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INFLUENCE OF PERIVASCULAR PEPTIDES ON ENDONEURIAL BLOOD FLOW AND MICROVASCULAR RESISTANCE IN THE SCIATIC NERVE OF THE RAT

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SUMMARY

1. A variety of vasoactive peptides has been identified in the axon terminals innervating vasa nervorum but their function is unknown. In mesenteric arterioles, substance P (SP) and calcitonin gene-related peptide (CGRP) have been postulated to have a role in tonic vasodilatation.

2. We explored the effect of epineurial capsaicin, SP, CGRP, spantide (SP antagonist), and hCGRP (8–37) (CGRP antagonist) on blood flow (EBF) and microvascular resistance (EMR) in the endoneurial compartment of the rat sciatic nerve, as measured by hydrogen clearance.

3. Epineurial capsaicin induced a prompt, intense and prolonged increase in EBF and lowering of EMR as compared to epineurial application of the carrier alone in a separate animal group. The hyperaemic response was also confirmed by studying serial clearance curves in individual animals.

4. Multifibre sciatic–tibial motor conduction was not changed by epineurial capsaicin.

5. When co-administered with capsaicin, hCGRP (8–37) completely blocked the hyperaemic response and increased EMR above the pooled control range. Spantide also blocked the capsaicin response.

6. When administered alone, both epineurial hCGRP (8–37) and spantide lowered EBF below and increased EMR above the control measurements in the same animals.

7. At 10^{-5} M epineurial CGRP, but not SP lowered EMR. Vasodilatation from intra-arterial administration of CGRP was much greater and was more prolonged compared with that induced by SP. hCGRP (8–37), but not spantide reduced the intra-arterial response to CGRP.

8. The findings suggest that epineurial peptidergic terminals mediate a vasodilatory response (particularly through CGRP) that increases blood flow in the 'downstream' endoneurial compartment. Physiological peptide release (blocked by SP and CGRP receptor antagonism) may be important in maintaining tonic vasodilatation.

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INTRODUCTION

A subpopulation of dorsal root ganglia sensory fibres are 'capsaicin sensitive' in that they release their contents and later degenerate with capsaicin treatment (Lembeck, 1983, 1988; Buck & Burks, 1986; Holzer, 1988; Wharton, Gulbenkian, Mulderry, Ghatei, McGregor, Bloom & Polak, 1986). These fibres may contain substance P (SP), calcitonin gene-related peptide (CGRP), other peptides or a combination (Lundberg, Franco-Cereceda, Hua, Hokfelt & Fischer, 1985; Gibbins, Furness, Costa, MacIntyre, Hillyard & Girgis, 1985; Uddman, Edvinsson, Ekblad, Hakanson & Sundler, 1986; Gulbenkian, Merighi, Wharton, Varndell & Polak, 1986; Ju, Hokfelt, Brodin, Fahrenkrug, Fischer, Frey, Elde & Brown, 1987; Merighi, Polak, Gibson, Gulbenkian, Valentino & Peirone, 1988). Antidromic stimulation of primary unmyelinated sensory fibres releases substance P (and CGRP) resulting in vasodilatation and plasma extravasation in the territory of innervation (Lembeck, 1983; Izumi & Karita, 1990).

Peripheral nerve is supplied by a plexus of blood vessels – vasa nervorum – innervated by unmyelinated nerve terminals (Hromada, 1963). Some of these terminals are noradrenergic, and mediate vasoconstriction but others are peptidergic and of unknown function (Appenzeller, Dhital, Cowen & Burnstock, 1984; Rechthand, Hervonen, Sato & Rapoport, 1986; Dhital & Appenzeller, 1988). Their content of SP and CGRP suggests that they may be vasodilatory, as in other vascular beds. The epineurial/perineurial and endoneurial vascular compartments of peripheral nerve have distinct morphological, physiological and functional features (Low, Lagerlund & McManis, 1989). Noradrenergic innervation of vasa nervorum is predominantly epineurial and perineurial, and has 'tonic' activity (Rechthand *et al.* 1986; Zochodne & Low, 1990; Zochodne, Huang, Ward & Low, 1990). There is evidence that epineurial adrenergic α-receptors determine 'downstream' endoneurial flow and that some segments of epineurial arterioles are particularly sensitive to applied noradrenaline (Zochodne & Low, 1990; Kihara & Low, 1990). However, peptidergic innervation could also be important in allowing neurogenic dilatation of epineurial/perineurial 'feeding' arterioles, and may provide 'tonic' dilatation as well. Indeed, 'tonic' peptidergic dilatation has been suggested in mesenteric vessels (Han, Naes & Westfall, 1990). Moreover, it could be that hyperaemic responses of peripheral nerve to stimulation or injury are peptide-mediated (Zochodne & Ho, 1990, 1991).

In the present study, we explored the action of substance P (SP) and calcitonin gene-related peptide (CGRP) on peripheral nerve endoneurial blood flow (EBF) and endoneurial microvascular resistance (EMR). We exploited the acute release of nerve terminal peptides that occurs from local capsaicin treatment and measured EBF using the technique of hydrogen clearance which permits serial recordings and pharmacological manipulation. Four major questions were addressed: (i) Does acute capsaicin-induced peptide release increase EBF and lower EMR? (ii) If so, is the effect mediated by SP or CGRP or both? (iii) Does capsaicin acutely injure the peripheral nerve trunk? (iv) Does blockade of SP or CGRP receptors influence normal EBF or EMR?

METHODS

Animals

The experiments were performed on 200–250 g male Sprague–Dawley rats housed in grouped wire cages with free access to rat chow and water.

Multifibre conduction studies

Under pentobarbitone anaesthesia (65 mg kg^{-1} I.P.), recordings were made of multifibre sciatic–tibial motor conduction as previously described (Zochodne, Ward & Low, 1988). The nerve was supramaximally stimulated at the sciatic notch and the knee, spanning the segment on which epineurial capsaicin was applied (see below). Subcutaneous temperature above the nerve segment studied was maintained at 37 ± 1 °C with a thermistor probe and temperature control feedback unit (TH8 and TCAT-1A, Sensortek, Clifton, NJ, USA). Compound motor action potentials from the dorsal foot muscles were recorded on a digital oscilloscope (Nicolet 310, Madison, WI) and stored on microdiskette. Latencies were measured to the onset of the negative deflection of the potential and amplitudes calculated from baseline to peak.

Animal preparation (EBF and EMR studies)

The animals were anaesthetized with sodium pentobarbitone by intraperitoneal injection (65 mg kg^{-1}). A ventral mid-line neck incision was made for placement of left carotid intra-arterial line (PE-50, Intramedic, Clay Adams, Parsippany, NJ) connected to a pressure transducer (P23ID Gould, Oxnard, CA) and tracheostomy. The animal was then paralysed using tubocurarine (1·5 mg kg^{-1} intra-arterial; Sigma Chemical Co., St Louis, MO) and ventilated (Roden ventilator 683, Harvard Bioscience, South Natick, MA) through flowmeters (NO32-416 and N112-026, Cole-Parmer, Chicago, IL) to permit regulation of inspired gases. The left sciatic nerve was exposed, covered with mineral oil and the microelectrode was inserted through an epineurial window into the endoneurium. A remote reference KCl–agar bridge electrode was inserted subcutaneously and sutured into place. The microelectrode was connected to a microsensor (Microsensor II, Diamond General, Ann Arbor, MI) for polarization and current detection. The exposed nerve preparation was maintained at 37 °C throughout using a thermistor probe connected to a control and feedback unit with an infra-red heating lamp (as above). Additional doses of pentobarbitone (20 mg kg^{-1}) and tubocurarine (0·8 mg kg^{-1}) were administered two hourly through the intra-arterial catheter to maintain a constant level of anaesthesia (as judged by the level and stability of mean arterial pressure recordings). Pentobarbitone has not been shown to significantly influence nerve blood flow (Sugimoto, Monafo & Eliasson, 1986). Continuous recordings of mean arterial pressure (MAP) and the microsensor current reading were made using two channels of a polygraph recorder (79E, Grass Instruments, Quincy, MA).

EBF and EMR

EBF was measured using an endoneurial microelectrode sensitive to hydrogen clearance as previously described (Tuck, Schmelzer & Low, 1984; Low & Tuck, 1984). In brief, the microelectrodes were made using an electrode puller (Model 720, Kopf Instruments, Tujunga, CA) and consisted of a glass housing with platinum core and tip of 3–5 μm diameter bevelled to a 45 deg angle and tested for linearity. Hydrogen was admixed (10–15%) with the ventilatory gas mixture until the hydrogen signal indicated endoneurial saturation. At this stage, the hydrogen was turned off and a clearance curve was recorded. The curve was considered acceptable if MAP was within a suitable range (e.g. > 100 mmHg). After hydrogen clearance was complete (usually within 20 min although the signal was routinely recorded for 30–40 min), arterial blood gases were withdrawn (identical volume replaced with heparanized Ringer solution) from the carotid line. The clearance curve was taken if the values were within an acceptable physiological range (e.g. P_{O_2} > 90 mmHg; P_{CO_2} 35–45 mmHg; pH 7·35–7·45) (see Table 1). The first clearance curve was routinely discarded because it is slightly faster than subsequent recordings (which remain stable), as previously documented (Day, Schmelzer & Low, 1989*b*; Zochodne & Ho, 1990). Hydrogen clearance curves were fitted to a mono- or biexponential model for calculation of EBF using a least-squares regression program (Systat Version 4.1, Evanston, IL). As in previous work (Low & Tuck, 1984;

Day, Lagerlund & Low, 1989*a*), nutritive blood flow was derived from the slow component of the wash-out curve. The equation $y = a\exp(bx)+c\exp(dx)$ described biexponential clearance curves (one term for monoexponential) with EBF $= -d \times 100$ where d is the rate constant for the slow wash-out component, b for the fast component, y = current and x = time. Composite flow was calculated as $F = [-w_1 b+(w_1-1)d] \times 100$ where $w_1 = a/(a+c)$. Percentage non-nutritive flow calculated as $w_1 \times 100$. EMR was calculated as MAP/EBF.

TABLE 1. Arterial blood gases

Experiment	n	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	pH
Parallel studies				
(i) Capsaicin (3·5 h)	6	132±3	41·3±0·7	7·36±0·01
Carrier (3·5 h)	6	128±6	41·8±1·8	7·36±0·01
Serial studies				
(ii) Capsaicin	8	147±5	40·2±0·9	7·38±0·01
+hCGRP (8–37)	4	167±8	38·4±1·5	7·43±0·02
+spantide	4	157±5	39·4±1·1	7·42±0·01
(iii) 10^{-5} M-CGRP	7	161±2	39·4±1·1	7·39±0·02
10^{-5} M-SP	3	152±9	39·1±0·4	7·37±0·01
(iv) hCGRP (8–37)	6	169±5	39·6±0·8	7·41±0·01
Spantide	6	145±14	40·8±1·8	7·37±0·01

Values are means±S.E.M. P_{O_2}, arterial oxygen tension; P_{CO_2}, arterial carbon dioxide tension.

Agents

Peptide doses were based on their reported action in other tissue beds (Otsuka & Yanagisawa, 1988; Han *et al.* 1990). Several reasons justified higher doses than those employed in direct pharmacological studies of excised and perfused vessel segments (Edvinsson, Ekman, Jansen, Ottosson & Uddman, 1987): (i) capsaicin releases local SP in concentrations greater than 10^{-8} M (Moskowitz, Brody & Liu-Chen, 1983); (ii) the epineurial arteriolar plexus has varying degrees of connective tissue overlying it, which we did not disrupt; (iii) direct application of lower doses of peptide were not effective, probably because of limited penetration to the site of vasoactive action; (iv) in similar published experiments considerably higher doses of epineurial noradrenaline were required to mimic the vasoconstrictor effects of local intra-arterial infusion of noradrenaline (Kihara & Low, 1990; Zochodne & Low, 1990); (v) the vessels were not pre-contracted, as in experiments on excised vessels (Edvinsson *et al.* 1987).

Substance P (SP), spantide ([D-Arg^1, D-Trp^{7,9}, Leu^1-SP), hCGRP (8–37) (human calcitonin gene-related peptide receptor antagonist) and CGRP (calcitonin gene-related peptide) were administered immediately after preparation or thawing (storage at −70 °C immediately following preparation). Samples were not thawed more than once. All solutions were applied to the epineurium in a constant volume of 0·20 ml. SP, spantide and CGRP were purchased from Sigma Chemical Co. (St Louis, MO, USA) and hCGRP (8–37) from Bachem (Torrance, CA). Evidence for CGRP antagonism of hCGRP (8–37) and SP antagonism of spantide has been published (Hakanson & Sundler, 1985; Otsuka & Yanagisawa, 1988; Chiba, Yamaguchi, Yamatani, Nakamura, Morishita, Inui, Fukase, Nuda & Fujita, 1989).

Protocol

Experiments were conducted in two formats. In parallel studies, two separate groups of animals (one treated with capsaicin, the other with carrier only) were compared (see below). In serial studies, comparisons were made before and after intervention in the same animal group. Figure 1 outlines the serial experimental protocol.

(i) *Parallel capsaicin studies.* In these experiments one group of rats underwent sciatic–tibial multifibre conduction recordings (see above) followed by exposure of the left sciatic nerve and application of 2% capsaicin. Capsaicin was dissolved in normal saline with 10% ethanol and 10% Tween 80 (Sigma Chemical Co., St Louis, MO). The thigh wound was resutured with capsaicin

in situ and conduction recordings repeated 3 h later. Following this, the thigh wound was reopened and measurements made (at 3·5 h) of EBF (see above). The second group of animals underwent identical studies but the carrier solution without capsaicin was administered.

(ii) *Serial capsaicin studies* (Fig. 1). After a normal clearance curve had been recorded (the second serial curve obtained, see above), capsaicin was applied to the epineurium and left *in situ* starting 3 min before and continued during the clearance curve. In separate animals, spantide or

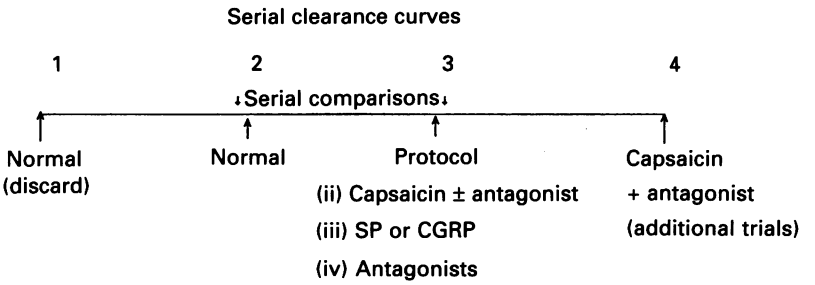

Fig. 1. Outline of experimental approach in the analysis of serial hydrogen clearance curves.

hCGRP (8–37) was applied 2 min before capsaicin administration (before the clearance curve) to study SP and CGRP antagonism respectively. The effects of spantide and hCGRP (8–37) were also studied in subsequent clearance curves after capsaicin had been administered and the evoked hyperaemia had been recorded.

(iii) *Serial studies of SP and CGRP* (Fig. 1). SP or CGRP were applied at varying concentrations to the epineurium after a normal clearance curve had been recorded (as in (ii)) and a subsequent curve was then recorded.

(iv) *Serial studies of spantide and hCGRP (8–37)* (Fig. 1). Spantide or hCGRP (8–37) at 10^{-4} M was applied to the epineurium after a normal clearance curve had been recorded (as in (ii)) and a subsequent curve was recorded.

(v) *Intra-arterial studies.* A femoral arterial catheter (PE-10, Intramedic, Parsipanny, NY) was inserted to the level of the aorto-iliac bifurcation (position checked at autopsy) to permit administration (by-passing a direct cardiac effect; Franco-Cereceda & Lundberg, 1985) of varying concentrations of SP and CGRP. Bolus doses of 0·20 ml were given through the catheter. The effects of spantide and hCGRP (8–37) on CGRP vasodilatation were also explored by this method.

Data analysis

Results were analysed by calculating means and standard errors and comparisons using Student's two-tailed (unless specified), unpaired or paired (for serial studies) *t* tests. Unpaired group comparisons were also made using a one-way analysis of variance (ANOVA). Statistical analyses were carried out using an IBM 55SX computer with appropriate software (Systat 4.1, Evanston, IL, Graphpad Instat, San Diego, CA). The null hypothesis was rejected for $P < 0·05$.

RESULTS

(i) *Parallel capsaicin studies*

The influence of epineurial capsaicin treatment on multifibre sciatic–tibial motor conduction was assessed by comparing studies before and 3 h after treatment both on the treated side and on the untreated contralateral side ($n = 6$). No significant change in conduction velocity was observed (Fig. 2). The ratio of distal/proximal

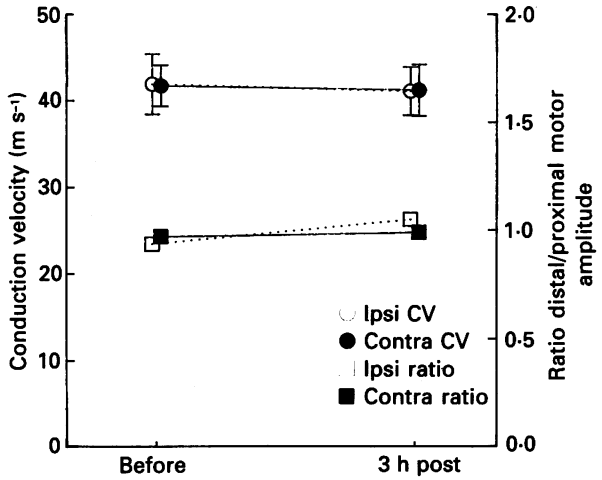

Fig. 2. Comparison of ipsilateral (capsaicin-treated) and contralateral (untreated) sciatic–tibial motor conduction before and 3 h after applying capsaicin to the sciatic nerve. Motor responses were recorded from the dorsal foot muscles. Proximal (sciatic notch) and distal (knee) stimulation sites spanned the treated nerve segments. No significant changes were noted in the conduction velocity (CV) or distal/proximal ratio of motor response amplitudes.

TABLE 2. Effects of capsaicin and carrier on endoneurial blood flow and microvascular resistance

Measurement	Capsaicin treated (3·5 h)	Carrier treated (3·5 h)	P
n	6	6	
EBF nutritive (ml 100 g^{-1} min^{-1})	25·0 ± 3·5	13·8 ± 1·0	0·008
Composite flow (ml 100 g^{-1} min^{-1})	28·7 ± 5·4	34·7 ± 8·7	n.s.
[% non-nutritive]	[6·9 ± 4·4]	[20 ± 12]	n.s.
MAP (mmHg)	114 ± 9	123 ± 6	n.s.
EMR (mmHg 100 g min ml^{-1})	5·03 ± 0·85	9·16 ± 0·82	0·006

Values are means ± S.E.M. EBF, endoneurial blood flow; MAP, mean arterial pressure; EMR, endoneurial microvascular resistance.

stimulated compound motor action potentials (spanning the site of capsaicin application) did not significantly differ from unity (Fig. 2). In control animals with application of carrier ($n = 6$) alone, EBF and EMR were not significantly different from published normals (Tuck, Schmelzer & Low, 1984; Rundquist, Smith, Michel, Ask, Oberg & Rapoport, 1985; Day *et al.* 1989*b*; Zochodne *et al.* 1990). Capsaicin application increased EBF by 80% (Fig. 3) and reduced EMR by 45% (Table 2). A second set of parallel experiments (three animals in each case) suggested that hyperaemia was also present after only 1 h but the differences between the capsaicin and carrier treated group were not statistically significant (Fig. 3). When capsaicin was applied with the nerve exposed and visualized by a dissecting microscope the subjective impression was that prompt vasodilatation of the epineurial plexus developed. Serial studies (see below) confirmed that the capsaicin hyperaemic response occurred as early as 3 min after application.

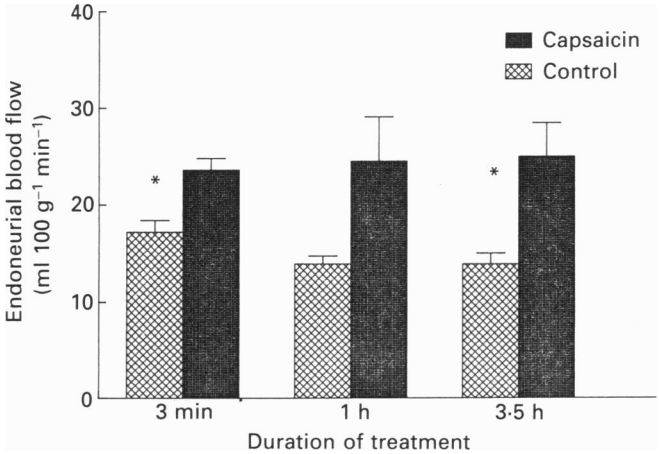

Fig. 3. Sciatic nerve endoneurial blood flow (EBF) in animals treated with epineurial capsaicin for varying periods. Control values are derived from preceding normal wash-out curves (3 min studies) or parallel application of the capsaicin carrier solution without capsaicin (1 and 3·5 h). * Difference significant ($P < 0.05$).

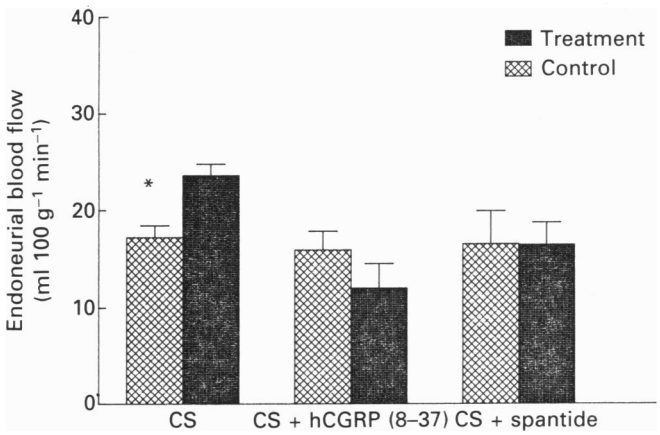

Fig. 4. Sciatic nerve endoneurial blood flow (EBF) before (controls) and after epineurial capsaicin (CS) (2%), capsaicin and hCGRP (8–37) (10^{-4} M) or capsaicin and spantide (10^{-4} M). * Difference significant ($P < 0.05$).

(ii) *Serial capsaicin studies*

In serial studies ($n = 8$) capsaicin increased EBF by 37% (Fig. 4) over previous measurements in the same animal and reduced EMR by 23% indicating vasodilatation (Table 3, Fig. 6). Application of hCGRP (8–37) (10^{-4} M) just before capsaicin ($n = 4$) not only completely blocked the capsaicin induced vasodilatation, but also increased EMR above the pooled control range indicating vasoconstriction (Table 3). EMR increased by 35% (Fig. 6). The effects of capsaicin and of capsaicin with hCGRP (8–37) on the hydrogen clearance curve are illustrated in Fig. 7. In four animals, capsaicin and hCGRP (8–37) (10^{-4} M) were applied after EBF had already been increased by capsaicin. EBF subsequently declined by an average of 38% ($P = 0.03$, Student's paired t test).

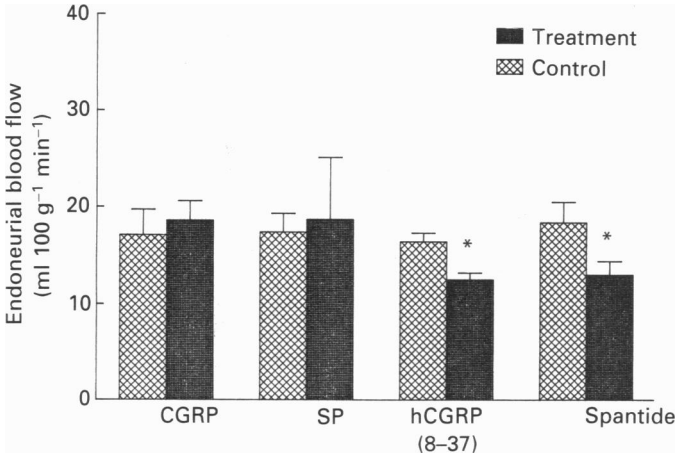

Fig. 5. Sciatic nerve endoneurial blood flow (EBF) before (controls) and after epineurial CGRP (10^{-5} M), SP (10^{-5} M), hCGRP (8–37) (10^{-4} M) or spantide (10^{-4} M). * Difference significant ($P < 0.05$).

TABLE 3. Influence of capsaicin with antagonists on endoneurial microvascular resistance

	n	EMR (mmHg 100 g min ml^{-1})	MAP (mmHg)
Pooled controls*	38	8·06 ± 0·50[a]	126 ± 3
Capsaicin	8	5·40 ± 0·26[b]	126 ± 5
+ hCGRP (8–37)	4	12·9 ± 2·3[c]	139 ± 7
+ spantide	4	8·5 ± 1·4[d]	130 ± 2

Values are means ± S.E.M. * Pooled control wash-out values from all serial experiments; see Figs 4 and 6 for control values of individual serial experiment groups and paired comparisons (intra-group). Unpaired inter-group comparisons: a *vs.* b, $P = 0.02$; a *vs.* c, $P = 0.007$; a *vs.* d, P = n.s.; b *vs.* c, $P = 0.0008$; b *vs.* d, $P = 0.012$ (Student's unpaired *t* test).

Application of spantide (10^{-4} M) just before capsaicin ($n = 4$) also blocked the EBF enhancement and the fall in EMR (Figs 4 and 6, Table 3). Spantide also reduced EBF in the epineurial bed of two of three animals that had already been treated with capsaicin.

Control EBF and EMR values did not significantly change in the parallel or serial experiments (all control groups compared by ANOVA) and MAP was not changed by any of the treatments. Table 3 gives the results of unpaired inter-group comparisons. Intra-group paired comparisons with the control values of each group are given in Figs 4 and 6.

(iii) *Serial studies on SP and CGRP*

At concentrations of less than 10^{-5} M, epineurial SP ($n = 3$) and CGRP ($n = 3$) had little influence on EBF. At 10^{-5} M-SP ($n = 3$) tended to increase EBF, but the changes were not statistically significant (Fig. 5). EMR did not change significantly (Fig. 6). CGRP ($n = 7$) at the same concentration reduced EMR (Fig. 6). Interpretation of results obtained with higher concentrations than 10^{-5} M-SP ($n = 3$)

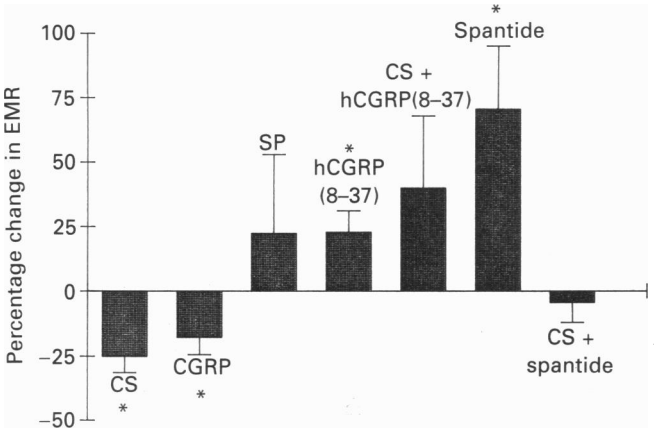

Fig. 6. Percentage change in endoneurial microvascular resistance (EMR) between a preceding 'normal' measurement and that following the intervention indicated. CS, capsaicin. * Difference from preceding measurement significant ($P < 0.05$).

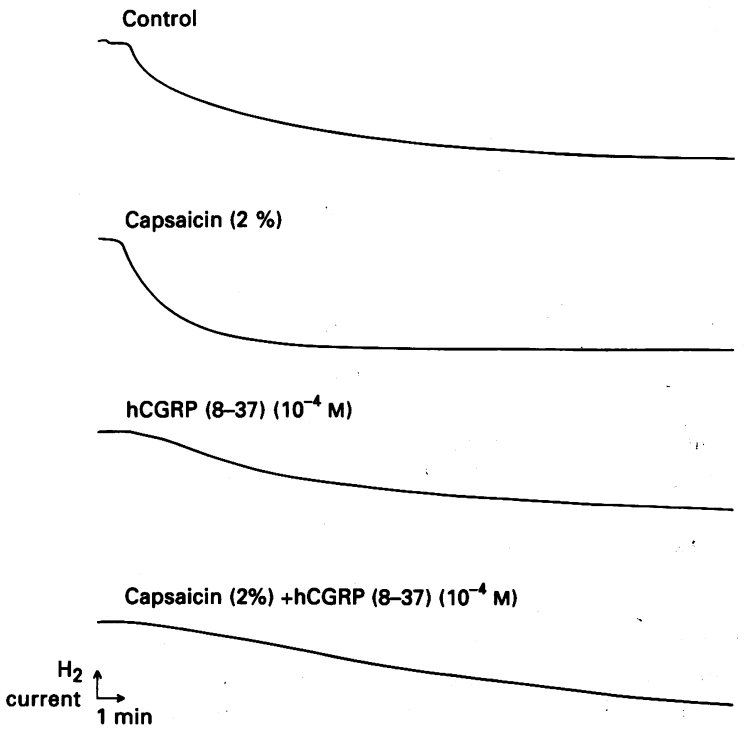

Fig. 7. Examples of changes in wash-out slopes of hydrogen from the sciatic nerve in different animals with epineurial administration of capsaicin and hCGRP (8–37). H_2 current measured in arbitrary units.

or CGRP ($n = 4$) was apparently complicated by a decline in MAP (probably resulting from systemic absorption of the peptide) and possible reflex adrenergic vasoconstriction.

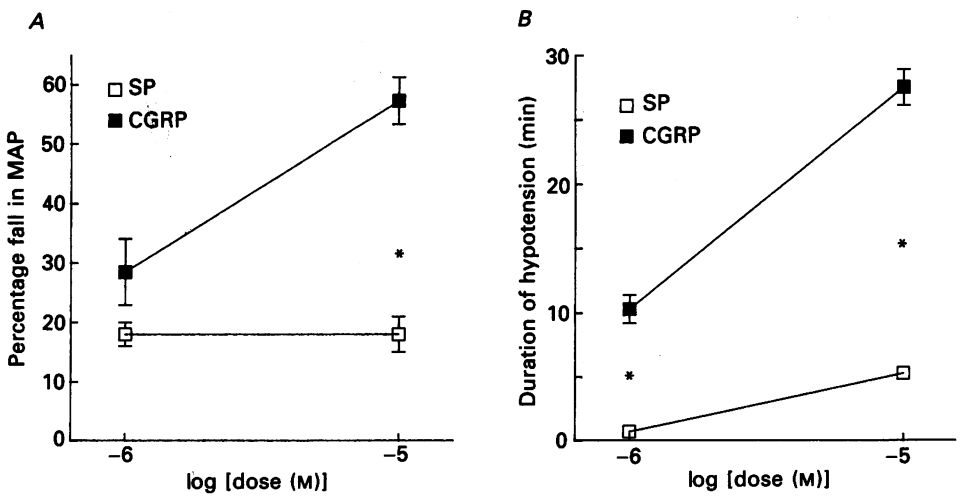

Fig. 8. *A*, percentage fall in MAP from 0·20 ml intra-arterial bolus doses of SP and CGRP. The peptides were delivered into the distal aorta and MAP recorded from a carotid catheter. * Difference significant ($P < 0·05$). *B*, the duration of hypotension from intra-arterial SP and CGRP (as in *A*). * Difference significant ($P < 0·05$).

TABLE 4. Influence of epineurial hCGRP (8–37) and spantide on endoneurial blood flow and microvascular resistance

Measurement	Pooled controls*	hCGRP (8–37)	Spantide
n	38	6	6
EBF nutritive (ml 100 g^{-1} min^{-1})	$17·0 \pm 0·7^{a}$	$12·5 \pm 0·7^{b}$	$13·0 \pm 1·4^{c}$
Composite flow (ml 100 g^{-1} min^{-1})	$30·3 \pm 2·4^{d}$	$13·8 \pm 1·2^{e}$	$15·0 \pm 2·1^{f}$
[% non-nutritive]	$[23·3 \pm 4·3]^{g}$	$[1·0 \pm 1·0]^{h}$	$1·1 \pm 1·1^{i}$
MAP (mmHg)	126 ± 3	128 ± 5	126 ± 7
EMR (mmHg 100 g min ml^{-1})	$8·06 \pm 0·50^{j}$	$10·3 \pm 0·6^{k}$	$10·2 \pm 1·2^{l}$

Values are means ± S.E.M. * Pooled control wash-out values from all serial experiments; see Figs 5 and 6 for control values of individual serial experiment groups and paired comparisons (intra-group). Unpaired inter-group comparisons: a, b, c, $P = 0·01$ (ANOVA); a *vs.* b, $P = 0·02$; a *vs.* c, $P = 0·03$; d, e, f, $P = 0·003$ (ANOVA); d *vs.* e, $P = 0·007$; d *vs.* f, $P = 0·01$; g, h, i, $P = 0·02$ (ANOVA); g *vs.* h, $P = 0·04$; g *vs.* i, $P = 0·04$; j, k, l, $P = 0·09$ (ANOVA); j *vs.* k, $P = 0·04$ (one-tailed); j *vs.* l, $P = 0·05$ (one-tailed).

(iv) *Serial studies of hCGRP (8–37) and spantide*

Studies were limited to a single high (10^{-4} M) concentration of each antagonist. Both spantide and hCGRP (8–37) alone ($n = 6$ each agent) decreased EBF and increased EMR compared to the previous control clearance measurements indicating vasoconstriction (Table 4, Figs 5 and 6). The changes were similar to those noted with co-administration of capsaicin and the antagonists (see (ii) above). Figure 7 illustrates the effect of 10^{-4} M-hCGRP on the hydrogen clearance curve. MAP was not influenced by either spantide or hCGRP (Table 4). Both reduced the fast component of the hydrogen clearance curve which is included in the calculation of 'composite flow' (Table 4).

(v) *Intra-arterial studies*

Intra-arterial CGRP resulted in a prompt dose-dependent fall in MAP with slow recovery. In contrast, the vasodilatory effect of SP was comparatively modest and brief (Fig. 8*A*, *B*). Prior administration of hCGRP (8–37) (10^{-4} M), but not spantide (10^{-4} M), reduced the MAP response to CGRP. The data are provided in Table 5.

TABLE 5. Influence of CGRP, SP and antagonists on mean arterial pressure

Agents	*n*	Pre-dose MAP (mmHg)	Percentage fall MAP	Duration (min)
CGRP (10^{-6} M)	8	117±6	36·0±6·4[a]	10·7±0·8
+hCGRP (8–37)	4	129±8	15·0±2·0[b]	14·3±3·5
+spantide	5	127±6	40·1±4·8	12·6±2·7
CGRP (5×10^{-6} M)	3	129±4	43·3±4·8	15·7±1·5
CGRP (10^{-5} M)	6	113±8	60·4±3·3[c]	29·3±1·6
+hCGRP (8–37)	4	134±4	36·4±11·2[d]	23·1±6·9
+spantide	5	115±7	48·4±4·0	20·0±1·9
SP (10^{-7} M)	2	105±11	7·0±2·0	0·5
SP (10^{-6} M)	4	109±6	22·3±2·6	2·0±0·8
SP (10^{-5} M)	4	116±2	22·0±2·6	5·8±0·4

Values are means±S.E.M. Concentration of hCGRP (8–37) and spantide was 10^{-4} M. a *vs.* b, $P = 0{\cdot}05$; c *vs.* d, $P = 0{\cdot}04$.

DISCUSSION

The mechanism of acute peptide release induced by capsaicin is not completely understood despite a search for an endogenous capsaicin analogue. Capsaicin appears to selectively depolarize afferent nerve terminals in the peripheral nervous system and dorsal horn of the spinal cord (Otsuka & Yanagisawa, 1988). There is now a body of evidence from varying tissues to indicate that SP and CGRP and probably other tachykinins are co-localized in afferent neurons (Lundberg *et al.* 1985; Gibbins *et al.* 1985; Gulbenkian *et al.* 1986; Merighi *et al.* 1988) and that capsaicin causes acute release of both from sensory perivascular fibres (Saria, Theodorsson-Norheim, Gamse & Lundberg, 1984; Zaidi, Bevis, Girgis, Lynch, Stevenson & MacIntyre, 1985; Uddman *et al.* 1986; Wharton *et al.* 1986; Izumi & Karita, 1990). The present studies provide evidence that capsaicin-sensitive peptide terminals exist on vasa nervorum. Also, they indicate that the released terminal contents cause vasodilatation.

The advantages of using the hydrogen clearance technique in nerve over other methods include its selectivity for the endoneurial nutritive flow compartment because of high spatial resolution (the microelectrode sensing sphere has a 30 μm radius; Stosseck & Lubbers, 1971). Hydrogen clearance also permits serial measurements of blood flow. Previous work (Low & Tuck, 1984; Rundquist *et al.* 1985; Sladky, Greenberg & Brown, 1985; Day *et al.* 1989*b*; Day & Lagerlund, 1989*a*; Zochodne & Low, 1990) has attested to its reliability, repeatability (provided the first curve is discarded), accuracy and comparability with other methods (see Low *et al.* 1989, for review). However, normal clearance curves require twenty or more minutes to complete and so provide little information about dynamic flow changes. Further, in each animal only three to four curves can be obtained (excluding the first)

which limits pharmacological dose–response data that can be acquired. Also, the normal connective tissue that covers portions of the epineurial plexus means that high doses of pharmacological agents must be applied (as in previously published work, Kihara & Low, 1990) with the possibility of inducing complicating systemic effects. Despite these potential limitations, this approach probably predicts '*in vivo*' microvessel behaviour better than '*in vitro*' study of excised vessel segments. The use of epineurial pharmacological agents allows the study of smaller vessels and provides information about the influence of epineurial arterioles on the endoneurial flow compartment. This experimental strategy has been previously employed in studies of adrenergic epineurial microvessel vasoconstriction (Kihara & Low, 1990).

There are several reasons why it is unlikely that capsaicin induced the release of peptides carried by unmyelinated fibres 'in transit' through the sciatic nerve trunk. Capsaicin was applied on the epineurium, and so had limited (and delayed) access to endoneurial unmyelinated fibres. Secondly, histofluorescence studies have not identified significant amounts of SP or CGRP in axons 'in transit' (Dhital & Appenzeller, 1988). Finally, if peptides had been released from endoneurial sites, then prompt transport to vasoactive sites would be required to explain the present findings; this is also an unlikely possibility. Further, we did not observe changes in conduction velocity nor evidence of conduction block across the treated segment indicating that our findings were not attributable to non-specific nerve trunk injury. Also, the absence of changes in MAP, argued against a generalized systemic effect except when doses of direct CGRP or SP application were greater than 10^{-5} M (as discussed in Results).

Our findings suggest that CGRP mediates a significant proportion of the vasodilatation induced by capsaicin. The experiments also identified a probable effect of SP (see below), but its vasodilator action was less convincing. Differences between the vasodilator actions of SP and CGRP have been observed in other vessel beds as well (Edvinsson, Gulbenkian, Wharton, Jansen & Polak, 1989). The importance of CGRP was supported by the effects of its antagonist hCGRP (8–37) in both blocking and reversing capsaicin-induced hyperaemia. As in previous work, we observed greater and much more prolonged vasodilatation with intra-arterial administration of CGRP than with SP (Brain, Williams, Tippins, Morris & MacIntyre, 1985; Ohlen, Linbom, Staines, Hokfelt, Cuello, Fischer & Hedqvist, 1987; Edvinsson *et al.* 1987). Finally, direct application of CGRP, but not SP at 10^{-5} M lowered microvascular resistance in the endoneurium. It is possible, however, that this was because by using the hydrogen clearance technique we missed a brief, early response to SP.

Despite the relatively modest evidence of a direct SP action, spantide, an SP antagonist, also largely blocked capsaicin hyperaemia. This was not a result of blockade of CGRP receptors because intra-arterial adminstration of spantide did not block CGRP vasodilatation. It is possible that larger amounts of the less potent SP are released by capsaicin or that there are more SP receptors on the microvessels. Neither possibility explains the complete blockade of capsaicin hyperaemia by hCGRP (8–37). An alternative hypothesis is that SP release by capsaicin helps to trigger the additional release of CGRP (perhaps alone or through histamine) that results in vasodilatation. For this hypothesis, both antagonists would interrupt the

vasodilator pathway. In the schema of Lembeck (1983), SP evoked by a variety of stimuli in turn causes release of histamine from mast cells which then stimulates neighbouring axons to 'enlarge' the vasodilated territory. We did not explore the role of histamine. SP depolarizes both secondary afferent fibres in the dorsal horn of the spinal cord and putative enteric axons (Otsuka & Yanagisawa, 1988; Bartho, Holzer, Leander & Lembeck, 1989) raising the possibility that it might depolarize peripheral afferents also, if it were initially released by capsaicin (perhaps by stimulating a receptor related to or identical to that stimulated by capsaicin). If capsaicin-evoked SP release were 'self amplifying' because of excitatory SP receptors on nerve terminals then spantide (and tetrodotoxin, see below) might be expected to interrupt this vasodilator loop, and to indirectly block the vasodilator response. The blockade of capsaicin-induced contraction in guinea-pig ileum (Chahl, 1989) that is induced by tetrodotoxin is consistent with this hypothesis since capsaicin-induced nerve terminal depolarization is not thought to be TTX-sensitive (Buck & Burks, 1986). Finally, it is possible that spantide activates an alternative receptor that mediates an overriding vasoconstriction or blocks the capsaicin receptor. The presence of more than one type of spantide-susceptible tachykinin receptor has been previously suggested (Chahl, 1985; Featherstone, Fosbraey & Morton, 1986; Buck & Shatzer, 1988; Nussbaumer, Yanagisawa & Otsuka, 1989).

Epineurial hCGRP (8–37) and spantide alone both reduced endoneurial blood flow and increased microvascular resistance at the concentrations employed. Composite flow, calculated by including a weighted fraction of the fast component of hydrogen clearance possibly representing epineurial, or arteriovenous shunt flow (Day *et al.* 1989*a*) was also reduced by both antagonists. Spantide-induced vasoconstriction and ischaemia have been suspected (but not proven) to result in the spinal cord necrosis that occurs after intrathecal administration of this agent (Freedman, Post, Kahrstrom, Ohlen, Mollenholt, Owman, Alari & Hokfelt, 1988; Freedman, Hokfelt, Post, Brodin, Sundstrom, Jonsson, Terenius, Leander, Fischer & Verhofstad, 1989). These effects may result from the removal of normal 'tonic' SP vasodilatation. Alternatively, a 'vasoconstrictor' receptor may have been responsible. Vasoconstriction to hCGRP (8–37) has been noted in the mesenteric vascular bed prompting the authors (Han *et al.* 1990) to suggest that CGRP plays an important role in normal physiological 'tonic' vasodilatation (similar to 'tonic' adrenergic activity).

As in previous work on the epineurial circulation (Kihara & Low, 1990; Zochodne & Low, 1990), the findings probably indicate that changes in blood flow in the 'downstream' endoneurial vasculature are determined by the 'feeding' epineurial and transperineurial vessels. Endoneurial microvessels are largely capillary (Bell & Weddell, 1984) with a limited capacity to respond to vasoactive agents, may not be innervated (Rechthand *et al.* 1986; Dhital & Appenzeller, 1988) and were not exposed to peptide action in the epineurial bathing preparation we employed. The hyperaemia induced by capsaicin may indicate that peptidergic terminals are the efferent loop of a local peripheral nerve trunk 'axon reflex'. This possibility requires further study.

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