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Involvement of capsaicin-sensitive neurones in the haemodynamic effects of exogenous vasoactive peptides: studies in conscious, adult Long Evans rats treated neonatally with capsaicin

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1 The regional haemodynamic effects of i.v. bolus injections of bradykinin (0.05 or 0.5 nmol), cholecystokinin (0.175 or 1.75 nmol), substance P (0.01 or 0.1 nmol) and calcitonin gene-related peptide (0.05 or 0.5 nmol) were assessed in conscious, adult Long Evans rats that had been treated neonatally with either capsaicin (50 mg kg^{-1}, s.c.) or vehicle.

2 In vehicle-treated rats, both doses of bradykinin were without effect on blood pressure, but caused tachycardia and hindquarters vasodilatation. Moreover, after the higher dose there were dilatations in the renal and superior mesenteric vascular beds. In capsaicin-treated rats the hindquarters vasodilator effects elicited by both doses of bradykinin were significantly reduced, while the tachycardia and responses in the renal and superior mesenteric vascular beds were unchanged.

3 In vehicle-treated rats, cholecystokinin caused dose-dependent increases in blood pressure accompanied by renal, superior mesenteric and hindquarters vasoconstriction followed, after the higher dose, by a hindquarters vasodilatation. The lower dose produced a tachycardia, while there was a bradycardia followed by a tachycardia after the higher dose. In capsaicin-treated rats, the pressor response, as well as the renal vasoconstrictor effects of cholecystokinin, were greater than in vehicle-treated rats, while the heart rate, superior mesenteric or hindquarters responses were not different.

4 In vehicle-treated rats, substance P produced a dose-dependent depressor response and tachycardia accompanied by dilatations in the renal and hindquarters vascular beds and constriction in the superior mesenteric vascular bed. In capsaicin-treated rats, the responses to the lower dose of substance P were not different from those in vehicle-treated rats, while the depressor response to the higher dose of substance P was slightly less than in vehicle-treated rats and the renal vasodilatation was absent.

5 In vehicle-treated rats, calcitonin gene-related peptide caused dose-dependent hypotensive and tachycardic effects associated with dilatations in renal and hindquarters vascular beds and a constriction in the superior mesenteric vascular bed. After the higher dose, the renal vasodilatation was followed by a modest vasoconstriction. In capsaicin-treated rats, the depressor responses to both doses of calcitonin gene-related peptide were slightly more prolonged than in vehicle-treated animals, whereas the heart rate and renal and mesenteric vascular conductance changes were not significantly different. However, there was a more sustained hindquarters vasodilator response to the higher dose of calcitonin gene-related peptide in the capsaicin-treated rats.

6 The results suggest that peripheral, capsaicin-sensitive neurones are involved in the cardiovascular responses to exogenous bradykinin and cholecystokinin in conscious rats. It does not appear that the extent of involvement of these neurones is underestimated on account of development of marked supersensitivity to the peptides they normally release, since responses to such peptides (e.g. substance P and calcitonin gene-related peptide) are relatively normal in capsaicin-treated rats.

Keywords: Regional haemodynamics; capsaicin; blood pressure; heart rate; peptides

Introduction

Capsaicin (8 methyl-N-vanillyl-6-nonenamide), the pungent algesic substance contained in many red peppers, is a valuable pharmacological tool for studying a specific subset of primary afferent neurones (Buck & Burks, 1986; Maggi & Meli, 1988). It is now well documented that some unmyelinated primary afferent neurones sensitive to capsaicin contain a variety of peptides, including substance P (SP) and calcitonin gene-related peptide (CGRP) (Skofitsch & Jacobowitz, 1985; Buck & Burks, 1986; Franco-Cereceda *et al.*, 1987), which have potent cardiovascular effects (Pernow, 1983; Sigrist *et al.*, 1986; Dipette *et al.*, 1987; Lappe *et al.*, 1987; Gardiner *et al.*, 1988; 1989a,b,c). In several animal species, it has been demonstrated that the peripheral endings of capsaicin-sensitive, primary afferents are widely distributed throughout peripheral tissues, usually in association with blood vessels (Furness *et al.*, 1982; Barja *et al.*, 1983; Rozsa *et al.*, 1984; Lundberg *et al.*, 1985; Uddmann *et al.*, 1986; Wharton *et al.*, 1986; Wharton & Gulbenkian, 1987; Martling *et al.*, 1988; Hottenstein *et al.*, 1991). This observation raises the possibility that these neurones may be involved with the regulation of vascular tone.

There is evidence indicating that the neuropeptides contained in the capsaicin-sensitive primary afferent neurones can be released in response to stimuli, such as the nonapeptide, bradykinin (BK), and that other peptides, such as cholecystokinin (CCK), cause activation of capsaicin-sensitive afferents (Geppetti *et al.*, 1988; 1990; McCann *et al.*, 1988; Manzini *et al.*, 1989). Hence, release of the neuropeptides contained in these afferent fibres could contribute, at least partly, to some of the *in vivo* effects of BK and CCK.

Capsaicin given to newborn rats is known to cause permanent and selective degeneration of up to 90% of peripheral unmyelinated primary sensory neurones (Nagy *et al.*, 1981). Parallel to the destruction of these unmyelinated fibres it has

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been shown by radioimmunoassay that the levels of SP and CGRP are substantially, and irreversibly, reduced in regions of the peripheral and central nervous system normally containing projections of primary sensory neurones (Gamse *et al.*, 1980; Cuello *et al.*, 1981; Nagy *et al.*, 1981; Skofitsch & Jacobowitz, 1985; Hammon & Ruda, 1989). Therefore, mechanisms dependent on the integrity of these sensory fibres, and possibly mediated by local release of the associated neuropeptides, might be significantly affected following this treatment.

The purpose of the present study was to assess the influence of capsaicin-sensitive primary afferent neurones on the regional haemodynamic effects of BK and CCK by comparing responses to these peptides in control rats and in rats treated neonatally with capsaicin. However, since degeneration of the neuropeptide-containing, capsaicin-sensitive primary afferents could alter sensitivity to neuropeptides contained in these fibres (for instance, SP and CGRP), the cardiovascular responses to exogenous SP and CGRP were also evaluated in the same control rats and rats treated neonatally with capsaicin that were challenged with BK and CCK.

Methods

Long Evans rats were anaesthetized with 1.5–2% halothane (in oxygen) on day 2 after birth and injected subcutaneously with either vehicle (10% ethanol, 10% Tween 80 in 0.9% w/v NaCl solution) or capsaicin (50 mg kg^{-1}) in a volume of 100 μl. After weaning (day 21–30 after birth) male rats were selected and housed in groups of 4 or 5 with free access to food and tap water.

At least 20 weeks after birth, rats (350–400 g) were anaesthetized with sodium methohexitone (Lilly, 60 mg kg^{-1}, i.p., supplemented as required) and had pulsed Doppler flow probes implanted as previously described (Haywood *et al.*, 1981; Gardiner *et al.*, 1988; 1990b,c). After operation, animals were given ampicillin trihydrate (Penbritin, Beecham, 7 mg kg^{-1}, i.m.) and housed singly in cages with free access to food and water.

At least 7 days after this operation, the animals that were healthy (i.e. gaining weight and feeding, drinking and grooming normally) were re-anaesthetized (sodium methohexitone; 40 mg kg^{-1}, i.p., with supplemental doses). The probe wires were soldered into a microconnector and the quality of the signals from the implanted probes was checked via a pulsed Doppler monitoring system (VF-1 mainframe, Crystal Biotech), modified to operate with a pulse repetition frequency of 125 kHz (Gardiner *et al.*, 1990a). Any animal in which good signals (signal: noise ratio > 20:1) were not obtained from all 3 probes was rejected from the study. Animals that met this criterion had 3 i.v. catheters (right jugular) and 1 intra-arterial catheter (distal abdominal aorta, via caudal artery) implanted. We used 3 i.v. catheters so the peptides could be injected through a fresh catheter on each experimental day (see below). The catheters ran subcutaneously and emerged at the same point as the pulsed Doppler probe leads. A microconnector (Microtech Inc. USA), soldered to the probe leads, was clamped in a harness worn by the rat and the catheters passed through a flexible spring (for protection) attached to the harness. Animals were left until the next day before experiments were begun.

Throughout the experiment, recordings were made of heart rate, phasic and mean blood pressures and renal, superior mesenteric and hindquarters Doppler shift signals. The phasic Doppler shift signals were monitored to ensure their acceptability (Gardiner *et al.*, 1990b,c). Heart rate, mean blood pressure and mean Dopple shifts were averaged (by eye) over 10 s at time points selected to represent the full profile of the effects of the peptide. Changes (absolute changes for the former two variables, percentages for the Doppler shifts) and vascular conductances were calculated relative to the pretreatment baseline value. Before every experiment baseline measurements were made over a period of 30 min. Animals (fully conscious and unrestrained) were allowed free access to water and food for the duration of the experiment.

Two groups of animals (vehicle-treated, $n = 9$ and capsaicin-treated, $n = 9$) were used on 3 consecutive days, during which they received i.v. injections of peptide vehicle (0.1 ml isotonic saline containing 1% bovine serum albumin (BSA)), bradykinin (BK) (0.05 or 0.5 nmol), substance P (SP) (0.01 or 0.1 nmol), calcitonin gene-related peptide (CGRP) (0.05 or 0.5 nmol) and cholecystokinin (CCK) (0.175 or 1.75 nmol), in random order, although low doses were always given before high doses. Injections of low doses of peptides were separated by at least 60 min; the intervals separating the high doses were sufficient to allow variables to return to baseline (up to 2 h). Measurements were made before, during and for 15 min following i.v. injections of vehicle or peptides. Since the results obtained with the two doses of each peptide were consistent, only the data relating to the effects of the higher doses are given in the Results.

Drugs and peptides

Substance P (Bachem), rat α-calcitonin gene-related peptide (Bachem), bradykinin (Bachem), cholecystokinin (26–33), sulphated (Bachem), and capsaicin (Sigma) were used. Peptides were dissolved in isotonic saline containing 1% BSA. All i.v. injections were given as 100 μl boluses which were flushed in with 100 μl isotonic saline (the dead spaces of the catheters).

Statistical analysis

Values are expressed as the mean ± s.e.mean; n is the number of animals. Results were analysed for statistical significance by a paired Student's t test or an analysis of variance (ANOVA) followed by Fisher's test. The paired Student's t test was used to evaluate the responses to injection of saline-BSA, while the ANOVA was used to compare the responses to peptide injections with those to saline-BSA injections, or to compare the responses to peptide injections in vehicle-treated and capsaicin-treated rats. In the text the word significant means $P < 0.05$ (at least).

Results

The baseline values for cardiovascular variables in vehicle- and capsaicin-treated rats are given in Table 1. The resting values in capsaicin-treated rats were not different from those in vehicle-treated animals. The i.v. injection of saline-BSA in vehicle-treated rats had no effect on mean arterial blood pressure, but caused a slight, but significant, increase in heart rate and hindquarters flow, while there were no significant changes in renal or superior mesenteric flows (Table 2). Hence, there was a dilatation of the hindquarters vascular bed but no significant effect on renal or superior mesenteric vascular conductances (Table 2).

Table 1 Baseline values for heart rate, blood pressure and regional vascular conductance in vehicle- or capsaicin-treated Long Evans rats

	Heart rate	*Mean blood pressure*	*Doppler shift* (kHz)			*Vascular conductance* ([kHz mmHg^{-1}] 10^3)		
Treatment	(beats min^{-1})	(mmHg)	*Renal*	*Mesenteric*	*Hindquarters*	*Renal*	*Mesenteric*	*Hindquarters*
Vehicle	381 ± 10	103 ± 3	10.0 ± 1.5	8.8 ± 1.1	5.0 ± 0.4	96 ± 13	87 ± 11	50 ± 5
Capsaicin	354 ± 17	109 ± 5	10.3 ± 1.4	7.9 ± 0.4	5.2 ± 0.6	94 ± 11	73 ± 5	50 ± 7

Values are mean ± s.e.mean; $n = 9$ for both groups.

Table 2 Peak cardiovascular changes following intravenous administration of saline-bovine serum albumin (1%) or peptides in vehicle-treated (Veh) or capsaicin-treated (Cap) Long Evans rats

		Δ *Heart rate* (beats min^{-1})	Δ *Mean blood pressure* (mmHg)	Δ *Doppler shift* (%)			Δ *Vascular conductance* (%)		
				Renal	*Mesenteric*	*Hindquarters*	*Renal*	*Mesenteric*	*Hindquarters*
Veh	Saline-BSA (1%)	+29 ± 10†	0 ± 3	+1 ± 1	−4 ± 5	+23 ± 6†	+1 ± 2	−5 ± 4	+24 ± 8†
Cap	Saline-BSA (1%)	+10 ± 7	+2 ± 1	−1 ± 2	−1 ± 3	+18 ± 6†	−3 ± 1	−3 ± 3	+16 ± 7
Veh	BK (0.5 nmol)	+98 ± 11*	−10 ± 8	+8 ± 4	+28 ± 11*	+49 ± 18*	+25 ± 11* −8 ± 1*	+44 ± 12* −7 ± 3*	+72 ± 15*
Cap	BK (0.5 nmol)	+98 ± 9*	+4 ± 7	+12 ± 3*	+14 ± 8*	+35 ± 10	+10 ± 7 −4 ± 2	+46 ± 14*	+30 ± 8#
Veh	CCK (1.75 nmol)	−60 ± 17* +51 ± 15*	+32 ± 2*	+9 ± 3*	−57 ± 3*	+42 ± 11*	−27 ± 3*	−67 ± 2*	−10 ± 14* +34 ± 7*
Cap	CCK (1.75 nmol)	−55 ± 19* +53 ± 8*	+45 ± 3*#	−10 ± 6	−62 ± 2*	+43 ± 6*	−38 ± 3*#	−74 ± 1*	−14 ± 13* +31 ± 6*
Veh	SP (0.1 nmol)	+128 ± 12*	−28 ± 2*	−13 ± 2*	−50 ± 4*	+73 ± 9*	+21 ± 5*	−30 ± 5* +14 ± 5*	+140 ± 12*
Cap	SP (0.1 nmol)	+133 ± 9*	−21 ± 2*#	−16 ± 4*	−51 ± 2*	+74 ± 9*	+6 ± 5#	−38 ± 4* +13 ± 7	+119 ± 10*
Veh	CGRP (0.5 nmol)	+157 ± 15*	−34 ± 2*	−19 ± 5*	−40 ± 7*	+38 ± 15	+65 ± 11* −13 ± 2*	+16 ± 18 −27 ± 9*	+100 ± 22*
Cap	CGRP (0.5 nmol)	+134 ± 13*	−37 ± 2*	−33 ± 3*#	−37 ± 4*	+34 ± 5	+43 ± 9* −18 ± 3*	+18 ± 6* −16 ± 3*	+99 ± 9*

Values are mean ± s.e.mean, $n = 9$ in both groups. BK = bradykinin, CCK = cholecystokinin, SP = substance P, CGRP = calcitonin gene-related peptide.
† $P < 0.05$ versus baseline, paired Student's t test for injection of saline-bovine serum albumin (saline-BSA). * $P < 0.05$ versus response to injection of saline-BSA, analysis of variance followed by Fisher's test. # $P < 0.05$ versus vehicle-treated responses, analysis of variance followed by Fisher's test. Some responses were biphasic; in those cases the first value represents the initial change and below is the later change.

In the capsaicin-treated rats the injection of saline-BSA 1% had no effect on blood pressure, heart rate or renal or superior mesenteric flows but caused a slight increase in hindquarters flow (Table 2). There were no significant changes in renal, superior mesenteric or hindquarters vascular conductances (Table 2). The cardiovascular responses following injection of saline-BSA in capsaicin-treated rats were not significantly different from those in vehicle-treated animals (Table 2).

Haemodynamic effects of bradykinin

Vehicle-treated rats BK (0.5 nmol) had no significant effect on blood pressure, but produced tachycardia (significant at 0.5–1 min). There were increases in superior mesenteric (significant at 0.5 min) and hindquarters flow (significant at 0.5–1 min), but no significant change in renal flow (Figure 1, Table 2). These responses were associated with initial increases in renal and superior mesenteric vascular conductances followed by modest decreases (Table 2). There was only vasodilatation in the hindquarters vascular bed (Table 2).

Capsaicin-treated rats BK (0.5 nmol) had no significant effect on blood pressure, but produced tachycardia (significant at 0.5–1 min) that was not different from that in vehicle-treated rats (Figure 1, Table 2). There was a modest increase in renal flow (significant at 0.5–1 min) and a marked increase in superior mesenteric flow (significant at 0.5 min). These responses were not significantly different from those seen in vehicle-treated rats. The increase in hindquarters flow was not different from that in vehicle-treated rats, or from the response following control injection of saline-BSA (Figure 1, Table 2). There was no significant change in renal vascular conductance, whereas the superior mesenteric vascular bed showed a dilatation (Table 2). The latter was not followed by a constriction, in contrast to that seen in vehicle-treated rats, but these changes were not significantly different from those in vehicle-treated rats (Table 2). There was an increase in hindquarters vascular conductance which was significantly less than the response evoked in vehicle-treated rats and not different from the response to control injection of saline-BSA (Table 2).

Haemodynamic effects of cholecystokinin

Vehicle-treated rats CCK (1.75 nmol) produced an increase in blood pressure (significant at 0.5–2 min) accompanied by an initial bradycardia (significant at 0.5–1 min) followed by a tachycardia (significant at 3–5 min) (Figure 2, Table 2). Renal and hindquarters flows increased (renal, significant at 2–3 min; hindquarters, significant at 1–4 min), while a decrease in superior mesenteric flow occurred (significant at 0.5–3 min) (Figure 2, Table 2). There were constrictions in renal (significant at 0.5 min) and superior mesenteric (significant at 0.5–3 min) vascular beds (Figure 3, Table 2). There was a modest constriction in the hindquarters vascular bed (significant at 0.5 min), followed by a dilatation (significant at 1–5 min) (Figure 3, Table 2).

Capsaicin-treated rats CCK (1.75 nmol) caused a pressor response (significant at 0.5–4 min) that was slightly greater, and an initial bradycardia (significant at 0.5–1 min) followed by a tachycardia (significant at 3–15 min), which were not different, from those seen in vehicle-treated rats (Figure 2, Table 2). There was no significant change in renal flow, while a reduction in superior mesenteric flow (significant at 0.5–3 min) and an increase in hindquarters flow (significant at 1–4 min) occurred (Figure 2, Table 2). These responses were not significantly different from those in vehicle-treated rats (Figure 2, Table 2). There were constrictions in renal (significant at 0.5–1 min), superior mesenteric (significant at 0.5–3 min) and hindquarters (significant at 0.5 min) vascular beds, followed by a dilatation in the hindquarters vascular bed (significant at 2–3 min) (Figure 3, Table 2). The response in the renal vascular bed was greater, while the responses in the two other vascular beds were not different from those in vehicle-treated rats (Figure 3, Table 2).

Haemodynamic effects of substance P

Vehicle-treated rats SP (0.1 nmol) produced a decrease in blood pressure (significant at 0.5–1 min) and a marked tachycardia (significant at 0.5–2 min) accompanied by reductions in renal (significant at 0.5 min) and superior mesenteric (significant at 0.5 min) flows and a substantial increase in hindquarters flow (significant at 0.5 min) (Figure 4, Table 2).

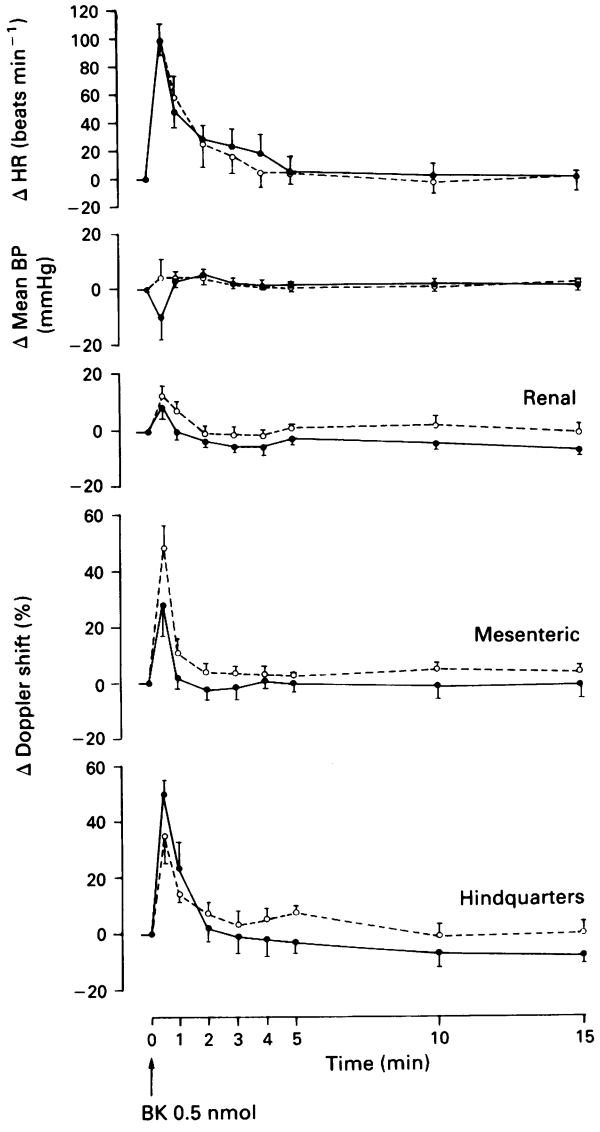

Figure 1 Cardiovascular responses to i.v. bolus injections of bradykinin (BK, 0.5 nmol) in vehicle- (●) or capsaicin-treated (○) Long Evans rats. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ vehicle-treated group ($n = 9$) versus capsaicin-treated group ($n = 9$), (analysis of variance followed by Fisher's test). HR = heart rate; BP = blood pressure.

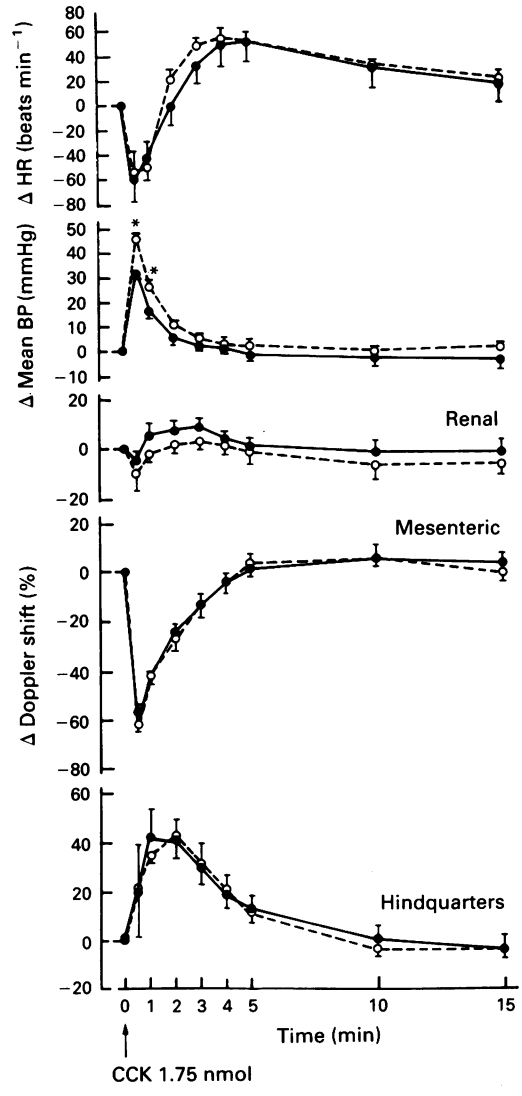

Figure 2 Cardiovascular responses to i.v. bolus injections of cholecystokinin (CCK, 1.75 nmol) in vehicle- (●) or capsaicin-treated (○) Long Evans rats. Values are mean with s.e.mean shown by vertical lines. * $P < 0.5$ vehicle-treated group ($n = 9$) versus capsaicin-treated group ($n = 9$), (analysis of variance followed by Fisher's test). HR = heart rate; BP = blood pressure.

These responses were associated with a moderate renal dilatation (significant at 0.5 min) but a marked dilatation in the hindquarters vascular bed (significant at 0.5 min) (Figure 5, Table 2). The superior mesenteric vascular bed showed an initial constriction (significant at 0.5 min) followed immediately by a dilatation (significant at 1 min) (Figure 5, Table 2).

Capsaicin-treated rats SP (0.1 nmol) caused a decrease in blood pressure (significant at 0.5 min) which was slightly, but significantly, less and a tachycardia (significant at 0.5–2 min), which was not different, from that in vehicle-treated rats (Figure 4, Table 2). There were reductions in renal (significant at 0.5 min) and superior mesenteric (significant at 0.5 min) flows and a substantial increase in hindquarters flow (significant at 0.5 min); these were not different from the responses seen in vehicle-treated animals (Figure 4, Table 2). The renal vasodilatation observed in vehicle-treated animals was absent, although there was a substantial dilatation in the hindquarters vascular bed (significant at 0.5 min) which was not different from that in vehicle-treated animals. As in vehicle-treated animals, the superior mesenteric vascular bed also showed an initial decrease (significant at 0.5 min) followed immediately by an increase in conductance (significant at 1 min) (Figure 5, Table 2).

Haemodynamic effects of calcitonin gene-related peptide

Vehicle-treated rats CGRP (0.5 nmol) caused a decrease in blood pressure (significant at 0.5–15 min) and a marked tachycardia (significant at 0.5–15 min) accompanied by a delayed reduction in renal flow (3–15 min) and an early-onset reduction in superior mesenteric flow (significant at 1–15 min); the increase in hindquarters flow was not different from the response following control injection of saline-BSA (Figure 6, Table 2). The renal vascular bed showed an early dilatation (significant at 0.5–2 min) followed by a late constriction (significant at 10–15 min) (Figure 7, Table 2). There was a delayed constriction in the superior mesenteric vascular bed (significant at 2–4 min) and a substantial dilatation in the hindquarters vascular bed (significant at 0.5–3 and 5–15 min) (Figure 7, Table 2).

Capsaicin-treated rats CGRP (0.5 nmol) caused a decrease in blood pressure (significant at 0.5–15 min) which was more prolonged, and a tachycardia (significant at 0.5–15 min) which was not different from that in vehicle-treated animals (Figure 6, Table 2). The reduction in renal flow (significant at 3–15 min) was slightly greater, while the reduction in the

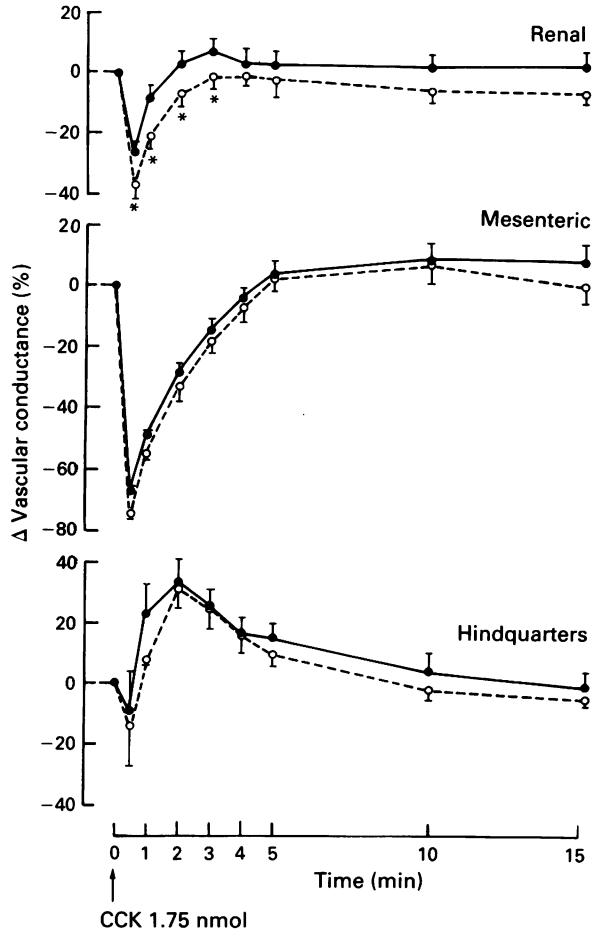

Figure 3 Changes in regional vascular conductances elicited by i.v. bolus injections of cholecystokinin (CCK, 1.75 nmol) in vehicle-treated (●) or capsaicin-treated (○) Long Evans rats. These data were derived from the data shown in Figure 2. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ vehicle-treated group ($n = 9$) versus capsaicin-treated group ($n = 9$), (analysis of variance followed by Fisher's test).

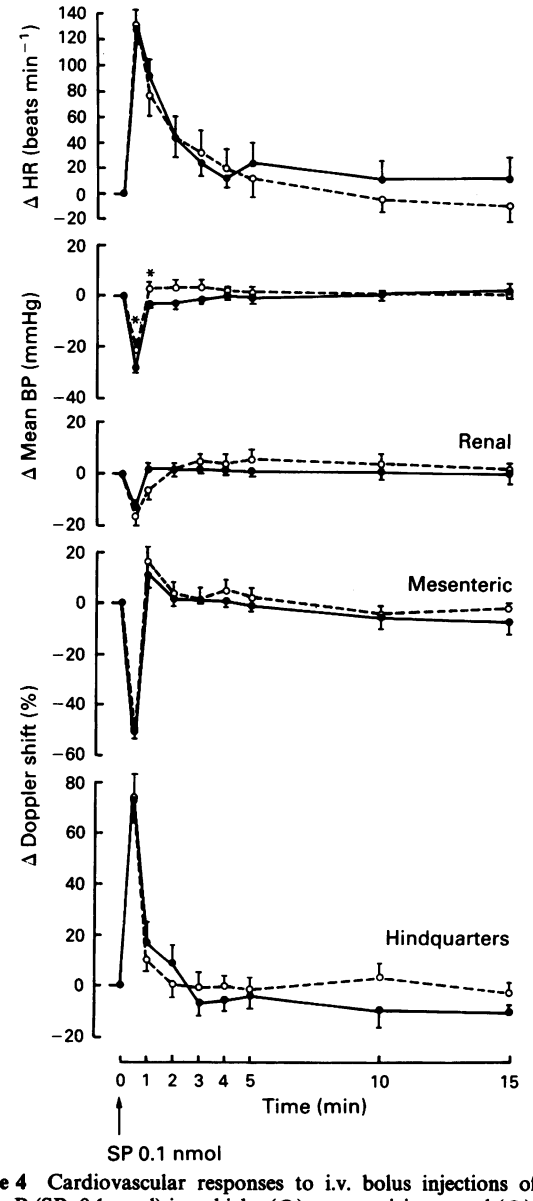

Figure 4 Cardiovascular responses to i.v. bolus injections of substance P (SP, 0.1 nmol) in vehicle- (●) or capsaicin-treated (○) Long Evans rats. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ vehicle-treated group ($n = 9$) versus capsaicin-treated group ($n = 9$), (analysis of variance followed by Fisher's test). HR = heart rate; BP = blood pressure.

superior mesenteric flow (significant at 1–15 min) was not different from that in vehicle-treated rats (Figure 6, Table 2). The increase in hindquarters flow was not different from that in vehicle-treated rats or from the response to saline-BSA (Figure 6, Table 2). There was a dilatation followed by constriction in the superior mesenteric vascular bed (significant at 3–10 min) and a marked hindquarters dilatation (significant at 0.5–15 min) (Figure 7, Table 2). The renal vascular bed showed an early dilatation (significant at 0.5–2 min) followed by a late constriction (significant at 5–15 min). The hindquarters dilatation was more prolonged than that seen in vehicle-treated rats (Figure 7), but the renal and mesenteric responses were not different from those in vehicle-treated rats (Figure 7, Table 2).

Discussion

The first objective of the present work was to delineate the influence of neonatal treatment with capsaicin on the cardiovascular responses to exogenous BK and CCK. Since there is evidence that some of the *in vivo* effects of these peptides are attributable to stimulation, and/or sensitization of neuropeptide-containing, capsaicin-sensitive sensory neurones, we reasoned that destruction of the majority of these primary afferents, by neonatal treatment with capsaicin, ought to affect responses to BK and CCK. Our second objective was to determine if neonatal treatment with capsaicin produced any supersensitivity to CGRP and/or SP, i.e. two peptides contained in capsaicin-sensitive neurones. Although we did not make any assessments of the extent of capsaicin-induced lesions in the present study, the protocol we followed has been found to cause a selective and permanent destruction of a large percentage of the peripheral, unmyelinated fibres (Nagy *et al.*, 1981).

In capsaicin-treated rats the resting values for mean arterial pressure, heart rate, and renal, superior mesenteric and hindquarters flows and vascular conductances were not significantly different from those of control rats. These results corroborate previous findings also in conscious rats (Bennett & Gardiner, 1985a,b).

Haemodynamic effects of bradykinin

In rats treated neonatally with capsaicin the heart rate and blood pressure responses to BK were similar to those seen in vehicle-treated animals. This finding could be interpreted as indicating a lack of involvement of capsaicin-sensitive neurones in the cardiovascular responses to BK. However, examination of the regional haemodynamic changes revealed that

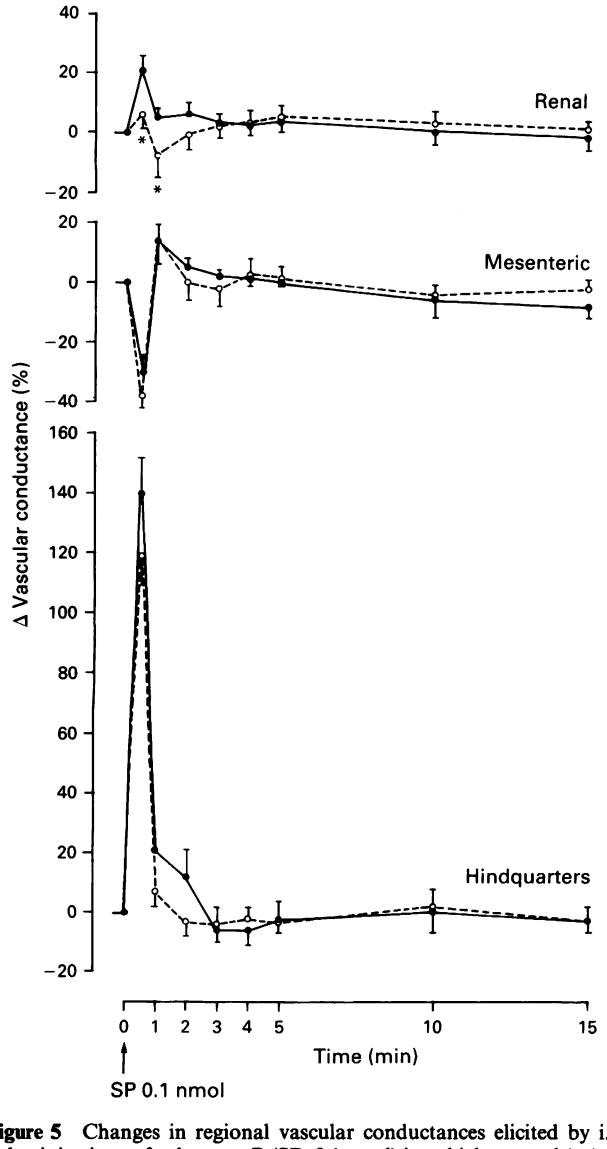

Figure 5 Changes in regional vascular conductances elicited by i.v. bolus injections of substance P (SP, 0.1 nmol) in vehicle-treated (●) or capsaicin-treated (○) Long Evans rats. These data were derived from the data shown in Figure 4. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ vehicle-treated group ($n = 9$) versus capsaicin-treated group ($n = 9$), (analysis of variance followed by Fisher's test).

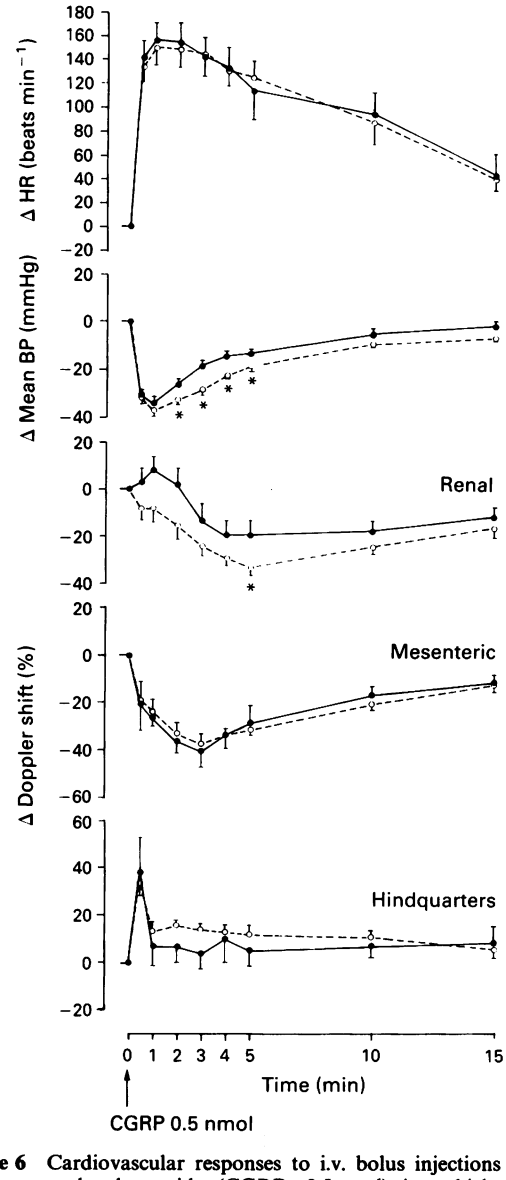

Figure 6 Cardiovascular responses to i.v. bolus injections of calcitonin gene-related peptide (CGRP, 0.5 nmol) in vehicle- (●) or capsaicin-treated (○) Long Evans rats. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ vehicle-treated group ($n = 9$) versus capsaicin-treated group ($n = 9$), (analysis of variance followed by Fisher's test). HR = heart rate; BP = blood pressure.

the increase in hindquarters vascular conductance elicited by BK was significantly smaller in capsaicin-treated, than in vehicle-treated, rats. This finding is compatible with the hindquarters vasodilator response in vehicle-treated animals being the result of stimulation of capsaicin-sensitive nerve fibres, evoking local release of vasodilator substances (e.g. SP, CGRP) from the nerve fibres. Previous studies have demonstrated that BK can cause the release of SP and CGRP from capsaicin-sensitive sensory fibres in the heart (Geppetti *et al.*, 1988; 1990; Manzini *et al.*, 1989). However, in conscious Long Evans rats, we have found that the hindquarters vasodilator effect of BK is converted into a vasoconstriction in the presence of a β_2-adrenoceptor antagonist (Gardiner *et al.*, unpublished). Therefore, one explanation of the present results is that the hindquarters vasodilator response to BK results from stimulation of capsaicin-sensitive neurones causing the release of adrenaline from the adrenal medulla (Feldberg & Lewis, 1964; Warashina *et al.*, 1990). This interpretation is supported by the findings of previous studies indicating that capsaicin-sensitive neurones innervating the adrenal medulla are involved in modulating the neurogenic secretion of catecholamines induced by selective stressors, such as histamine, insulin, cold and capsaicin itself (Khalil *et al.*, 1984; 1987; Watanabe *et al.*, 1988; Zhou *et al.*, 1990; Zhou & Livett, 1991). While it is feasible that bradykinin could act directly on adrenal medullary chromaffin cells to release adrenaline, it is not obvious why neonatal treatment with capsaicin should influence such a process.

It is interesting to note that besides the hindquarters vasodilator effects of BK, none of the other cardiovascular responses were significantly affected by the neonatal treatment with capsaicin. Thus, it is likely that in vehicle-treated rats, the overall action of BK would be the resultant not only of the stimulation of capsaicin-sensitive sensory nerve endings, but also of direct effects, as well as indirect effects via the release of other mediators (such as histamine, prostaglandins and nitric oxide) (Johnson & Erdos, 1973; Goldberg *et al.*, 1976; Warren *et al.*, 1987; Gardiner *et al.*, 1990c).

Haemodynamic effects of cholesytokinin

In rats neonatally-treated with capsaicin the heart rate responses to CCK were not different from, while the pressor

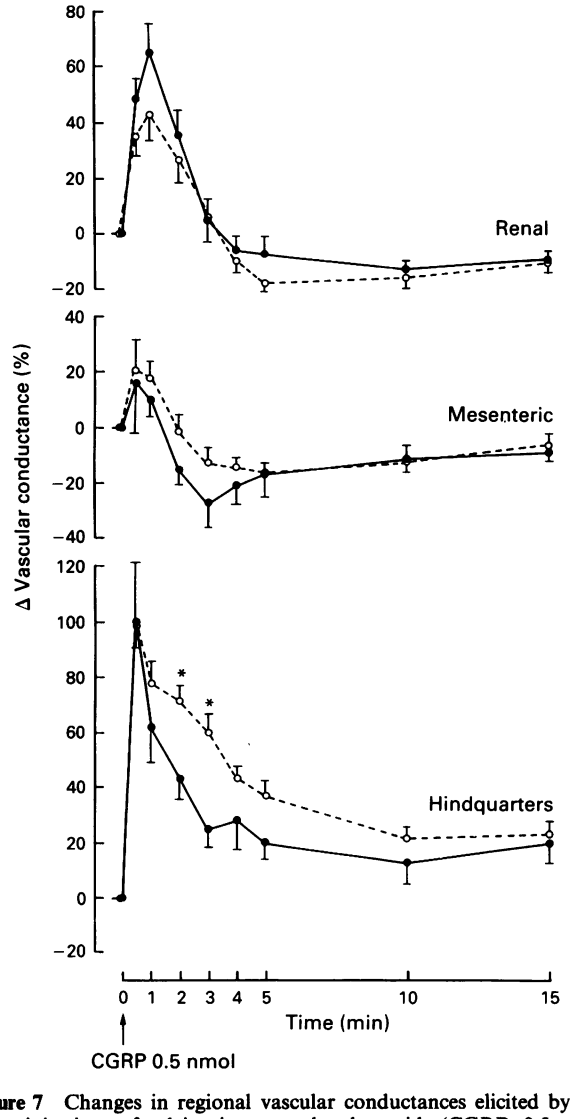

Figure 7 Changes in regional vascular conductances elicited by i.v. bolus injections of calcitonin gene-related peptide (CGRP, 0.5 nmol) in vehicle-treated (●) or capsaicin-treated (○) Long Evans rats. These data were derived from the data shown in Figure 6. Values are mean with s.e.mean shown by vertical lines. * $P < 0.05$ vehicle-treated group ($n = 9$) versus capsaicin-treated group ($n = 9$), (analysis of variance followed by Fisher's test).

responses elicited by both doses of CCK were slightly greater than, those in vehicle-treated rats. Hence, these results suggest that capsaicin-sensitive neurones modulate the pressor response to exogenous CCK in control animals. The blood pressure and heart responses to CCK in capsaicin-treated rats were associated with a greater renal vasoconstriction, while the superior mesenteric and hindquarters vasoconstrictor effects were not different from those seen in vehicle-treated rats. However, the possibility that the greater renal vasoconstrictor response to CCK was responsible for the potentiation of the pressor response in capsaicin-treated rats seems unlikely since, under both conditions (i.e., in vehicle- and in capsaicin-treated rats) the pressor response to CCK was not accompanied by any significant changes in renal flow (Table 2). Therefore, these results suggest the decrease in renal vascular conductance was autoregulatory, rather than indicating direct or indirect effects of CCK on the renal vasculature. The potentiation of the pressor response to CCK in capsaicin-treated rats raises the possibility that there was a greater increase in cardiac output in these animals than in vehicle-treated rats. However, we cannot exclude the possibility that in the capsaicin-treated rats CCK evoked greater vasoconstrictor responses in vascular beds not examined.

Haemodynamic effects of substance P

Carter & Lightman (1983) observed an increased sensitivity of the SP receptors in the nucleus tractus solitarius in capsaicin-treated rats. It has also been reported that neonatal treatment with capsaicin causes a supersensitivity to SP in laminae I and II of the lumbar spinal cord (Mantyh & Hunt, 1985). These observations constitute a rational basis for investigating the effects of neonatal treatment with capsaicin on the cardiovascular responses to SP.

Administration of SP produced a dose-related depressor response and tachycardia in both vehicle- and capsaicin-treated rats, accompanied by dilatations in the renal and hindquarters vascular beds and constriction in the superior mesenteric vascular bed (Miller & Scott, 1988; Gardiner *et al.*, 1989c). In capsaicin-treated rats, the depressor response to SP was slightly less, and the renal vasodilatation was absent compared to vehicle-treated rats. However, the magnitudes of the tachycardia and the dilatations in the superior mesenteric and hindquarters vascular beds observed in capsaicin-treated rats were not different from those in vehicle-treated rats. It is likely that the inhibition of the renal vasodilatation in capsaicin-treated rats was responsible for the slight attenuation of the hypotensive response to SP. Although the explanation of these effects is unclear, the present results do not indicate any increase in sensitivity of the vasculature to SP in capsaicin-treated rats.

Haemodynamic effects of calcitonin gene-related peptide

The characteristics of the cardiovascular responses to CGRP we observed in vehicle-treated rats are consistent with those of previous studies carried out in conscious, untreated rats (Gardiner *et al.*, 1988; 1989a,b), and as in those studies, the lack of a mesenteric vasodilator response to CGRP was probably due to activation of reflex and neurohumoral mechanisms.

In rats treated neonatally with capsaicin the depressor response to CGRP was more prolonged than in vehicle-treated rats, but the heart rate and renal and mesenteric conductance changes were not significantly different from those in vehicle-treated rats. However, the prolongation of the depressor response to the high dose of CGRP in the capsaicin-treated rats was associated with a significant prolongation of the rise in hindquarters vascular conductance. Thus, although the potentiation of the depressor and hindquarters vasodilator responses to CGRP in capsaicin-treated rats might have been due to neonatal treatment with capsaicin causing a small increase in CGRP sensitivity, this effect was not sufficient to show up as an increase in all regional vasodilator responses to the peptide.

Conclusions

From the present results it appears that neuropeptide-containing, capsaicin-sensitive neurones make a contribution to the cardiovascular responses to exogenous BK and CCK in conscious Long Evans rats. Considering the cardiovascular responses to exogenous SP and CGRP, it is feasible that release of these peptides, from capsaicin-sensitive neurones, following administration of BK or CCK contributes to the haemodynamic effects of the latter peptides. However, we have been unable to inhibit the haemodynamic responses to exogenous BK by pretreatment with the CGRP antagonist, human α-CGRP [8–37] (Gardiner, S.M. *et al.*, unpublished observations). Nonetheless, further studies are required to assess the possibility that local release of neuropeptides contributes to the effects of BK or CCK, since capsaicin-sensitive sensory neurones are known to contain vasoactive peptides in addition to SP and CGRP.

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