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Involvement of β_2-adrenoceptors in the regional haemodynamic responses to bradykinin in conscious rats

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1 Bradykinin can release neuronal calcitonin gene-related peptide (CGRP) and adrenal medullary catecholamines, both of which could contribute to its cardiovascular effects *in vivo*. Therefore, in the main experiment, regional haemodynamic responses to bolus injections of bradykinin (3 nmol kg^{-1}, i.v.) were assessed in the same chronically-instrumented, conscious, Long Evans rats in the absence and in the presence of human α-CGRP [8-37] or ICI 118551, antagonists of $CGRP_1$-receptors and β_2-adrenoceptors, respectively. The selected doses of these antagonists caused specific inhibition of responses mediated by exogenous human α-CGRP and β_2-adrenoceptor agonists, respectively.

2 Bradykinin administered alone as an i.v. bolus had a slight pressor effect accompanied by a marked tachycardia. There were early (at about 30 s) increases in flow and conductance in the mesenteric vascular bed, and delayed (at about 90 s), but qualitatively similar, changes in the hindquarters vascular bed. There were only slight increases in flow and conductance in the renal vascular bed.

3 Human α-CGRP [8-37] had no statistically significant effects on the responses to bolus doses of bradykinin. However, in the presence of ICI 118551, the pressor effect of bradykinin was significantly enhanced while its tachycardic effect was significantly suppressed. The hindquarters vasodilator effect of bradykinin was converted to a vasoconstriction and there was a slight renal vasoconstriction, but the mesenteric vasodilator effect of bradykinin was unchanged by ICI 118551.

4 In subsidiary experiments, in other animals, it was found that infusion of bradykinin (36 nmol kg^{-1} min^{-1}) elicited a pattern of haemodynamic responses similar to that seen with bolus injections and, as in the latter case, the hindquarters hyperaemic vasodilatation was inhibited by ICI 118551. In the presence of mecamylamine (at a dose sufficient to block reflex heart rate responses to rises or falls in arterial blood pressure) bolus injection or infusion of bradykinin still elicited increases in renal, mesenteric and hindquarters blood flow. However, in additional experiments in adrenal demedullated rats ($n = 4$) the hindquarters hyperaemic effect of bradykinin was absent, although the mesenteric hyperaemic effect remained.

5 The results indicate that the increase in hindquarters blood flow following administration of bradykinin *in vivo* is largely due to activation of β_2-adrenoceptors by catecholamines released subsequent to direct stimulation of the adrenal medulla by the peptide. However, the bradykinin-induced increase in mesenteric blood flow does not depend on this mechanism.

Keywords: Bradykinin; calcitonin gene-related peptide; β_2-adrenoceptors; regional haemodynamics

Introduction

Bradykinin has complex cardiovascular effects *in vivo* (Regoli & Barabé, 1980) that may be direct or indirect. There is evidence that bradykinin causes endothelium-dependent vasorelaxation through release of prostanoids and nitric oxide from endothelial cells (Furchgott, 1983; Palmer *et al.*, 1987). However, in a recent study we found that inhibition of nitric oxide synthesis with the arginine analogue, N^G-nitro-L-arginine methyl ester (L-NAME), left intact a large component of the regional vasodilator responses to bradykinin in conscious rats (Gardiner *et al.*, 1990c). Hence, *in vivo*, it is likely that factors besides nitric oxide contribute to the vasodilator effects of bradykinin.

There is evidence that bradykinin can cause release of catecholamines from the adrenal medulla (Feldberg & Lewis, 1964; Warashina *et al.*, 1990) and of calcitonin gene-related peptide (CGRP) from a specific subset of neurones that are also sensitive to capsaicin (Gepetti *et al.*, 1990). In conscious rats, infusions of CGRP (Gardiner *et al.*, 1989a, b) or β_2-adrenoceptor agonists, including adrenaline (Gardiner *et al.*, 1991a, b), cause marked hyperaemia, particularly in the hindquarters vascular bed where bradykinin also elicits an increase in blood flow. Thus, release of CGRP and/or adrenaline could contribute to the hindquarters haemodynamic effects of bradykinin *in vivo*. Therefore, in the main experiment in the present work we examined the influence of human α-CGRP [8-37], an antagonist of the cardiovascular actions of CGRP (Dennis *et al.*, 1990; Han *et al.*, 1990; Gardiner *et al.*, 1990d; Maggi *et al.*, 1991), and of ICI 118551, an antagonist of β_2-adrenoceptors (Bilski *et al.*, 1983), on the regional haemodynamic effects of bolus injections of bradykinin in conscious rats. We used this protocol since our previous experiments had been concerned with the haemodynamic responses to bolus injections of bradykinin in rats with functional baroreflexes (Gardiner *et al.*, 1990c; Kiff *et al.*, 1991a). However, in subsidiary experiments we also assessed the haemodynamic effects of infusions of bradykinin in the absence and presence of ICI 118551, and the effects of bolus injections and infusions of bradykinin before and after treatment with the ganglion blocker, mecamylamine. Finally, we assessed the haemodynamic effects of bradykinin in animals that had undergone bilateral adrenal demedullation.

Methods

Male, Long Evans rats (350–450 g) were anaesthetized (sodium methohexitone 60 mg kg^{-1}, i.p. supplemented as

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required) and had pulsed Doppler probes (Haywood *et al.*, 1981) implanted to monitor renal, mesenteric and hindquarters blood flows. Animals were given ampicillin (Penbritin, Beecham, 7 mg kg^{-1}, i.m.) after surgery and returned to individual home cages with free access to food and water. At least 7 days later they were re-anaesthetized (sodium methohexitone 40 mg kg^{-1}, i.p.) and had intravenous catheters (3 in the right jugular vein) and an intra-arterial catheter (distal abdominal aorta via the ventral caudal artery) implanted. The probe wires and catheters passed subcutaneously and emerged at the back of the neck. A harness fitted to the rat was connected to a spring through which the catheters ran and to which the probe connector was taped. Animals were returned to their home cages and left to recover for at least 24 h before experiments were begun (Gardiner *et al.*, 1990b, c). During the experiment, animals were connected to a modified pulsed Doppler VF-1 mainframe (Crystal Biotech, Holliston, U.S.A.) (Gardiner *et al.*, 1990a).

All measurements were made in conscious, freely-moving animals, and the following experiments were carried out.

(1) *Effectiveness and selectivity of the antagonistic actions of human a-CGRP [8-37] and ICI 118551*

In previous studies we have shown that human α-CGRP [8-37] (1.5 μmol kg^{-1} min^{-1}) inhibits the haemodynamic actions of infusions of human α-CGRP without influencing responses to isoprenaline (Gardiner *et al.*, 1990d). Furthermore, on the basis of our previous experiences, we judged that ICI 118551 (670 nmol kg^{-1} bolus, 335 nmol kg^{-1} h^{-1} infusion) would be relatively selective for β_2-adrenoceptors (Gardiner & Bennett, 1988a, b, c). However, in order to ensure that our earlier findings with human α-CGRP [8-37] and ICI 118551 were reproducible, and hence applicable to the present studies, we carried out the following experiments.

In some animals ($n = 2$) we assessed cardiovascular responses to bolus injections of human α-CGRP (0.125 nmol kg^{-1}) and adrenaline (1.05 nmol kg^{-1}) before, during (at 20 min), and 60 min after infusion of human α-CGRP [8-37] (1.5 μmol kg^{-1} h^{-1}).

In other animals ($n = 2$) we assessed haemodynamic responses to 3 min infusions of salbutamol (2.1 nmol kg^{-1} min^{-1}), isoprenaline (0.24 nmol kg^{-1} min^{-1}) and sodium nitroprusside (0.14 μmol kg^{-1} min^{-1}) in the presence of atropine (2.7 μmol kg^{-1} bolus, 1.4 μmol kg^{-1} h^{-1} infusion) and again in the presence of ICI 118551 (670 nmol kg^{-1} bolus, 335 nmol kg^{-1} h^{-1} infusion) and atropine. At the end of this experiment we confirmed that the tachycardic effects of sodium nitroprusside were abolished by mecamylamine (20 min after the onset of infusion at 50 μmol kg^{-1} h^{-1}).

(2) *Haemodynamic effects of bolus injections of bradykinin*

This protocol constituted the main experiment. In a group of 8 animals, following a baseline period of 30 min, 2 bolus injections of bradykinin (3 nmol kg^{-1}, i.v.) were given separated by at least 10 min. Sixty min after the second injection an infusion of human α-CGRP [8-37] (1.5 μmol kg^{-1} min^{-1}) (Gardiner *et al.*, 1990d, and see above) was begun and the animals were re-challenged with bradykinin (3 nmol kg^{-1}, i.v.), 10 and 20 min into the infusion. Sixty min after the infusion of human α-CGRP [8-37] had been stopped, a fifth injection of bradykinin was given before primed infusion of ICI 118551 (670 nmol kg^{-1} bolus; 335 nmol kg^{-1} h^{-1} infusion) (Gardiner & Bennett, 1988a, b, c and see above) was started. Bradykinin was injected 60 min after the start of the infusion of ICI 118551. A separate group of animals ($n = 4$) received both the fifth and sixth injections of bradykinin in the absence of ICI 118551 to ensure there was no desensitization to the peptide at this stage in the experiment.

After bradykinin injection, measurements were made at about 30 s (to coincide with the peaks in mean arterial blood pressure, heart rate and mesenteric blood flow), at about 90 s (to coincide with the peak in hindquarters flow) and at about 3 min (when most variables were back to baseline). At each of these time points average values for heart rate, mean arterial blood pressure, and mean renal, mesenteric and hindquarters Doppler shift signals were obtained over a 10 s period. The baseline values were measured over the 20 s preceding the injection of bradykinin. Vascular conductances were calculated from the mean arterial blood pressure and mean Doppler shift signals; % changes in the latter were taken as indices of changes in regional blood flows (Haywood *et al.*, 1981).

(3) *Haemodynamic effects of infusions of bradykinin*

In order to ensure that the cardiovascular responses to bolus injections of bradykinin were not a function of the mode of administration of the peptide we assessed the effects of 3 min infusions of bradykinin (36 nmol kg^{-1} min^{-1}) as well as bolus injections (3 nmol kg^{-1}) in a separate group of animals ($n = 2$). These experiments were carried out before and 60 min after the onset of administration of ICI 118551 (670 nmol kg^{-1} bolus, 335 nmol kg^{-1} h^{-1} infusion).

(4) *Haemodynamic effects of bolus injections and infusions of bradykinin following ganglion blockade*

In order to determine the effects of ganglion blockade on responses to bradykinin we gave bolus injections and infusions of the peptide before and 20 min after onset of an infusion of mecamylamine (50 μmol kg^{-1} h^{-1}). This dose of mecamylamine is 10 fold higher than that used by Wright & Fozard (1990) in anaesthetized rats, but we found their dose in conscious rats did not block the reflex tachycardic effects of sodium nitroprusside (see above).

The experiments involving assessment of the effects of mecamylamine on responses to bradykinin were carried out in the same animals as those in the protocol 3, but on another day. (In pilot experiments we ensured that the effects of ICI 118551 on responses to bradykinin had disappeared within 24 h).

(5) *Haemodynamic effects of bolus injections and infusions of bradykinin in adrenal-demedullated rats*

In a further group of Long Evans rats ($n = 4$) bilateral adrenal demedullation (Gardiner & Bennett, 1988a, b) was carried out at the time the pulsed Doppler probes were implanted. Subsequently, these animals received bolus injections and infusions of bradykinin (as above). After the experiment these animals were killed with an overdose of sodium pentobarbitone (250 mg kg^{-1}) and their adrenal glands were removed. Microscopical examination confirmed the absence of adrenal medullae in all cases, the cores of the glands being occupied by calcified deposits and the remains of blood clots.

Drugs

Human α-CGRP [8-37] was synthesized at Celltech Ltd, using routine procedures (Gardiner *et al.*, 1990d). Bradykinin was obtained from Bachem (UK), ICI 118551 (erythro-(±)-1-[7-methylindan-4-yloxy]-3-isopropyl-aminobutan-2-ol) hydrochloride was a gift from ICI Pharmaceuticals plc, and atropine methyl nitrate, (±)-adrenaline hydrochloride, (±)-isoprenaline hydrochloride, salbutamol hemisulphate, sodium nitroprusside and mecamylamine hydrochloride were obtained from Sigma. Human α-CGRP [8-37] and bradykinin were dissolved in sterile saline (157 mmol l^{-1} NaCl) containing 1% bovine serum albumin (Sigma) and kept frozen (− 80°C) before use. ICI 118551 was dissolved in sterile saline by gentle warming. Atropine, adrenaline, isoprenaline, salbutamol, sodium nitroprusside and mecamylamine were dissolved in sterile saline at room temperature. In the case of

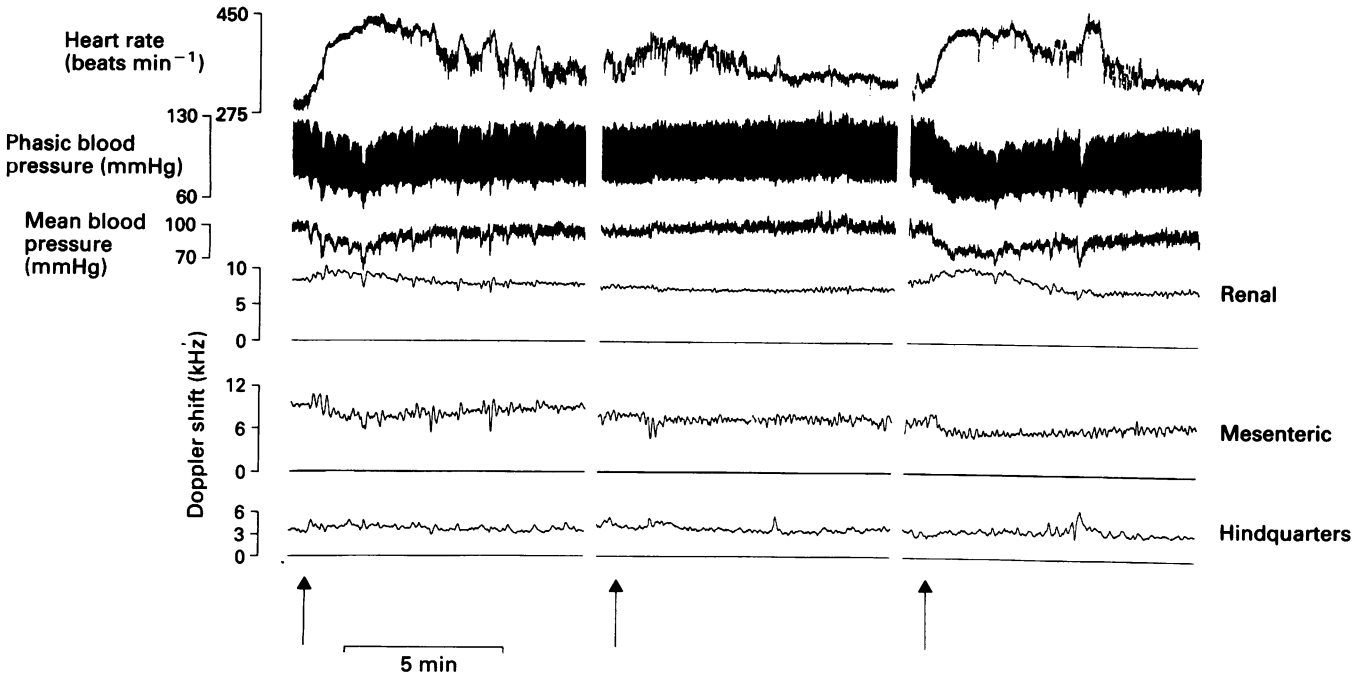

Figure 1 Original tracings showing cardiovascular effects of bolus injections (at arrows) of human α-calcitonin gene-related peptide (α-CGRP) (0.125 nmol kg^{-1}) before (left-hand panel), 20 min after the start of infusion of human α-CGRP [8-37] (1.5 μmol kg^{-1} h^{-1}) (centre panel) and 60 min after the end of infusion of human α-CGRP [8-37] (right-hand panel) in the same conscious, Long Evans rat. The results are representative of 2 experiments.

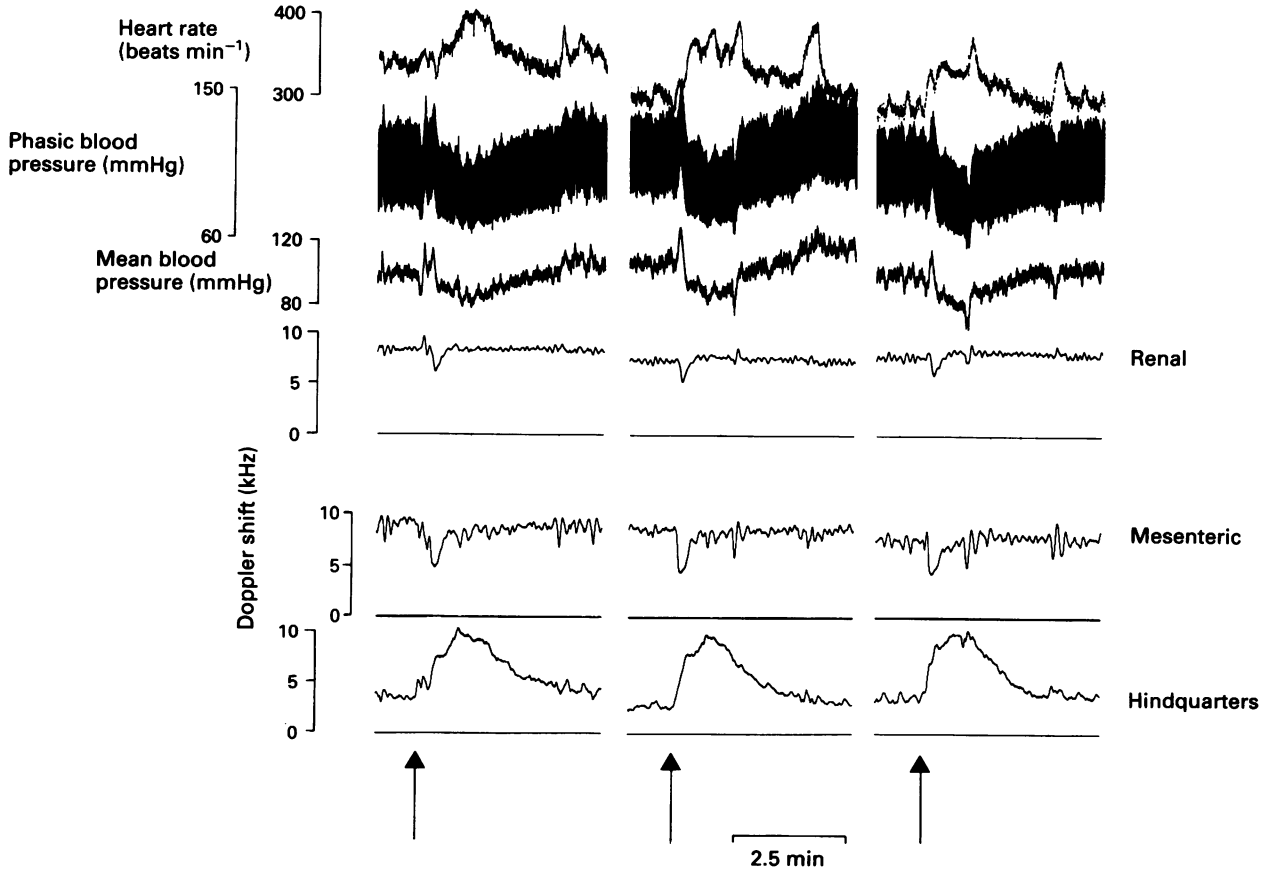

Figure 2 Original tracings showing cardiovascular effects of bolus injections (at arrows) of adrenaline (1.05 nmol kg^{-1}) before (left-hand panel), 20 min after the onset of infusion of human α-calcitonin gene-related peptide [8-37] (α-CGRP [8-37]) (1.8 μmol kg^{-1} h^{-1}) (centre panel) and 60 min after the end of infusion of human α-CGRP [8-37] (right-hand panel) in the same conscious, Long Evans rat. The results are representative of 2 experiments.

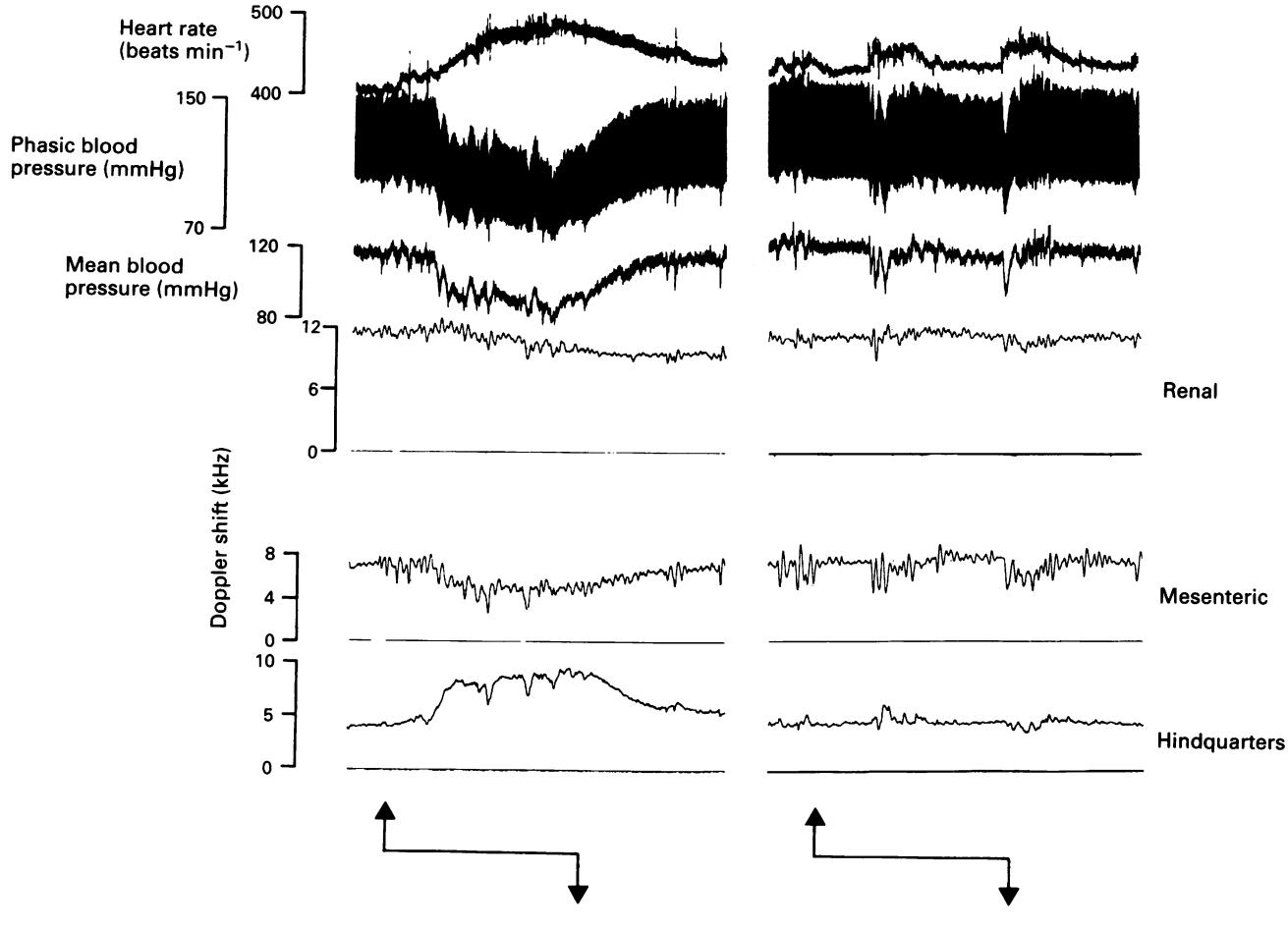

Figure 3 Original tracings showing cardiovascular effects of 3 min infusions (between arrows) of salbutamol (2.1 nmol kg^{-1} min^{-1}) before (left-hand panel) and 60 min after the start of ICI 118551 administration (670 nmol kg^{-1} bolus, 335 nmol kg^{-1} h^{-1} infusion) (right-hand panel) in the same conscious, atropine-pretreated, Long Evans rat. The results are representative of 2 experiments.

adrenaline, isoprenaline and salbutamol the saline contained ascorbic acid (1 μmol l^{-1}).

All bolus injections were given in 0.1 ml and flushed in with 0.15 ml. Infusions were given at 0.3 ml h^{-1}. Administration of vehicle solutions alone in these volumes had no consistent cardiovascular effects.

Data analysis

Within-group analysis of responses to bolus injections of bradykinin was by Friedman's test (Theodorsson-Norheim, 1987). Comparison of responses to bolus injections of bradykinin under different conditions was made by considering areas under or over curves (AUC, AOC, respectively) (Gardiner *et al.*, 1990b) and applying Wilcoxon's ranks sums test; a P value < 0.05 was taken as significant.

In the case of the subsidiary experiments statistical analysis was not carried out because the group sizes were small.

Results

(1) *Effectiveness and selectivity of the antagonistic actions of human α-CGRP [8-37] and ICI 118551*

During infusion of human α-CGRP [8-37] (1.5 μmol kg^{-1} min^{-1}) the haemodynamic effects of human α-CGRP (0.125 nmol kg^{-1}) were abolished, although a slight tachycardia persisted (Figure 1). Following cessation of the infusion of human α-CGRP [8-37], bolus injection of human α-CGRP once again elicited the usual effects (Figure 1). In the same animals human α-CGRP [8-37] had no effect on haemodynamic responses to bolus injections of adrenaline (Figure 2).

In other animals pretreated with atropine, infusion of salbutamol caused hypotension, tachycardia and falls in renal and mesenteric blood flows, together with a marked increase in hindquarters blood flow (Figure 3). All these effects were blocked by ICI 118551 (Figure 3).

Infusion of isoprenaline caused effects similar to those seen with salbutamol (Figure 4). ICI 118551 blocked all these effects except for the tachycardia, which was reduced (Figure 4).

All the cardiovascular changes seen during infusion of sodium nitroprusside were unaffected by ICI 118551 (data not shown).

(2) *Haemodynamic effects of bolus injections of bradykinin*

The bolus dose of bradykinin used caused a small, but significant pressor effect (AUC, 18 ± 4 units), accompanied by a marked, significant tachycardia (AUC, 179 ± 10 units) (Figures 5 and 6). There was a slight, but significant rise in renal blood flow (AUC, 21 ± 4 units), whereas mesenteric and hindquarters flows showed marked, significant increases (AUC, 79 ± 15 and 91 ± 10 units, respectively) (Figures 5 and 6). The peak in mesenteric blood flow occurred about 30 s after administration of bradykinin, while the hindquarters hyperaemia did not peak until about 90 s after bradykinin injection (Figures 5 and 6). Similar patterns of change were seen in the vascular conductances, with a slight renal vasodilatation (AUC, 20 ± 6 units), an early, substan-

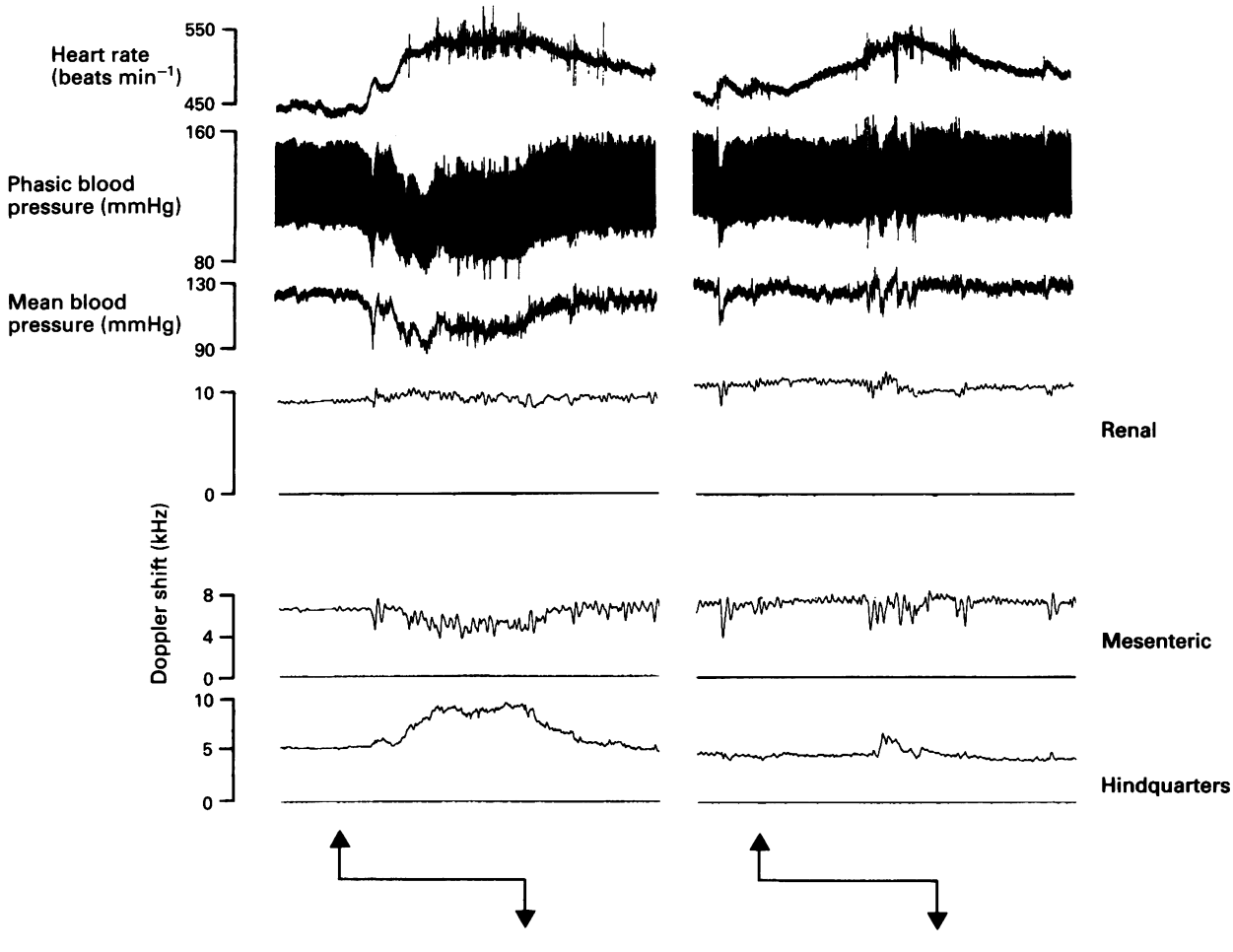

Figure 4 Original tracings showing cardiovascular effects of 3 min infusions (between arrows) of isoprenaline (0.24 nmol kg^{-1} min^{-1}) before (left-hand panel) and 60 min after the start of ICI 118551 administration (670 nmol kg^{-1} bolus, 335 nmol kg^{-1} h^{-1} infusion) (right-hand panel) in the same conscious, atropine-pretreated, Long Evans rat. The results are representative of 2 experiments.

tial, mesenteric vasodilatation (67 ± 6 units) and a delayed, but even greater, hindquarters vasodilatation (AUC, 102 ± 14 units) (Figure 6). A second dose of bradykinin elicited similar responses to the first (AUC, heart rate = 213 ± 35 units; mean blood pressure = 21 ± 4 units; renal flow = 26 ± 6 units; mesenteric flow = 82 ± 10 units; hindquarters flow = 101 ± 17 units; renal conductance = 19 ± 6 units; mesenteric conductance = 63 ± 11 units; hindquarters conductance = 120 ± 21 units) (Figure 6).

During infusion of human α-CGRP [8-37], injections of bradykinin elicited similar patterns of response to those seen in the absence of human α-CGRP [8-37] (Figure 6). Although following infusion of the latter for 20 min there was a tendency for the injection of bradykinin to elicit an enhanced mesenteric vasodilatation (AUC, 86 ± 13 units) and a diminished hindquarters vasodilatation (AUC, 78 ± 11 units), these differences did not reach significance. However, following discontinuation of the infusion of human α-CGRP [8-37] these trends reversed and the responses to bradykinin were then numerically closer to those under control conditions (AUC, heart rate = 233 ± 17 units; mean blood pressure = 24 ± 5 units; renal flow = 20 ± 5 units; mesenteric flow = 98 ± 11 units; hindquarters flow = 101 ± 17 units; renal conductance = 10 ± 5 units; mesenteric conductance = 72 ± 11 units; hindquarters conductance = 106 ± 20 units) (Figure 6).

In the presence of ICI 118551, injection of bradykinin elicited a significantly enhanced pressor effect (AUC, 43 ± 6 units) accompanied by a reduced tachycardia (AUC, 146 ± 13 units) (Figures 5 and 6). The increase in renal flow was unchanged (AUC, 20 ± 4 units) but the mesenteric hyperaemia was significantly increased (AUC, 127 ± 11 units) (Figure 6). However, the increase in hindquarters flow was abolished (AUC, 15 ± 5 units) (Figures 5 and 6). Thus, there was a renal vasoconstriction (AOC, 22 ± 6 units) and a hindquarters vasoconstriction (AOC, 33 ± 8 units), rather than the vasodilatations seen in the absence of ICI 118551, whereas the mesenteric vasodilator response to bradykinin (AUC, 81 ± 15 units), was unchanged (Figure 6).

In the 4 animals that received the sixth injection of bradykinin in the absence of ICI 118551 the responses did not differ from those seen to the fifth injection of bradykinin (data not shown).

(3) *Haemodynamic effects of infusions of bradykinin*

Infusion of bradykinin caused hypotension, tachycardia and increases in renal, mesenteric, and, particularly, hindquarters blood flow (Figure 7). In the presence of ICI 118551 the hypotensive effect of bradykinin infusion was inhibited, as was the tachycardia. Renal blood flow rose as before, whereas the mesenteric hyperaemia was increased. However, the increase in hindquarters blood flow was almost abolished (Figure 7).

(4) *Haemodynamic effects of bolus injection and infusions of bradykinin following ganglion blockade*

In the presence of ganglion blockade there were substantial changes in haemodynamic status (Figures 8 and 9).

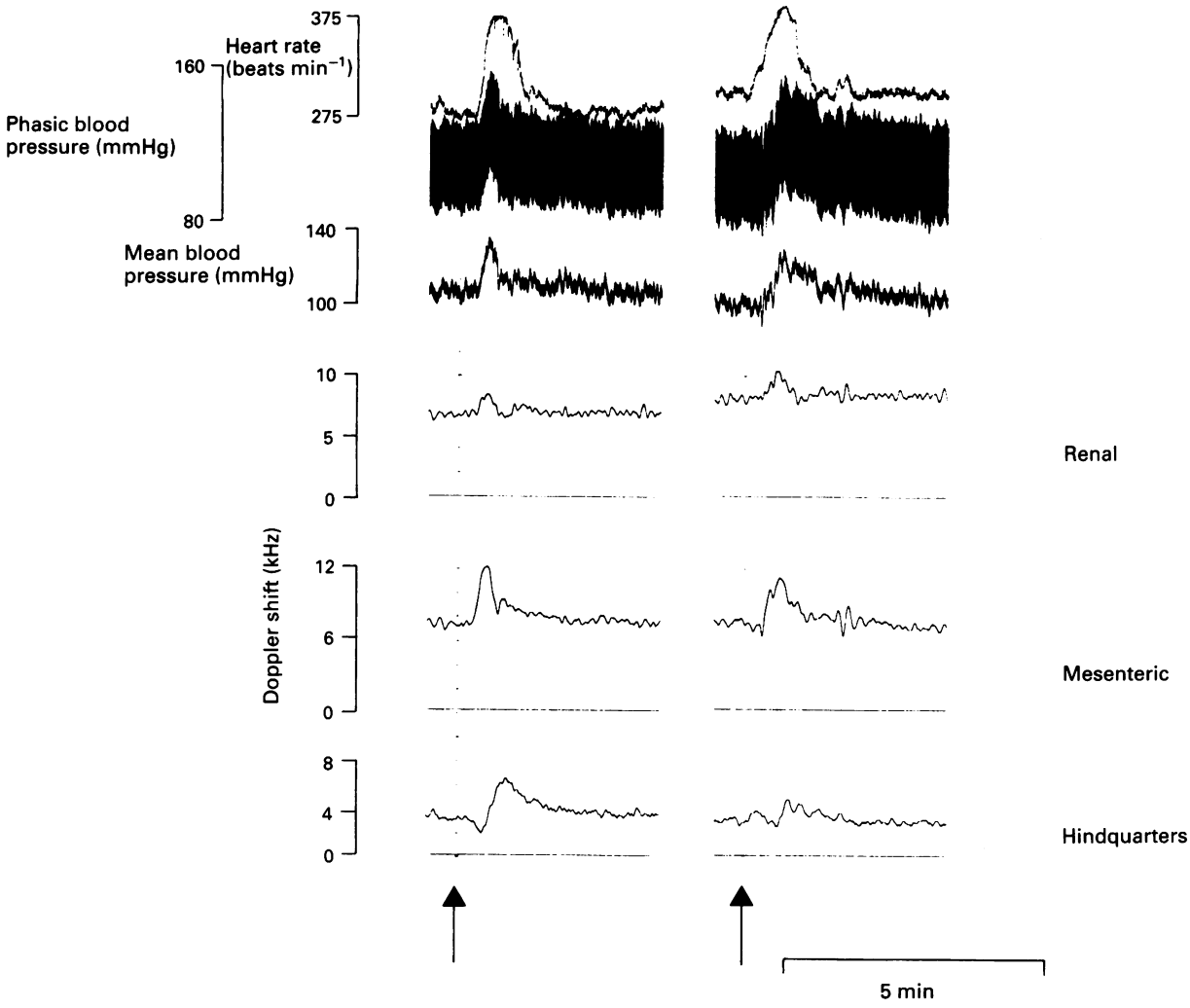

Figure 5 Original tracings showing cardiovascular effects of bolus injections (at arrows) of bradykinin (3 nmol kg^{-1}) before (left-hand panel) and 60 min after the start of ICI 118551 administration (670 nmol kg^{-1} bolus, 335 nmol kg^{-1} h^{-1} infusion) (right-hand panel) in the same conscious, Long Evans rat.

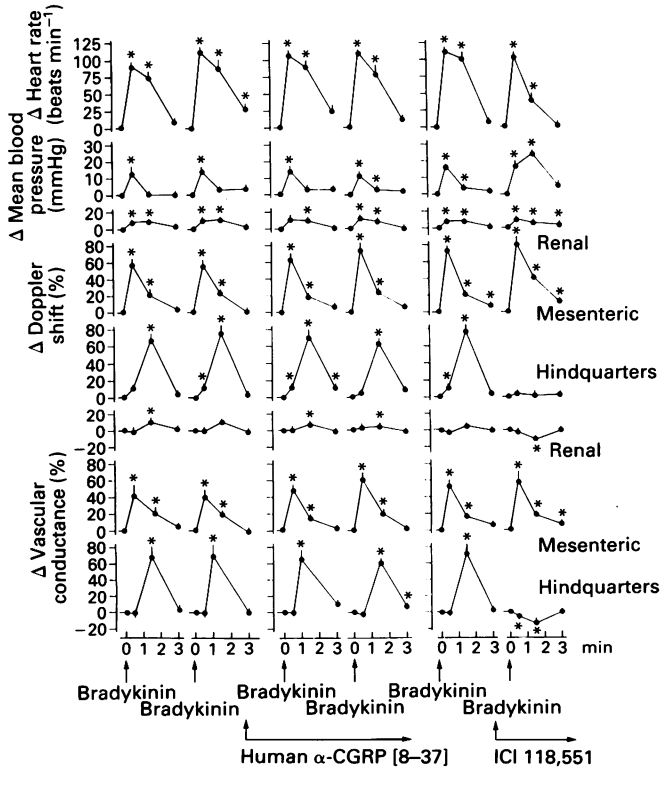

Figure 6 Cardiovascular responses to i.v. bolus injections of bradykinin (3 nmol kg^{-1}). The left-hand panels show responses to control injections; the middle panels show responses to bradykinin injection 10 to 20 min after the start of infusion of human α-calcitonin gene-related peptide [8-37] (α-CGRP [8-37]) (1.5 μmol kg^{-1} h^{-1}); the right-hand panels show responses to bradykinin injection before and 60 min after start of a primed infusion of ICI 118551 (670 nmol kg^{-1} bolus, 335 nmol kg^{-1} h^{-1} infusion). All responses were measured in the same conscious, Long Evans rats ($n = 8$) and the sequence of the experiment was as shown (from left to right). Values are mean and vertical bars show s.e.mean; $^*P < 0.05$ versus baseline (Friedman's test).

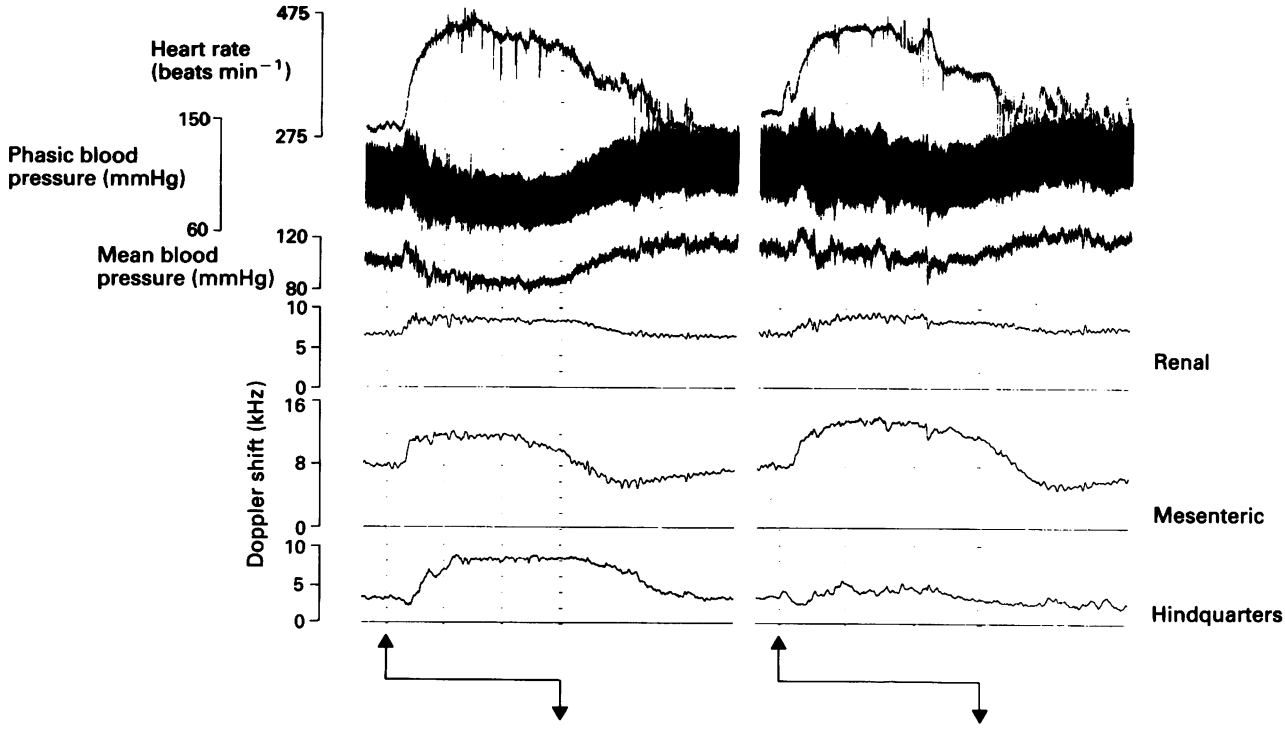

Figure 7 Original tracings showing cardiovascular effects of infusions (between arrows) of bradykinin (36 nmol kg^{-1} min^{-1}) before (left-hand panel) and 60 min after the start of ICI 118551 administration (670 nmol kg^{-1} bolus, 335 nmol kg^{-1} h^{-1} infusion) (right-hand panel) in the same conscious, Long Evans rat. The results are representative of 2 experiments.

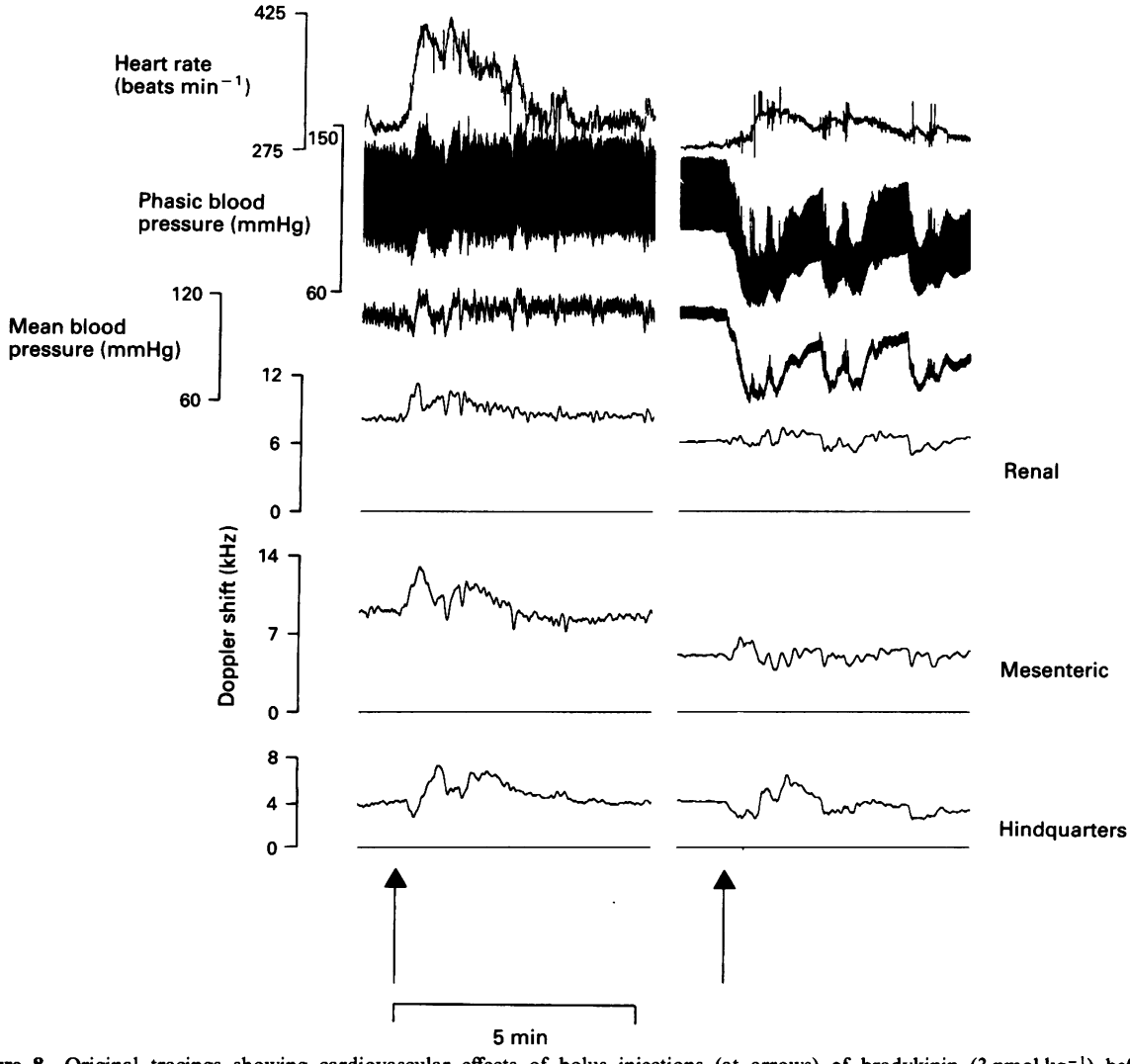

Figure 8 Original tracings showing cardiovascular effects of bolus injections (at arrows) of bradykinin (3 nmol kg^{-1}) before (left-hand panel) and 20 min after the start of mecamylamine administration (50 μmol kg^{-1} h^{-1} infusion) (right-hand panel) in the same conscious, Long Evans rat. The results are representative of 2 experiments.

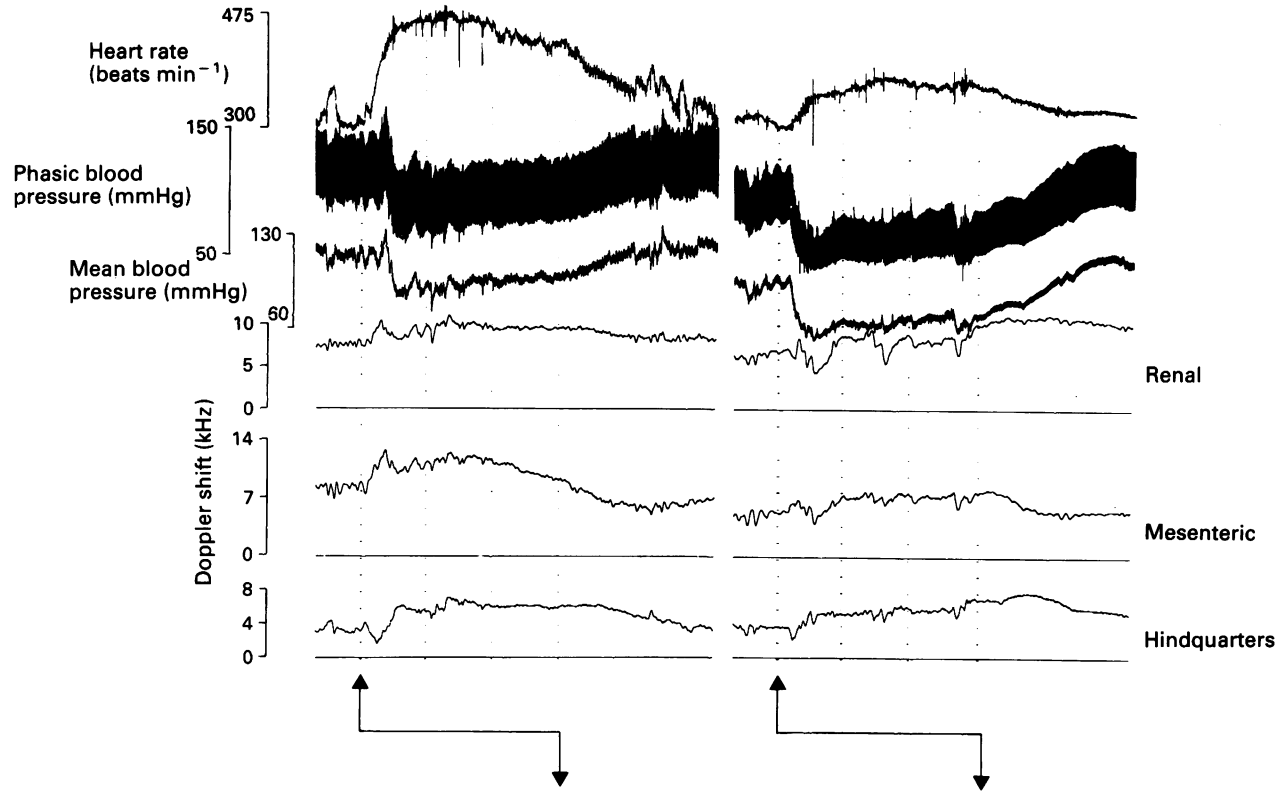

Figure 9 Original tracings showing cardiovascular effects of infusions (between arrows) of bradykinin (36 nmol kg^{-1} min^{-1}) before (left-hand panel) and 20 min after the start of mecamylamine administration (50 μmol kg^{-1} h^{-1} infusion) (right-hand panel) in the same conscious, Long Evans rat. The results are representative of 2 experiments.

Moreover, under these conditions the hypotensive effect of bradykinin was enhanced while its chronotropic effect was reduced (Figures 8 and 9). Because of the marked difference in the hypotensive response to bradykinin in the intact and ganglion-blocked state there were substantial differences in the patterns of change in regional blood flows (Figures 8 and 9). Nonetheless, there were still increases in renal, mesenteric and hindquarters blood flows following administration of bradykinin in the ganglion-blocked state, particularly when the peptide was infused (Figure 9). Thus, the hindquarters hyperaemic effect of bradykinin that was inhibited by ICI 118551 was not dependent upon ganglionic transmission.

(5) *Haemodynamic effects of bolus injections and infusions of bradykinin in adrenal demedullated rats*

Bolus injection of bradykinin caused transient hypotension and slight tachycardia (Figure 10). There was little change in renal blood flow, but a clear increase in mesenteric blood flow; however, the normal hindquarters hyperaemic response was absent (Figure 10).

A similar pattern of haemodynamic changes was seen during infusion of bradykinin in the adrenal demedullated rats (Figure 10).

Discussion

In the present work we assessed the possibility that release of CGRP (Gepetti *et al.*, 1990) and/or adrenal medullary catecholamines (Feldberg & Lewis, 1964) by bradykinin contributed to the regional haemodynamic effects of this peptide.

Human α-CGRP [8-37] has been shown to antagonize the cardiovasular effects of endogenous CGRP in the mesenteric vascular bed isolated from the rat (Han *et al.*, 1990) and the left atrium of the guinea-pig (Maggi *et al.*, 1991). Furthermore, it has marked inhibitory effects on the haemodynamic responses to infusions of human α-CGRP administered i.v. in conscious rats (Gardiner *et al.*, 1990d). In the present work we demonstrated that human α-CGRP [8-37] also blocked the haemodynamic effects of bolus injections of human α-CGRP, without affecting those to adrenaline. However, pretreatment with human α-CGRP [8-37] failed to affect significantly the responses to bolus doses of bradykinin in the present study, although there was a trend towards a diminution in the hindquarters, and an increase in the mesenteric, vasodilator responses to bradykinin in the presence of human α-CGRP [8-37]. Moreover, when infusion of human α-CGRP [8-37] was stopped these trends reversed. These findings make it difficult to dismiss the possibility that release of endogenous CGRP contributed to the haemodynamic responses to bradykinin in some animals (5/8 showed the changes described above). Although use of a higher dose of human α-CGRP [8-37] might, in theory, have produced a more clear-cut answer, this peptide at doses higher than used here has substantial effects itself (Gardiner *et al.*, 1990d).

ICI 118551 blocked the haemodynamic and tachycardic effects of salbutamol in atropine-pretreated rats, and blocked the haemodynamic, while only attenuating the chronotropic, effects of isoprenaline in the same animals. These results are consistent with specific inhibition of β_2-adrenoceptor-mediated vasodilatation (particularly in the hindquarters) by the selected dose of ICI 118551. This action, by suppressing the hypotensive responses to salbutamol and isoprenaline, would remove the stimulus for reflex tachycardia, although we cannot exclude the possibility that ICI 118551 antagonized any putative cardiac β_2-adrenoceptor effects (Brodde, 1991) of salbutamol or isoprenaline. However, the finding that ICI 118551 blocked all the effects of salbutamol while leaving a residual tachycardic response to isoprenaline, and having no influence on the positive chronotropic response to sodium nitroprusside in atropine-pretreated rats,

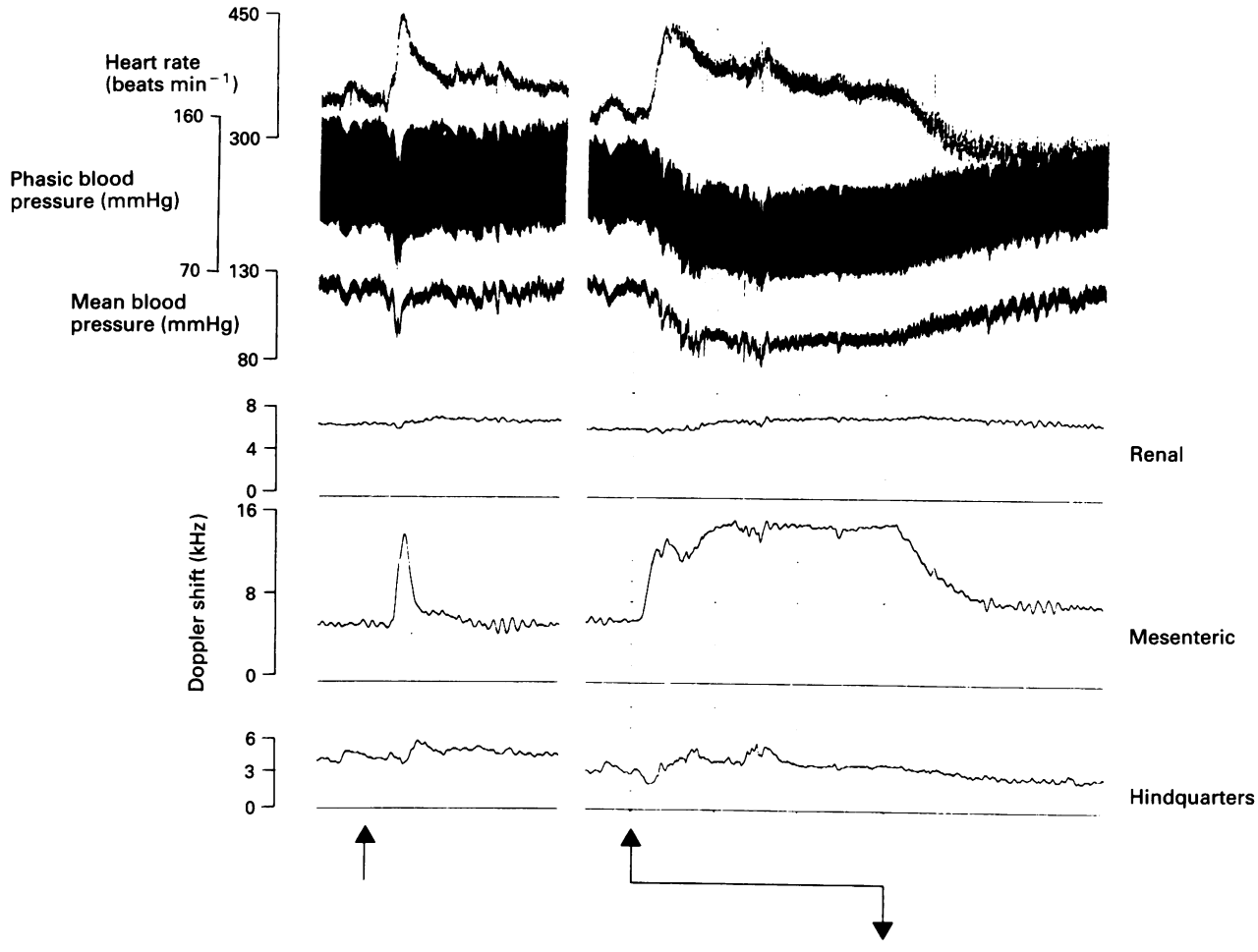

Figure 10 Original tracings showing cardiovascular effects of bolus injection (3 nmol kg^{-1}) (at arrow, left-hand panel) or infusion (36 nmol kg^{-1} min^{-1}) (between arrows, right-hand panel) of bradykinin in the same conscious, adrenal demedullated Long Evans rat. The results are representative of 4 experiments.

indicates that the dose of ICI 118551 used caused effective antagonism of β_2-adrenoceptor-mediated effects, and left cardiac β_1-adrenoceptor-mediated responses intact.

In the presence of ICI 118551, there was complete loss of the substantial hindquarters and the small renal vasodilator effects of bolus injections of bradykinin. Indeed, under these conditions, hindquarters and renal vasoconstrictions occurred in response to bradykinin. These results are consistent with activation of β_2-adrenoceptors causing the renal and hindquarters vasodilator responses to bolus injections of bradykinin under normal conditions. The difference in magnitude of the vasodilator responses in the two vascular beds is as would be predicted on the basis of their relative vasodilator responses to the β_2-adrenoceptor agonist, salbutamol (Gardiner *et al.*, 1991a), or to other interventions that activate β_2-adrenoceptors (Gardiner & Bennett, 1988c).

The blockade of the hindquarters hyperaemic effect of bradykinin by ICI 118551 was probably the major factor contributing to the enhanced pressor response to bolus doses of the peptide, and, under these circumstances, the attentuation of the tachycardic response to bradykinin was most likely baroreflex-mediated.

Infusions of bradykinin produced clear increases in blood flows in all three vascular beds studied. However, even under those circumstances, ICI 118551 caused marked attentuation of the hindquarters hyperaemic effects of bradykinin, although renal and mesenteric responses were not diminished. Thus, both the results with bolus injections and those with infusions of bradykinin indicate that the major component of the hindquarters hyperaemic vasodilator response, was β_2-adrenoceptor-mediated. The most likely explanation of this effect was that bradykinin released adrenaline from the adrenal medullae, and the adrenaline caused the hindquarters vasodilatation (Gardiner *et al.*, 1991b). This proposal is consistent with the finding that the hindquarters hyperaemic vasodilator effect of bradykinin was absent in adrenal demedullated rats, whereas the mesenteric vasocilator response was intact. The inability of mecamylamine to prevent the hindquarters hyperaemic response to bradykinin indicates the latter was probably acting directly on the chromaffin cells to release adrenaline (Warashina *et al.*, 1990).

In recent studies we observed that L-NAME reduced the hindquarters vasodilator action of bolus doses of bradykinin (Gardiner *et al.*, 1990c) and, furthermore, the hindquarters vasodilator effect of bradykinin was attenuated selectively in rats with streptozotocin-induced diabetes mellitus (Kiff *et al.*, 1991a). In addition, in the latter animals, the hindquarters vasoconstrictor response to L-NAME was diminished relative to that in control rats (Kiff *et al.*, 1991b). We interpreted these findings, together, as showing a particular involvement of nitric oxide in the hindquarters vasodilator effect of bradykinin, and a selective impairment of this process in streptozotocin-treated rats. At first sight, that interpretation seems to be at variance with the present findings. However, we now know that the hindquarters vasodilator effects of the β_2-adrenoceptor agonist, salbutamol (Gardiner *et al.*, 1991a)

and of adrenaline (Gardiner *et al.*, 1991b) are inhibited by L-NAME. So, these various findings can be reconciled by the proposition that the hindquarters vasodilator response to bradykinin that is mediated by adrenaline release involves nitric oxide at some point, and it is this process that is abnormal in streptozotocin-treated rats.

The mechanisms responsible for the mesenteric hyperaemic effects of bradykinin *in vivo* remain to be determined since other experiments (Gardiner *et al.*, 1990c) have shown this effect still occurs in the presence of L-NAME.

References

BILSKI, A.J., HALLIDAY, S.E., FITZGERALD, J.D. & WALE, J.L. (1983). The pharmacology of a β_2-selective adrenoceptor antagonist (ICI 118551). *J. Cardiovasc. Pharmacol.*, **5**, 430–437.

BRODDE, O.-E. (1991). β_1- and β_2-adrenoceptors in the human heart: properties, function, and alterations in chronic heart failure. *Pharmacol. Rev.*, **43**, 203–242.

DENNIS, T., FOURNIER, A., CADIEUX, A., POMERLEAU, F., JOLICOEUR, F.B., ST PIERRE, S. & QUIRION, R. (1990). $hCGRP_{8-37}$, a calcitonin gene-related peptide antagonist revealing calcitonin gene-related receptor heterogeneity in brain and periphery. *J. Pharmacol. Exp. Ther.*, **254**, 123–128.

FELDBERG, W. & LEWIS, G.P. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. *J. Physiol.*, **171**, 98–108.

FURCHGOTT, R.F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circ. Res.*, **53**, 557–573.

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

GARDINER, S.M. & BENNETT, T. (1988b). Adrenoceptors and cardiovascular regulation in conscious, unrestrained, Long Evans and Brattleboro rats. *J. Auton. Nerv. Syst.*, **24**, 193–203.

GARDINER, S.M. & BENNETT, T. (1988c). Regional hemodynamic responses to adrenoceptor antagonism in conscious rats. *Am. J. Physiol.*, **225**, H813–H824.

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

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

GARDINER, S.M., COMPTON, A.M., BENNETT, T. & HARTLEY, C.J. (1990a). Can pulsed Doppler technique measure changes in aortic flow in conscious rats? *Am. J. Physiol.*, **259**, H448–H456.

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

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

GARDINER, S.M., COMPTON, A.M., KEMP, P.A., BENNETT, T., BOSE, C., FOULKES, R. & HUGHES, B. (1990d). Antagonistic effect of human α-CGRP [8-37] on the *in vivo* regional haemodynamic actions of human α-CGRP. *Biochem. Biophys. Res. Commun.*, **171**, 938–943.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991a). 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.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991b). Effects of N^G-nitro-L-arginine methyl ester on vasodilator responses to adrenaline or BRL 38227 in conscious rats. *Br. J. Pharmacol.*, **104**, 731–737.

GEPETTI, P., TRAMONTANA, M., SANTICIOLI, P., DEL BIANCO, E., GIULIANI, S. & MAGGI, C.A. (1990). Bradykinin-induced release of calcitonin gene-related peptide from capsaicin-sensitive nerves in guinea-pig atria: mechanism of action and calcium requirements. *Neuroscience*, **38**, 687–692.

HAN, S.-P., NAES, L. & WESTFALL, T.C. (1990). Inhibition of periarterial nerve stimulation-induced vasodilation of the mesenteric arterial bed by CGRP [8-37] and CGRP receptor desensitization. *Biochem. Biophys. Res. Commun.*, **168**, 786–791.

HAYWOOD, J.R., SHAFFER, R., 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.

KIFF, R.J., GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1991a). Selective impairment of hindquarters vasodilator responses to bradykinin in conscious Wistar rats with streptozotocin-induced diabetes mellitus. *Br. J. Pharmacol.*, **103**, 1357–1362.

KIFF, R.J., GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1991b). The effect of endothelin-1 and N^G-nitro-L-arginine methyl ester on regional haemodynamics in conscious rats with streptozotocin-induced diabetes mellitus. *Br. J. Pharmacol.*, **103**, 1321–1326.

MAGGI, C.A., CHIBA, T. & GIULLIANI, S. (1991). Human α-calcitonin gene-related peptide [8-37] as an antagonist of exogenous and endogenous calcitonin gene-related peptide. *Eur. J. Pharmacol.*, **192**, 85–88.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.

REGOLI, D. & BARABÉ, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol Rev.*, **32**, 1–46.

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

WARASHINA, A., FUJIWARA, N. & SHIMOJI, K. (1990). Bradykinin-induced calcium mobilization and catecholamine secretion in rat adrenal medullary cells. *Biomed. Res.*, **11**, 219–229.

WRIGHT, C.E. & FOZARD, J.R. (1990). Differences in regional vascular sensitivity to endothelin-1 between spontaneously hypertensive and normotensive Wistar-Kyoto rats. *Br. J. Pharmacol.*, **100**, 107–113.

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