 (Figures 6 and 7) Figure 6 shows the effects of CCK in a single rat, and Figure 7 shows the group mean results.

CCK caused a bradycardia (significant at 0.5–1 min, $P < 0.05$) followed by a tachycardia (significant at 5–10 min, $P < 0.05$). The bradycardia was accompanied by a marked increase in blood pressure (significant at 0.25–1 min, $P < 0.05$). Renal flow was increased slightly (significant at 1–2 min, $P < 0.05$; maximum $+6 \pm 2\%$) while initial decreases in mesenteric (significant at 0.25–1 min, $P < 0.05$) and hindquarters (significant at 0.25–0.5 min, $P < 0.05$) flows were followed by increases (mesenteric significant at 5–10 min, $P < 0.05$; hindquarters significant at 1–2, 10 min, $P < 0.05$). There were vasoconstrictions in renal (significant at 0.25–0.5 min, $P < 0.05$), mesenteric (significant at 0.25–2 min, $P < 0.05$) and hindquarters (significant at 0.25–0.5 min, $P < 0.05$) vascular beds, followed by vasodilatations (renal (significant at 2–5 min, $P < 0.05$); mesenteric (significant at 5–10 min, $P < 0.05$); hindquarters (significant at 2 min, $P < 0.05$)).

Pretreatment with vehicle did not cause any changes in baseline values or in the responses to CCK ($n = 8$, data not shown).

In rats pretreated with L364,718, CCK caused an initial tachycardia (significant at 1 min, $P < 0.05$), i.e. the opposite of that which was seen in untreated rats (significant at 0.25–

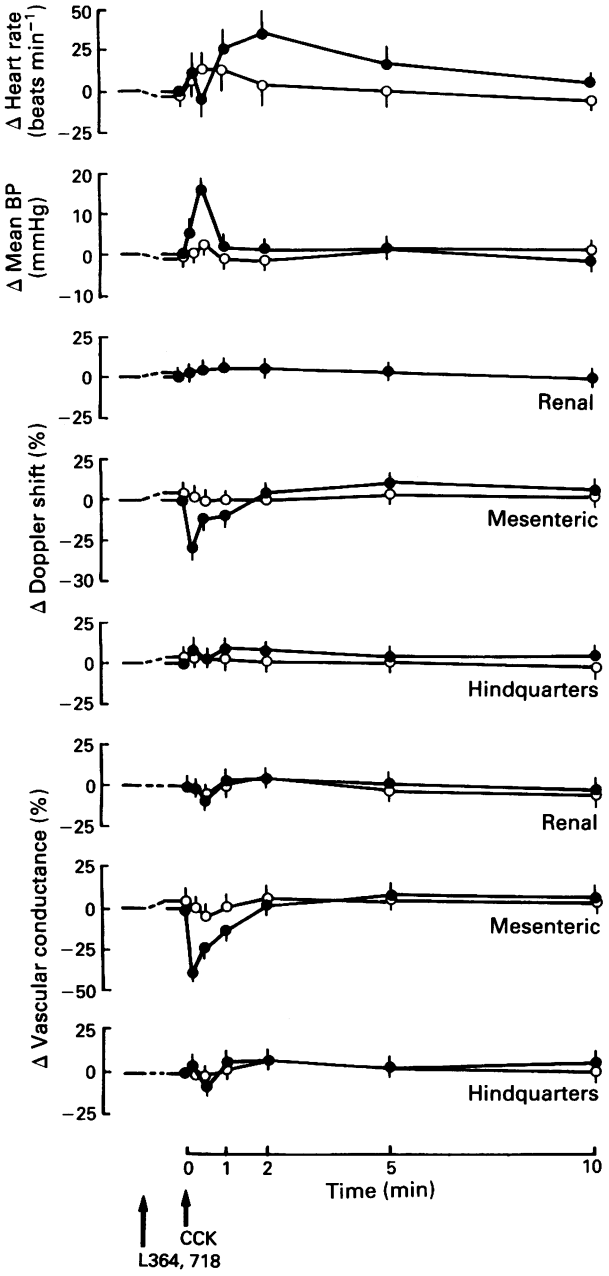

Figure 5 Haemodynamic responses to cholecystokinin (CCK, 0.5 $\mu g\,kg^{-1}$, bolus, i.v.) in conscious, unrestrained, Long Evans rats, in the absence, (●), or in the presence of L364,718, (○). The dotted line shows the response to L364,718. Values are mean ($n = 8$) with s.e.mean shown by vertical lines. All statistics are given in the text.

1 min, $P < 0.05$). The pressor response (significant at 0.5 min, $P < 0.05$) was smaller (significant at 0.25–1 min, $P < 0.05$) than in untreated rats. Changes in renal and hindquarters vascular conductances were not significant, while the mesenteric vasoconstriction (significant at 0.5 min, $P < 0.05$) was smaller (significant at 0.25–1 min, $P < 0.05$) than in untreated rats, and the subsequent mesenteric vasodilatation, seen in untreated rats, did not occur.

Haemodynamic responses to CCK (5.0 $\mu g\,kg^{-1}$) in the presence of a vasopressin V_1-receptor antagonist Pretreatment with the vasopressin V_1-receptor antagonist did not change baseline values or the haemodynamic responses to CCK ($n = 8$, data not shown).

Haemodynamic responses to CCK (5.0 $\mu g\,kg^{-1}$) in the presence of phentolamine (Figure 8) Seventy-five min after the onset of phentolamine administration there was a marked tachycardia

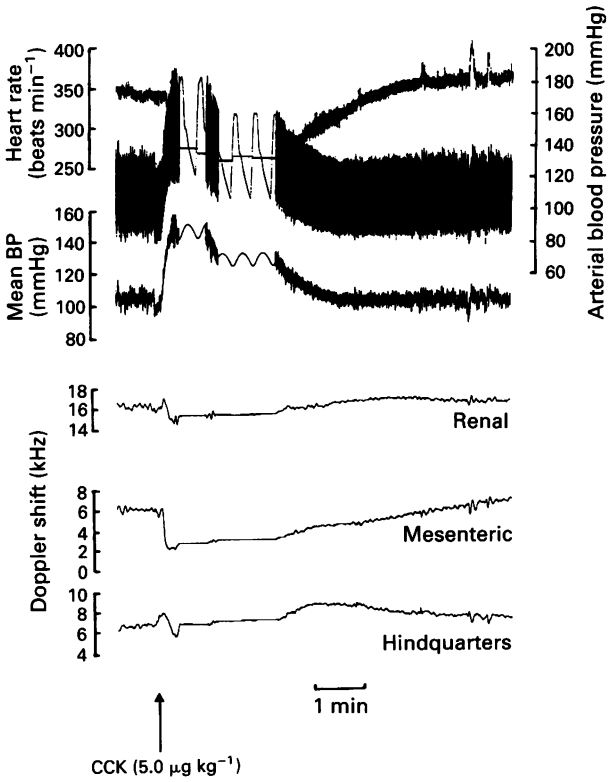

Figure 6 Haemodynamic responses to cholecystokinin (CCK, 5.0 $\mu g\,kg^{-1}$, bolus, i.v.) in a conscious, unrestrained, Long Evans rat.

and reductions in blood pressure and in renal and mesenteric flows. There were renal and mesenteric vasoconstrictions, and a hindquarters vasodilatation (all significant, $P < 0.05$).

In the presence of phentolamine, CCK caused a bradycardia (significant at 0.5 min, $P < 0.05$) and an increase in blood pressure (significant at 0.5 min, $P < 0.05$) that were less (significant at 0.5 min, $P < 0.05$) than in untreated rats. Furthermore, there was a subsequent hypotension (significant at 1–2 min, $P < 0.05$), which did not occur in untreated rats. The renal vasoconstriction observed in untreated rats was absent, but a vasodilatation (significant at 1–2 min, $P < 0.05$) occurred. Changes in mesenteric vascular conductance were absent, and there was no hindquarters vasoconstriction, but the hindquarters vasodilatation (significant at 1–2 min, $P < 0.05$) was greater (significant at 1–2 min, $P < 0.05$) than in untreated rats.

Haemodynamic responses to CCK (5.0 $\mu g\,kg^{-1}$) in the presence of phentolamine and propranolol (Figure 9) Seventy-five min after the onset of phentolamine and propranolol administration there was bradycardia, hypotension, and decreases in renal and mesenteric flows, together with hindquarters hyperaemia and vasodilatation, (all significant, $P < 0.05$).

In the presence of phentolamine and propranolol, CCK caused a rise in mean blood pressure (significant at 0.5–1 min, $P < 0.05$) and a bradycardia (significant at 0.5–1 min, $P < 0.05$) followed by a tachycardia (significant at 5–10 min, $P < 0.05$); the bradycardia and the pressor effect were less (significant at 0.5–1 min and 0.25–1 min, respectively $P < 0.05$) than in untreated animals. All vascular beds showed vasoconstrictions but the mesenteric (significant at 0.25–0.5 min, $P < 0.05$) and the hindquarters (significant at 0.5 min, $P < 0.05$) vasoconstrictions were less than in untreated rats. The late hindquarters vasodilatation seen in untreated rats was absent.

Haemodynamic responses to CCK (5.0 $\mu g\,kg^{-1}$) in the presence of phentolamine, propranolol and atropine (Figure 10) Seventy-five min after the onset of atropine adminis-

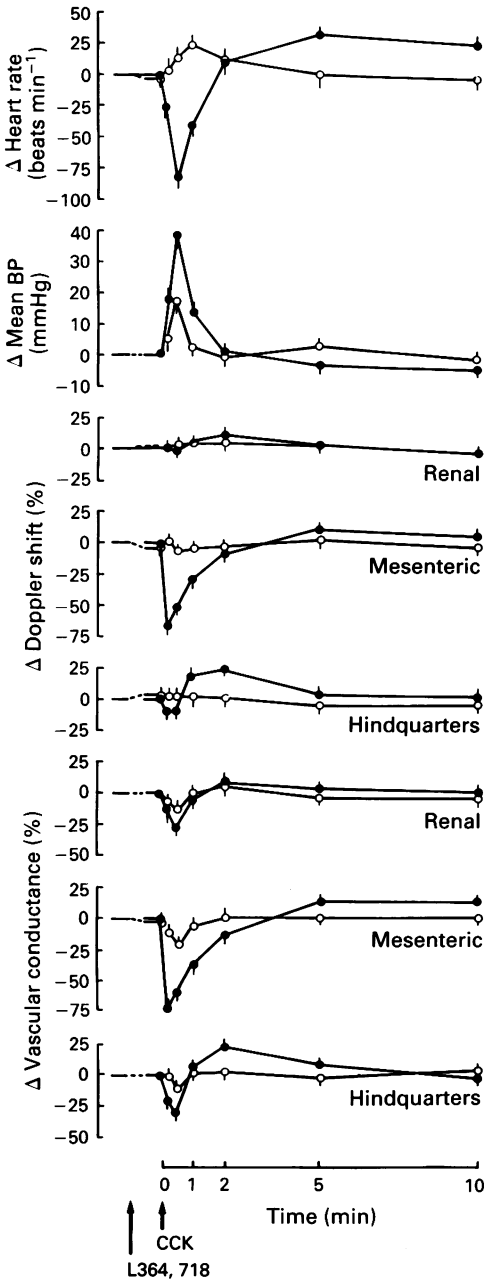

Figure 7 Haemodynamic responses to cholecystokinin (CCK, 5.0 $\mu g\,kg^{-1}$, bolus, i.v.) in conscious, unrestrained, Long Evans rats, in the absence, (●), or in the presence of L364,718, (○). The dotted line represents the response to L364,718 before CCK was given. Values are mean ($n = 8$) with s.e.mean shown by vertical lines. All statistics are given in the text.

tration in the presence of phentolamine and propranolol, heart rate and blood pressure were not different from baseline, but there were rises in mesenteric and hindquarters flows, together with vasodilatations (all significant, $P < 0.05$).

In the presence of phentolamine, propranolol and atropine, CCK caused a bradycardia (significant at 0.5–1 min, $P < 0.05$) and a rise in blood pressure (significant at 0.5 min, $P < 0.05$) that were smaller (significant at 0.5–1 min and 0.5 min, respectively $P < 0.05$) than in untreated rats. Renal (significant at 0.5 min, $P < 0.05$) and mesenteric (significant at 0.5–2 min, $P < 0.05$) vasoconstrictions occurred, but the latter was smaller (significant at 0.25–0.5 min, $P < 0.05$) than in untreated rats. The hindquarters vascular bed showed no initial vasoconstriction or subsequent vasodilatation, unlike untreated rats (significantly different at 0.5 and 2 min, $P < 0.05$).

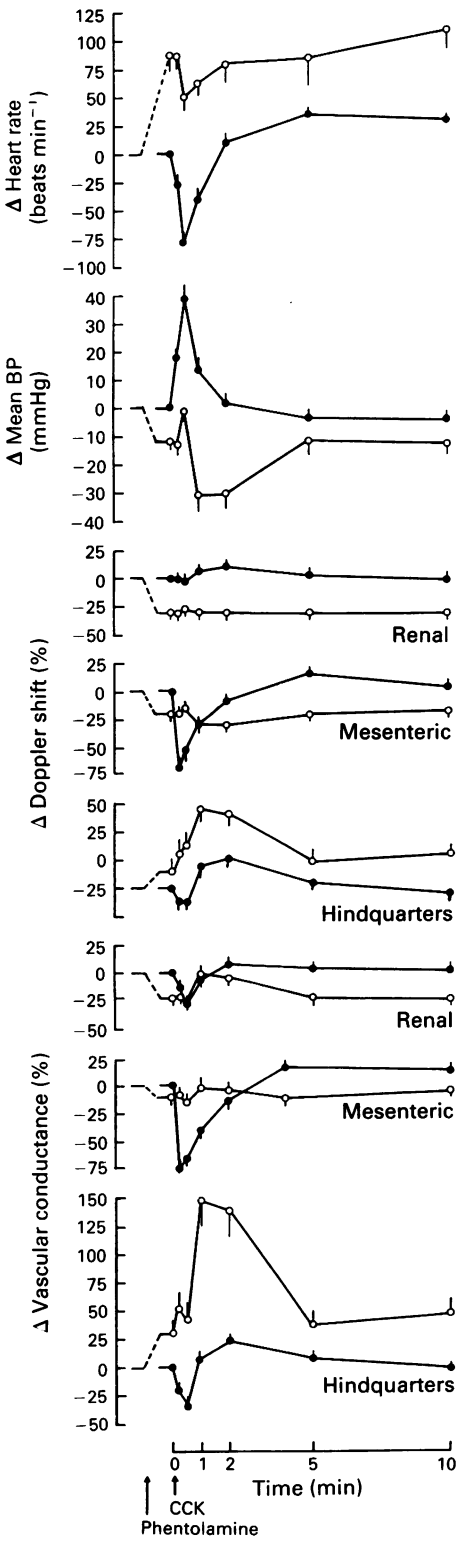

Figure 8 Haemodynamic responses to cholecystokinin (CCK, 5.0 $\mu g\,kg^{-1}$, bolus, i.v.) in conscious, unrestrained, Long Evans rats, in the absence, (●), or in the presence of phentolamine, (○). The dotted line represents the response to phentolamine. Values are mean ($n = 8$) with s.e.mean shown by vertical lines. All statistics are given in the text.

Discussion

The present work has shown that i.v. administration of the neuropeptides, bombesin and CCK, can have substantial haemodynamic effects in conscious Long Evans rats. However, such experiments do not necessarily provide information about putative physiological effects of bombesin and CCK.

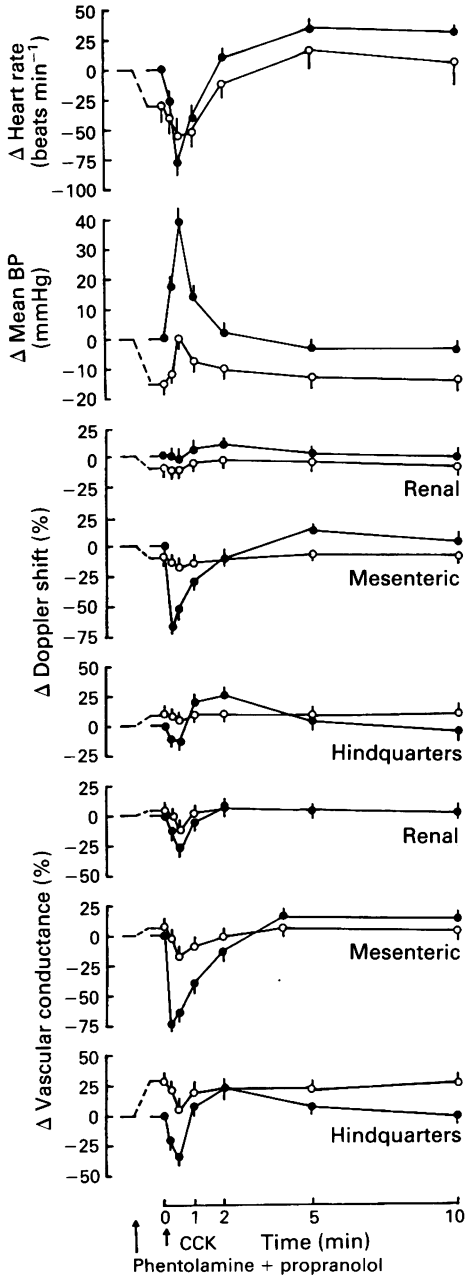

Figure 9 Haemodynamic responses to cholecystokinin (CCK, 5.0 $\mu g\,kg^{-1}$, bolus, i.v.) in conscious, unrestrained, Long Evans rats, in the absence, (●), or in the presence of phentolamine and propranolol, (○). The dotted line represents the response to phentolamine and propranolol. Values are mean ($n = 8$) with s.e.mean shown by vertical lines. All statistics are given in the text.

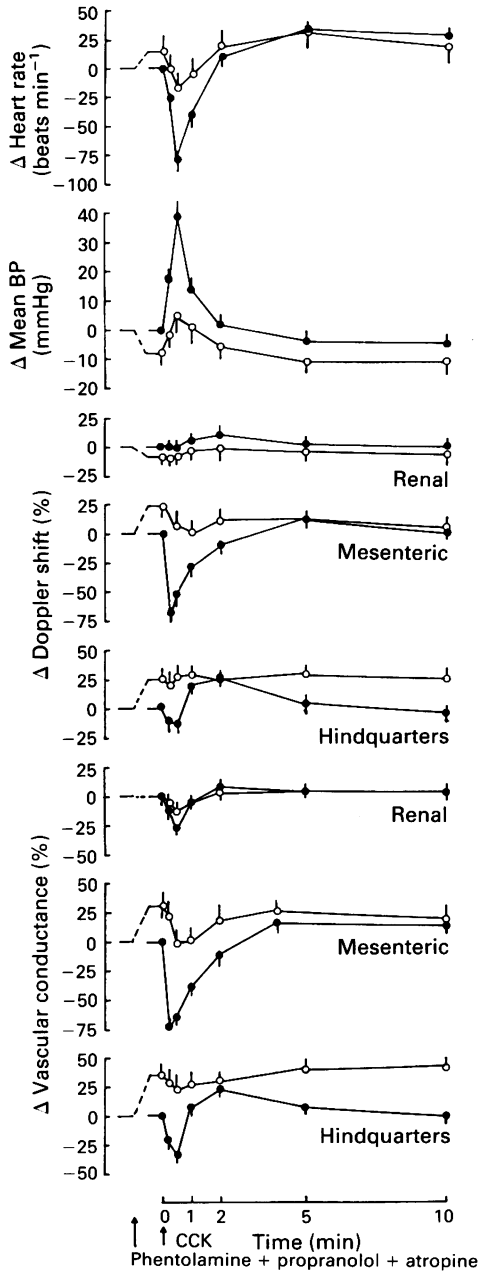

Figure 10 Haemodynamic responses to cholecystokinin (CCK, 5.0 $\mu g\,kg^{-1}$, bolus, i.v.) in conscious, unrestrained, Long Evans rats, in the absence, (●), or in the presence of phentolamine, propranolol and atropine, (○). The dotted line represents the response to phentolamine, propranolol and atropine. Values are mean ($n = 8$) with s.e.mean shown by vertical lines. All statistics are given in the text.

Indeed, there is no evidence that CCK or the mammalian equivalent of bombesin are circulating cardiovascular hormones. Furthermore, it is not possible to assess the extent to which any paracrine effects of such endogenous peptides would be simulated by i.v. administration of the exogenous peptides. Thus, the major aim of our study was to characterize the regional haemodynamic profiles of the peptides given i.v. and then, with the use of pharmacological interventions, to attempt to delineate the mechanisms contributing to the effects seen.

Haemodynamic effects of bombesin

In this study, in conscious, Long Evans rats, bombesin (2.5 $\mu g\,kg^{-1}$, i.v.) caused a tachycardia, an increase in blood pressure, a short-lasting renal vasoconstriction and a sustained mesenteric vasodilatation; there was an initial hindquarters vasodilatation followed by a vasoconstriction (Table 3). As noted above (see Methods and Table 1), bombesin did not cause dose-dependent effects possibly because, in the conscious intact rats investigated, baroreflex mechanisms were acting to oppose its actions (see below). However, Erspamer *et al.* (1972) also noted a variable dose-dependency in the pressor effects of bombesin in anaesthetized rats.

Phentolamine attenuated the tachycardic response to bombesin, and converted its pressor action into a depressor effect (Table 3). The latter would have been expected to cause a greater baroreflex-mediated tachycardia, and hence the reduced tachycardia observed may have been due to the fact that in the presence of phentolamine heart rate was increased already. It is possible also that cardiac α-adrenoceptors (Bennett & Kemp, 1978; Flavahan & McGrath, 1982; Tung *et al.*, 1982; 1985) were involved in the tachycardic responses to bombesin.

Table 3 Patterns of cardiovascular changes elicited by bombesin (2.5 $\mu g\,kg^{-1}$) under the conditions of the experiments described in the Results

Condition	*Bombesin alone*	*Phentolamine + bombesin*	*Phentolamine + propranolol + bombesin*	*Phentolamine + propranolol + atropine + bombesin*
Heart rate	↑+++	↑++	↑+	↑++
Mean blood pressure	↑++	↓+++	↑++	↑++
Renal flow	↓+++	↓++	↓+++	↓+++
Mesenteric flow	↑+++	↑+	↑+++	↑+++
Hindquarters flow	↑++	↑++	↓++	↑+
Renal conductance	↓+++	↓+	↓+++	↓++
Mesenteric conductance	↑++	↑++	↑+++	↑++
Hindquarters conductance	↑++↓+	↑+++	↓++	↑+↓+

The directions of the arrows for each variable represent the directions of the changes; biphasic changes are represented by a sequence of arrows in different directions. The relative magnitudes of the changes are indicated by + signs.

The increase in heart rate following bombesin was further attenuated by propranolol in the presence of phentolamine (Figure 3, Table 3), consistent with the findings of Bayorh & Feuerstein (1985). However, the duration of the tachycardia was prolonged by atropine in the presence of phentolamine and propranolol, possibly due to suppression of reflex vagal influences. Bearing in mind the changes in the blood pressure profiles in these various conditions (see below), it is likely that the heart changes elicited by bombesin were influenced by circulating catecholamines (Fisher *et al.*, 1985) and by cardiac baroreflexes exerting actions through adrenoceptors and muscarinic receptors (to different extents in different experiments). Following antagonism of these receptors (accepting competitive antagonists were used) there remained a sizeable (about half that seen in the unblocked conditions) and prolonged tachycardic response to bombesin (Table 3). It is feasible that this was due to a direct cardiac action of the peptide and/or an indirect effect mediated through non-adrenergic, non-cholinergic (NANC) mechanisms, possibly involving histamine (Bayorh & Feuerstein, 1985).

The pressor response to bombesin in the presence of phentolamine and propranolol was similar to that seen in the unblocked state (Table 3) (albeit against a different profile of haemodynamic changes). Thus, it appears likely that the marked hypotensive response to bombesin in the presence of phentolamine alone (Table 3) was due to an enhancement of β-adrenoceptor-mediated vasodilator responses (as a consequence of antagonism of prejunctional, α-adrenoceptor autoinhibitory effects, in the presence of suppression of post-junctional α-adrenoceptor vasoconstrictor influences), rather than to unmasking of the normal degree to which vasodilator β-adrenoceptor-mediated mechanisms were involved in the unblocked state. This is consistent with the substantial augmentation of the hindquarters vasodilator response to bombesin seen in the presence of phentolamine (Table 3). However, it is clear also that the hindquarters response to bombesin alone involved an early vasodilator component that was susceptible to blockade by propranolol (Table 3). It is feasible this was due to neurally released and/or circulating catecholamines (Fisher *et al.*, 1985) acting on post-junctional β_2-adrenoceptors, since there is evidence that in the hindquarters vascular bed this dilator mechanism is particularly well-developed (Gardiner & Bennett, 1988).

As mentioned above, in the presence of phentolamine and propranolol the pressor effect of bombesin was little different from normal. However, this was against a background of diminished tachycardia and an abolition of the hindquarters vasodilatation, together with persistence of the vasoconstrictor response to bombesin in this vascular bed (Table 3). Thus, it appears bombesin could have had NANC vasoconstrictor effects in the hindquarters vascular bed, although, since flow did not fall below baseline (in the presence of phentolamine, propranolol and atropine) it is feasible the vasoconstriction was autoregulatory. However, skeletal muscle does not usually show well-developed autoregulation (Heistad & Abboud, 1974).

In the renal vascular bed phentolamine alone caused some attenuation of the vasoconstriction following administration of bombesin, but the degree to which this reflected the involvement of α-adrenoceptor mechanisms in the change in renal vascular conductance following bombesin alone is difficult to assess since the marked fall in blood pressure following bombesin administration in the presence of phentolamine (Table 3) would have enhanced baroreflex-mediated sympathetic efferent outflow to the kidney. However, in the presence of phentolamine, propranolol and atropine there was only slight attenuation of the bombesin-induced renal vasoconstriction (even though blood pressure did not fall; Table 3), so NANC mechanisms must have featured large in this response. While it is feasible the renin-angiotensin system was involved (Melchiorri *et al.*, 1971), the time course of change of renal flow and conductance following bombesin administration makes it more likely these changes were due to a direct renal action of bombesin (Melchiorri *et al.*, 1971; Erspamer & Melchiorri, 1973) and/or to the involvement of neural mechanisms influencing the vasculature of the kidney through NANC pathways. The marked actions of bombesin on renal blood flow could contribute to its reported antidiuretic effect (Melchiorri *et al.*, 1971; Erspamer & Melchiorri, 1973).

Under all conditions the relatively slow-onset, persistent, mesenteric vasodilator response to bombesin was unaffected (Table 3) (although the increase in flow was reduced when bombesin caused a fall in blood pressure in the presence of phentolamine). These observations are consistent with bombesin exerting direct, and/or indirect, NANC effects to increase mesenteric vascular conductance. It is feasible that such an influence was mediated through release of other gut hormones (Walsh, 1989) and a possible candidate is corticotropin-releasing hormone (CRH), since this peptide exerts profound superior mesenteric vasodilator effects (Gardiner *et al.*, 1988b). However, CRH is not likely to have been responsible for the putative NANC effects of bombesin in the renal (or hindquarters) vascular beds, since exogenous CRH has little effect in these regions (Gardiner *et al.*, 1988b).

Overall, the present results are consistent with bombesin influencing cardiac activity through sympatho-adrenal mechanisms and also exerting NANC effects on the heart and renal (constriction), mesenteric (dilatation) and , possibly, hindquarters (constriction) vascular beds. In the hindquarters vascular bed early vasodilatation was probably due to circulating adrenaline (Fisher *et al.*, 1985) acting on β_2-adrenoceptors.

The work of Guarini and colleagues (1989) indicated that, following haemorrhage, the pressor effects of bombesin were due to CCK release since the CCK antagonist, L364,718,

Table 4 Patterns of cardiovascular changes elicited by cholecystokinin (CCK, 5.0 $\mu g kg^{-1}$) under the conditions of the experiments described in the Results

Condition	*CCK alone*	*Phentolamine + CCK*	*Phentolamine + propranolol + CCK*	*Phentolamine + propranolol + atropine + CCK*
Heart rate	↓+++ ↑++	↓+	↓++ ↑++	↓+
Mean blood pressure	↑+++	↑+ ↓++	↑++	↑++
Renal flow	↑+	→	→	→
Mesenteric flow	↓+++ ↑+	→	→	↓+
Hindquarters flow	↓+ ↑++	↑+++	→	→
Renal conductance	↓++ ↑+	↑++	↓+	↓+
Mesenteric conductance	↓+++ ↑++	→	↓+	↓++
Hindquarters conductance	↓++ ↑++	↑+++	↓+	↓+

The directions of the arrows for each variable represent the directions of the changes; biphasic changes are represented by a sequence of arrows in different directions. The relative magnitudes of the changes are indicated by + signs. Where an arrow is shown horizontal there was no significant change in that variable under that condition.

abolished the influence of bombesin on blood pressure. In the present work, in conscious normotensive rats, L364,718, in the same dose as used by Guarini *et al.* (1989), had no effect on the cardiovascular actions of bombesin. Hence it is unlikely that endogenous CCK was responsible for the NANC effects of bombesin in the present experiments. However, these results do not preclude the possibility that bombesin releases CCK (Ghatei *et al.*, 1982; Guarini *et al.*, 1989; Walsh, 1989) in amounts insufficient to influence haemodynamic status in conscious, Long Evans rats.

Haemodynamic effects of CCK

CCK caused dose-dependent pressor effects associated with renal, mesenteric and hindquarters vasoconstrictions, followed (after the higher dose) by vasodilatations. After the low dose, heart rate increased, but after the high dose there was a bradycardia followed by a tachycardia (Table 4).

The bradycardic effect of the high dose of CCK was attenuated in the presence of phentolamine, as was the pressor action of the peptide (Table 4) indicating the bradycardia may have been dependent, in part, on baroreflex mechanisms as observed in the pentobarbitone-anaesthetized dog (Koyama *et al.*, 1990). However, the occurrence of a bradycardia in the presence of phentolamine, propranolol and atropine (Table 4) suggests that, in the rat, CCK has direct, and/or indirect, NANC, negative chronotropic effects, as reported by Marker & Roberts (1988). The late tachycardic effect of the higher dose of CCK occurred independently of changes in blood pressure, and it was blocked by atropine (Table 4), thus it is feasible that the increase in heart rate was due to a non-baroreflex-mediated inhibition of vagal tone. Since the low dose of CCK had only a tachycardic effect and since only tachycardia was seen following the higher dose of CCK in the presence of L364,718, it seems that a direct and/or indirect action of CCK to inhibit cardiac vagal tone was exerted at a lower dose than its NANC negative chronotropic effects. Central administration of CCK causes tachycardia in chloralose-anaesthetized cats (Pagani *et al.*, 1982), so may be the atropine-sensitive tachycardia seen here was mediated centrally, although it is equally feasible it was due to an action of CCK on vagal afferent function (Zarbin *et al.*, 1981).

The marked pressor effect of CCK was substantially reduced in the presence of phentolamine and there was a pronounced secondary depressor response that was absent in the additional presence of propranolol (Table 4). As with bombesin, the likely explanation of these findings is that β-adrenoceptor-mediated vasodilator effects (see below) were augmented in the presence of phentolamine and the cardiovascular effects of CCK under these conditions probably were not a true representation of the contribution of β-adrenoceptor-mediated mechanisms to the responses to CCK alone. However, the attenuation of the pressor effects of CCK in the presence of phentolamine and propranolol indicates that a large part of the increase in blood pressure following CCK administration alone was probably due to increased sympathetic efferent activity (Koyama *et al.*, 1990) causing α-adrenoceptor-mediated vasoconstriction, in contrast to the mechanisms involved in the pressor response to bombesin (see above).

In response to CCK, the hindquarters showed a vasoconstriction followed by a vasodilatation, associated with a reduction and an increase in flow, respectively (Table 4). In the presence of phentolamine, CCK caused a marked hyperaemic vasodilatation in the hindquarters that was absent in the additional presence of propranolol (Table 4). It is likely the hindquarters vasodilator response to CCK was responsible for the fall in blood pressure seen in the presence of phentolamine. The hindquarters vasoconstriction that occurred in response to CCK in the presence of phentolamine and propranolol was not associated with a reduction in blood flow below resting levels (Table 4) and hence it could have been an autoregulatory change. Thus it appears CCK did not exert NANC effects in the hindquarters vascular bed. Collectively, these findings indicate CCK caused hindquarters vasoconstriction through activation of postjunctional α-adrenoceptor-mediated mechanisms and, following blockade of these and prejunctional, autoinhibitory α-adrenoceptors, β-adrenoceptor-mediated hindquarters vasodilatation was augmented. The different patterns of hindquarters response to CCK (vasoconstriction followed by vasodilatation) and bombesin (vasodilatation followed by vasoconstriction) are consistent with the possibility that the effects of CCK on the hindquarters were mediated largely through activation of sympathetic efferent outflow to that vascular bed whereas those of bombesin were due in greater part to adrenal medullary adrenaline release (Fisher *et al.*, 1985).

Under all conditions, there were only slight changes in renal blood flow following administration of CCK (Table 4) and, therefore, all the changes in vascular conductance seen could have been autoregulatory. This picture contrasts sharply with that observed following bombesin administration and indicates that CCK did not exert any specific direct and/or indirect effects on the renal vasculature as assessed here. However, it is feasible that CCK influenced intrarenal blood flow, for example, without affecting total renal blood flow.

In the presence of phentolamine, CCK caused no significant reductions in mesenteric blood flow or vascular conductance (Table 4). Therefore, the mesenteric vasoconstriction following CCK administration in the absence of phentolamine (Table 4) was probably mediated through α-adrenoceptors. However,

there was a slight enhancement of the mesenteric vasoconstrictor effect of CCK in the presence of phentolamine and propranolol, compared to that in the presence of phentolamine alone. This might indicate that any non-adrenergic vasoconstrictor effects of CCK in the latter condition were masked by β-adrenoceptor-mediated vasodilatation. In the presence of phentolamine, propranolol and atropine, CCK caused mesenteric vasoconstriction (Table 4) that was likely to have been responsible for the increase in blood pressure, since there was a bradycardia and the changes in renal and hindquarters vascular conductances were probably autoregulatory (see above). The possibility that this NANC mesenteric vasoconstrictor effect of CCK was due to release of vasopressin seems unlikely since pretreatment with a V_1-receptor antagonist did not significantly affect the haemodynamic responses to CCK. However, it is feasible under those conditions other mechanisms adjusted for the absence of vasopressin. In this context it is of interest that there was a tendency towards a reduction in the peak mesenteric vasoconstriction following CCK administration in the presence of the V_1-receptor antagonist (data not shown). A definite answer should come from a comparison of the cardiovascular responses to CCK in the presence of phentolamine, propranolol and atropine with those in the additional presence of a V_1-receptor antagonist, or with those in Brattleboro rats.

The CCK receptor antagonist, L364,718, abolished the cardiovascular effects of the low dose of CCK and reduced substantially the effects of the higher dose. In fact, there were minimal changes in renal, mesenteric or hindquarters blood flows in response to the higher dose of CCK in the presence of L364,718 and, hence, all changes in vascular conductances could have been autoregulatory. Under these conditions the pressor effect of CCK was most likely due to an increase in cardiac output consequent upon inhibition of cardiac vagal tone (see above). The effectiveness of the dose of L364,718 used against the cardiovascular actions of CCK indicates a major involvement of peripheral A-type receptors (Dourish *et al.*, 1989), although it is feasible the atropine-sensitive, tachycardic effects of CCK were mediated through central B-type receptors (Lotti & Chang, 1989). The substantial effects of L364,718 on the cardiovascular actions of exogenous CCK, and the failure of L364,718 to modify the actions of exogenous bombesin are not consistent with the proposal that release of endogenous CCK is responsible for the cardiovascular actions of exogenous bombesin (Guarini *et al.*, 1989). In addition, the markedly different haemodynamic changes following administration of exogenous bombesin and CCK do not support that proposal.

Finally, during the course of the present experiments several important observations were made regarding the influence of phentolamine, propranolol and atropine on cardiovascular status in conscious Long Evans rats. Administration of phentolamine caused a marked tachycardia, but only a slight fall in blood pressure, accompanied by renal and mesenteric vasoconstrictions and hindquarters vasodilatation. In the additional presence of propranolol the marked tachycardia gave way to a bradycardia, in spite of a further reduction in blood pressure; renal and mesenteric vasoconstrictions were abolished while the hindquarters vasodilatation was unaffected. These results are consistent with antagonism of prejunctional autoinhibitory α-adrenoceptors causing augmentation of sympathetic efferent effects on cardiac β-adrenoceptors. The apparent lack of such a component in the hindquarters vasodilator response to phentolamine is consistent with the hindquarters β_2-adrenoceptor-mediated vasodilator mechanisms (discussed above) being more dependent on adrenal medullary activation than on sympathetic efferent input to that vascular bed. The selective hindquarters vasodilatation following phentolamine indicates the existence of α-adrenoceptor-mediated tone in that vascular bed, but it is likely that any renal and mesenteric vasodilatations due to inhibition of α-adrenoceptor-mediated tone were masked by concurrent vasoconstrictions consequent upon activation of the renin-angiotensin system (i.e. effects antagonized by propranolol). This proposition is consistent with the potent renal and mesenteric vasoconstrictor effects of angiotensin II and its relative lack of effect on the hindquarters vascular bed (Gardiner *et al.*, 1988a).

The resting bradycardia seen in the presence of phentolamine and propranolol was abolished by atropine in spite of the fact that blood pressure was below baseline. Hence it appears that some cardiac vagal tone existed in conscious, Long Evans rats even in the presence of a relative hypotension.

Administration of atropine in the presence of phentolamine and propranolol caused mesenteric vasodilatation (compare Figures 9 and 10). It is possible this was due to activation of an endothelium-dependent mechanism (Thomas *et al.*, 1988), and in this context it is notable that inhibition of nitric oxide (the major endothelium-dependent relaxing factor: Moncada *et al.*, 1988) has particularly potent effects on the mesenteric vascular bed (Gardiner *et al.*, 1990b). An alternative, or additional, explanation for the mesenteric vasodilator effects of atropine is that the latter inhibited vasopressin release (Iitake *et al.*, 1986; Bisset & Chowdrey, 1988; Shoji *et al.*, 1989). Although atropine methyl nitrate does not cross the blood-brain-barrier readily, it is feasible that an inhibitory effect on vasopressin release could have been exerted centrally at a site outside the blood-brain-barrier (Gregg, 1985). Hence, it is likely that in the presence of phentolamine, propranolol and atropine the relative maintenance of blood pressure was dependent largely on NANC activation of the renin-angiotensin system together with vasopressin release (Winn *et al.*, 1985).

The authors thank Merck, Sharp and Dohme Ltd. for the generous gift of L364,718 and Prof. Manning for the gift of the vasopressin V_1-receptor antagonist.

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(Received March 29, 1990
Revised August 16, 1990
Accepted September 26, 1990)