Involvement of substance P present in primary afferent neurones in modulation of cutaneous blood flow in the instep of rat hind paw

¹Norifumi Yonehara, Ji-Qiang Chen, Yasuo Imai & Reizo Inoki

Department of Pharmacology, Faculty of Dentistry, Osaka University, Suita 565, Osaka, Japan

1 The participation of small-diameter afferent fibres in the microcirculatory haemodynamics of cutaneous tissue was examined by studies on the effects of antidromic stimulation of primary afferent neurones on cutaneous blood flow (CBF) and tachykinin release into the subcutaneous space in the instep of the hind paw of rats.

2 Antidromic stimulation of the sectioned sciatic nerve induced a biphasic flow response, an initial transient decrease followed by an increase, with no alteration in the blood pressure.

3 Neither phase was affected by pretreatment with phentolamine (0.1 mg kg^{-1} , i.a.), propranolol (0.5 mg kg^{-1} , i.a.), atropine (0.5 mg kg^{-1} , i.a.), methysergide (0.5 mg kg^{-1} , i.a.) or mepyramine (10 mg kg^{-1} , i.a.) plus cimetidine (10 mg kg^{-1} , i.a.), but both were significantly inhibited by pretreatment with capsaicin (50 mg kg^{-1} , s.c.).

4 Spantide ($1\text{--}2 \text{ } \mu\text{mol kg}^{-1}$, i.a.), a substance P (SP) antagonist, reduced the basal CBF, and also inhibited both phases of the biphasic flow response evoked by antidromic stimulation of the sectioned sciatic nerve.

5 Intra-arterial infusion of SP ($0.5 \text{ } \mu\text{mol kg}^{-1}$, i.a.) induced a biphasic flow response similar to that elicited by antidromic stimulation of the sectioned sciatic nerve.

6 Antidromic stimulation of the sectioned sciatic nerve caused a marked increase in SP release into the subcutaneous perfusate of the instep of the rat hind paw, but no detectable increase in neurokinin A release.

7 We suggest that SP and its receptors are mainly responsible for the vascular response induced by stimulation of the sectioned sciatic nerve, and that small-diameter afferent fibres containing SP tonically regulate vascular tone in cutaneous microvessels.

Keywords: Antidromic stimulation of the sciatic nerve; cutaneous blood flow; substance P; rat hind paw; primary afferent neurones

Introduction

Vasodilatation (heat, redness), swelling (oedema), and pain, which are the principal signs of inflammation, can all be produced by activity in unmyelinated primary afferent neurones. In a sense, then, primary afferent neurones are not merely pathways for passive signals indicating that tissue damage has occurred, but may play an active role in body defences by participating in the inflammatory process. Recent pathophysiological studies in man and laboratory animals have indicated that unmyelinated sensory neurones containing substance P (SP) may contribute to the pathogenesis of thermal injury (Saria, 1984; Yonehara *et al.*, 1987), polyarthritis (Lembeck & Gamse, 1982), respiratory-tract irritation (Lundberg & Saria, 1983), etc. For example, in rats with experimental arthritis, an increase in the concentration of SP was observed in peripheral nerves with branches innervating inflamed joints (Levine *et al.*, 1984), and administration of capsaicin to rats before or after the onset of arthritis diminished paw swelling and tenderness (Colpaert *et al.*, 1983). Furthermore, thermal injury produced by heat stimulation at $45\text{--}55^\circ\text{C}$ was found not only to excite pain afferents (Fleischer *et al.*, 1983) but also to cause vascular protein leakage in the skin through release of SP from the peripheral endings of capsaicin-sensitive, primary afferent neurones (Saria & Lundberg, 1983; Yonehara *et al.*, 1987). In addition to these pathophysiological findings, which indicate involvement of a neurogenic component in the inflammatory re-

sponse, recent immunohistochemical studies have shown that SP-positive activity is present in nerve axons and terminals in most peripheral organs including skin (Hökfelt *et al.*, 1975; Cuello *et al.*, 1978). SP immunofluorescent fibres in the skin were found in unmyelinated primary sensory neurones terminating around the small blood vessels in the dermis and epithelium. Moreover, electrical stimulation of the small-diameter afferent fibres was shown to induce release of SP in the periphery (Olgart *et al.*, 1977; White & Helme, 1985), and to produce the same cutaneous vasodilatation and plasma extravasation (Lembeck *et al.*, 1982), as after intradermal or intra-arterial administration of SP (Hagermark *et al.*, 1978; Lembeck & Holzer, 1979). These findings suggest that afferent impulses from nerve terminals of small-diameter afferent fibres generated by noxious stimuli may cause release of SP, which may finally control the inflammatory response through regulation of the microcirculation.

The aim of this study was to demonstrate more clearly the participation of small-diameter afferent fibres in microcirculatory haemodynamics in cutaneous tissue. For this, we examined the effects of antidromic stimulation of primary afferent neurones on cutaneous blood flow (CBF) by the noninvasive technique of laser Doppler flowmetry and on SP release into the subcutaneous (s.c.) space in the instep of the hind paw of rats. In addition, since recently another tachykinin, neurokinin A (NKA), which is chemically related to SP, was discovered in the peripheral nervous system, especially in small-diameter afferent fibres containing SP, we also determined the release of NKA into the subcutaneous space.

¹ Author for correspondence.

Methods

Measurement of blood flow

Male Sprague-Dawley rats (140–160 g body weight) were anaesthetized with urethane (780 mg kg⁻¹, i.p.) and then paralyzed by an injection of alcuronium (1 mg kg⁻¹, s.c.) with supplementary injections of the same dose when necessary. The trachea was cannulated and artificial ventilation was carried out during experiments. In most experiments the sciatic nerve was cut centrally and placed on bipolar stimulating electrodes insulated with enamel. The blood pressure was monitored continuously through a cannula inserted into the common carotid artery by means of a pressure transducer. The cutaneous blood flow in the instep of the hind paw was measured with a laser Doppler flowmeter (ALF 2100, ADVANCE). The instep of the hind paw was depilated with commercial depilatory cream, and then the laser probe was placed at a site 5 mm from the caput of the os metatarsal tertium to basis. The experiment was started 1 h later to allow time for the effects of surgery and the depilatory cream to wear off.

The change of blood flow was calculated according to the following formula: $(C-B)/A \times 100$, where A and B represent the blood flows just before intra-arterial infusion of drugs or saline and just before electrical stimulation of the sciatic nerve, respectively and C represents the minimal or maximal blood flow after electrical stimulation.

Release of substance P and neurokinin A into the subcutaneous space

Male Sprague-Dawley rats (140–160 g body weight) were anaesthetized with urethane (780 mg kg⁻¹, i.p.). A double polyethylene tube about 5 cm in length (the inner tube of 0.5 mm diameter was 5 mm longer than the external one of 3 mm diameter) was introduced into the s.c. space of the instep. Perfusion was carried out through this double coaxial tube with a peristaltic pump at a rate of 0.1 ml min⁻¹ with saline containing the aminopeptidase inhibitor bestatin (3 mg/100 ml) and the dipeptidyl carboxypeptidase inhibitor, captopril (0.1 mM). Fractions of perfusate were collected through the outer tube for periods of 10 min with a fraction collector and the tubes were placed in an ice bath. Each 10-min sample (1 ml volume) was lyophilized, and then radioimmunoassayed for SP and NKA with the methods described by Yonehara *et al.* (1986) and Takano *et al.* (1986), respectively. The antiserum of SP cross-reacted with neurokinin A (1%) and neurokinin B (NKB) (0.2%). The detection limit of SP was 1 fmol. On the other hand, the detection limit of NKA was 3 fmol. The NKA antiserum showed no cross-reactivity with SP, but cross-reacted with kassinin (1.5%) and NKB (5.4%).

Electrical stimulation

The sciatic nerve was sectioned proximally, and the end was placed on bipolar, enamel-insulated, stimulating electrodes under liquid paraffin. The sciatic nerve was stimulated for a period of 30 s or 10 min with a 10 V square wave (frequency 2 Hz, duration 1 ms).

Drug administration

Drugs were dissolved in saline and administered by the intra-arterial (i.a.) or s.c. route. Solutions were infused i.a. on the side of measurement, via an arterial cannula inserted into the contralateral common iliac artery. Infusion was carried out continuously for a period of 5 min with the microinjection pump (CMA/100, Carnegie Medicin, Sweden) from 10 min before electrical stimulation. The i.a. infusion rate was 12 μ l min⁻¹, and the dead volume of the inserted arterial cannula was 10 μ l.

Denervation of the hind paw

A solution of 1% capsaicin solution in Tween 80 was prepared by dissolving 50 mg of crystalline capsaicin in 0.5 ml ethanol, adding 0.5 ml Tween 80, and adjusting the final volume to 5 ml with saline. Capsaicin at a dose of 50 mg kg⁻¹ was injected s.c. into rats within a week after their birth, as described by Jancsó *et al.* (1967). Control rats were treated with the solvent only. Two months later, animals showing at least 40% increase in latency in response to noxious thermal stimulation (52°C) compared with control rats, were used for experiments.

Substances used

The following compounds were used: atropine sulphate (Nakarai Chem., Ltd., Japan); phentolamine mesylate (Ciba-Geigy, Japan); propranolol (Sumitomo Chem. Ind., Japan); mepyramine maleate, cimetidine and capsaicin (Sigma, U.S.A.); methysergide bimalate (Sandoz, Switzerland); SP, NKA and spantide (Peptide Institute, Inc., Japan); [¹²⁵I]-[Tyr⁸]SP (New England Nuclear Co., U.S.A.); N-[propionyl-³H]propionylated NKA (Amersham, U.K.); NKA antiserum (donation by Prof. H. Kamiya and Dr Y. Takano, Fukuoka University, Japan).

Results

Effect of electrical stimulation of the sciatic nerve on cutaneous blood flow

Antidromic stimulation of the intact sciatic nerve for 30 s caused an immediate increase in CBF which reached a peak ($256.0 \pm 1.1\%$, $n = 50$) 30 to 60 s after the beginning of stimulation and lasted for 9–12 min. The blood pressure also increased. On the other hand, stimulation of the distal end of the sectioned sciatic nerve led to a biphasic flow response with no alteration of the blood pressure. This biphasic response consisted of an initial transient decrease, followed by an increase, that is, CBF decreased immediately after the start of stimulation ($-28.1 \pm 1.1\%$, $n = 171$) and then increased near the end of stimulation. The increase was maximal 1 to 2 min after the start of stimulation ($84.8 \pm 13.5\%$, $n = 172$) and lasted for 10–12 min (Figure 1). Therefore, in all subsequent experiments, the sciatic nerve was sectioned to avoid a systemic influence on the effect of sciatic nerve stimulation as far as possible. On repeated stimulations, reproducible responses of the CBF to electrical stimulation of the sectioned sciatic nerve were obtained until, at least, the seventh stimulation when a resting period of 1 h was allowed between stimulations.

Involvement of the autonomic nervous system, histamine and 5-hydroxytryptamine in the changes in CBF induced by electrical stimulation of the sectioned sciatic nerve

Intra-arterial infusion of phentolamine (0.1 mg kg⁻¹), propranolol (0.5 mg kg⁻¹) or atropine (0.5 mg kg⁻¹) did not modify the basal CBF, or the changes of the CBF induced by antidromic stimulation of the sectioned sciatic nerve (Table 1). Similar results were obtained when mepyramine (10 mg kg⁻¹) together with cimetidine (10 mg kg⁻¹) or methysergide (0.5 mg kg⁻¹) was administered in the same way (Table 2). The size of the antidromic vasodilatation was not affected by systemic administration of mepyramine plus cimetidine (each 10 mg kg⁻¹, s.c.) 30 min previously. In all these experiments, the blood pressure was unaffected by i.a. infusion of drugs alone or in combination with antidromic stimulation of the sectioned sciatic nerve.

Figure 1 Representative laser Doppler flowmetry traces (upper) and systemic blood pressure traces (lower) following antidromic stimulation of the intact (left) or the sectioned (right) sciatic nerve. Electrical stimulation of the sciatic nerve was performed with a 10 V square wave for 30 s at the time indicated by the solid line (ST).

Table 1 Involvement of the autonomic nervous system in changes in cutaneous blood flow (CBF) induced in the rat hind paw by electrical stimulation of the sectioned sciatic nerve for 30 s

Test compound	Dose (i.a.)	First phase (%) (minimum CBF)	Second phase (%) (maximum CBF)
Saline		-33.2 ± 4.7	88.3 ± 18.5
Phentolamine	0.1 mg kg^{-1}	-25.7 ± 10.2	79.1 ± 18.1
Saline		-27.0 ± 2.0	81.2 ± 12.4
Propranolol	0.5 mg kg^{-1}	-27.7 ± 5.7	87.0 ± 18.2
Saline		-29.0 ± 7.4	83.4 ± 21.2
Atropine	0.5 mg kg^{-1}	-35.2 ± 7.7	78.5 ± 22.7

CBF just before i.a. infusion of saline or test compounds was considered as 0% (see Methods). Ten animals were used for each compound. Values are mean $\pm$ s.e.mean.

Table 2 Effect of mepyramine plus cimetidine and methysergide on changes in cutaneous blood flow (CBF) induced in the rat hind paw by electrical stimulation of the sectioned sciatic nerve for 30 s

Test compound	Dose (i.a.)	First phase (%) (minimum CBF)	Second phase (%) (maximum CBF)
Saline		-15.6 ± 4.9	79.1 ± 11.5
Cimetidine	10 mg kg^{-1}		
+ Mepyramine	10 mg kg^{-1}	-16.6 ± 6.3	93.6 ± 20.1
Saline		-22.9 ± 3.3	81.2 ± 19.1
Methysergide	0.5 mg kg^{-1}	-21.7 ± 4.0	76.0 ± 18.4

CBF just before i.a. infusion of saline or test compounds was considered as 0% (see Methods). Ten animals were used for each compound. Values are mean $\pm$ s.e.mean.

Effects of capsaicin and spantide

Pretreatment of newborn rats with capsaicin (50 mg kg^{-1} , s.c.) and i.a. infusion of spantide ($1\text{--}2 \mu\text{mol kg}^{-1}$) reduced the basal blood flow, and significantly inhibited both phases of change in the CBF; the phase of decrease and the phase of increase of the CBF induced by electrical nerve stimulation (Figures 2 and 3, and Table 3). On the other hand, as shown in Figure 3, blood pressure, particularly systolic pressure,

gradually decreased following i.a. infusion of spantide. The stimulation of the sectioned sciatic nerve subsequent to administration of spantide, however, produced very little alteration of the blood pressure.

Effect of i.a. infusion of substance P

Infusion of SP into the common iliac artery induced a similar biphasic response to that induced by antidromic stimulation

of the sectioned sciatic nerve; that is, the CBF decreased, immediately after the start of infusion of SP, to a minimum within 30 s, and then increased to a maximum about 1 min after the start of infusion. Subsequently, the CBF decreased even when SP infusion was continued for 5 min (Figure 4).

Effect of electrical stimulation of the sectioned sciatic nerve on the substance P and neurokinin A levels in the subcutaneous perfusates

The SP concentration in the perfusate was initially high, but gradually decreased with time to a steady level after 30 min, which was maintained for at least 4 h. The SP level in the resting state was $1.24 \pm 0.1 \text{ fmol } 10 \text{ min}^{-1}$ ($n = 28$). Antidromic stimulation of the sectioned sciatic nerve caused marked increase in SP release (Figure 5). On the other hand,

both NKA levels in the resting state and during antidromic stimulation of the sectioned sciatic nerve were below the detection limit of the assay.

Discussion

Changes in tissue blood flow observed by laser Doppler flowmetry are dependent on the blood pressure and the

Figure 2 Effect of pretreatment with capsaicin on changes in cutaneous blood flow (CBF) induced in the rat hind paw by electrical stimulation of the sectioned sciatic nerve for 30 s. Capsaicin (50 mg kg^{-1} , s.c.) was administered within a week after birth, i.e., 2 months before the experiment. Changes are shown in the CBF induced by antidromic stimulation in vehicle- (open columns) and capsaicin-treated (hatched columns) animals, at times A and B in the upper trace. CBF just before antidromic stimulation was taken as 0% (see Methods). Columns and vertical lines are means $\pm$ s.e. of values for 10 rats. $\star P < 0.05$ vs. change in CBF in vehicle-treated animals in each phase.

Figure 3 Representative laser Doppler flowmetry traces (upper) and systemic blood pressure traces (lower) on antidromic stimulation of the sectioned sciatic nerve after i.a. infusion of saline (a) or spantide (b).

Table 3 Effect of spantide on changes in cutaneous blood flow (CBF) induced in the rat hind paw by electrical stimulation of the sectioned sciatic nerve for 30 s

Test compound	Dose (i.a.)	First phase (%) (minimum CBF)	Second phase (%) (maximum CBF)
Saline		-27.0 ± 3.7	72.5 ± 11.2
Spantide	$1 \mu\text{mol kg}^{-1}$	$-11.0 \pm 2.5^*$	$41.6 \pm 8.3^*$
Saline		-22.3 ± 3.1	71.0 ± 5.3
Spantide	$2 \mu\text{mol kg}^{-1}$	$-5.3 \pm 1.7^*$	$12.5 \pm 6.4^*$

CBF just before i.a. infusion of saline or spantide was taken as 0% (see Methods). Ten animals were used for each dose of spantide. Values are mean $\pm$ s.e.mean. $\star P < 0.05$ vs. change in CBF in saline-treated animals in each phase (Student's t test for paired values).

Figure 4 Representative laser Doppler flowmetry traces (upper) and systemic blood pressure traces (lower) following antidromic stimulation of the sectioned sciatic nerve (a) and i.a. infusion of substance P (SP) (b).

diameter of the blood vessels. In the present study, an increase in blood pressure was observed following electrical stimulation of the intact sciatic nerve, but on stimulation of the cut end of the sciatic nerve sectioned centrally, the increase in blood pressure disappeared without a change in the increase of CBF. A reproducible response of the CBF to electrical stimulation of the sectioned sciatic nerve was obtained until, at least, the seventh stimulation when a resting period of 1 h was allowed between stimulations. These findings suggest that vasoactive substances involved in regulation of blood flow in cutaneous microvessels are present in the sciatic nerve. Cutaneous nerves are known to have two distinct functional categories of axons: primary afferents with cell bodies in the dorsal root ganglion and sympathetic post-ganglionic fibres with cell bodies in sympathetic ganglia (Fields, 1987). Furthermore, experiments have shown that vasoconstriction and vasodilatation in peripheral tissues are mediated by sympathetic α -adrenoceptors, and sympathetic β -adrenoceptors and parasympathetic nerves, respectively. In

Figure 5 Effect of electrical stimulation of the sectioned sciatic nerve on the release of substance P into the subcutaneous perfusate. The nerve was stimulated with a 10 V square wave at the times indicated by the hatched columns. Columns and vertical lines represent means $\pm$ s.e. of values for 14 rats. $\star P < 0.05$ vs. the mean value for the last three fractions before stimulation (Student's *t* test for paired values).

the present study, if the reduction of CBF observed immediately after the start of stimulation of the cut sciatic nerve was mediated through sympathetic α -adrenoceptors, pretreatment with phentolamine, an α -adrenoceptor blocker, should have inhibited this reduction of CBF. So our finding that phentolamine did not block the response indicates that the mechanism of the effect on cutting the sciatic nerve did not involve activation of sympathetic α -adrenoceptors. On the other hand, the increase in the CBF after its decrease was not affected by pretreatment with propranolol (β -adrenoceptor blocker) or atropine (antimuscarinic receptor drug), indicating that the autonomic nervous system is not involved in this vasodilatation on antidromic stimulation. A similar phenomenon has been observed in the dental pulp of cats; namely, pretreatment with propranolol or atropine was found not to influence the increase in pulpal blood flow produced by electrical stimulation of the inferior alveolar nerve (Gazelius & Olgart, 1980). Couture & Cuello (1984), however, demonstrated that the vasodilatation induced in the rat inferior lip by electrical stimulation of the trigeminal nerve was unaffected by pretreatment with guanethidine plus phentolamine and propranolol, but was significantly reduced by atropine. These observations indicate that the influence of the autonomic nervous system on vasodilatation induced by antidromic stimulation may vary in different species or tissues.

The laser Doppler flow meter used in the present study emits light of a 2mW He-Ne laser (wavelength 632.8 nm) which penetrates approximately 1 mm into the skin. Thus the measured changes in blood flow occur in the microvasculature. In the skin, small diameter nerve fibres containing SP are found in the dermis and are sometimes seen to be in association with small blood vessels (Hököfelt *et al.*, 1975; Cuello *et al.*, 1978). The nerve fibres can also be seen to branch from the dermal fibres into the epithelium. Electrical stimulation of the sciatic nerve resulted in a significant increase in SP-like immunoreactivity in the perfusate of the subcutaneous space in the instep of the rat hind paw, as

shown in Figure 5, or in blister fluid of the rat hind paw, and this increase was abolished by neonatal pretreatment of rats with capsaicin (White & Helme, 1985). Treatment of newborn rats with capsaicin results in selective and permanent degeneration of small-diameter afferent fibres and significant decrease in the SP content (Jancsó *et al.*, 1980) and capsaicin-treated animals show significantly increased latencies in response to a noxious thermal stimulus in either the tail-flick or hot-plate test (Nagy *et al.*, 1980). In the present study, the changes in the blood flow in the microvessels of the skin induced by electrical stimulation of the sectioned sciatic nerve were significantly inhibited by capsaicin and spantide. The latter compound has been shown to be one of the best SP antagonists in peripheral tissues (Håkanson & Sundler, 1985; Rosell, 1985). In this connection, Rosell *et al.* (1983, 1985) have observed that, when tested on the hamster urinary bladder, which contracts at low concentrations of NKA and eledoisin but is rather insensitive to SP and physalaemin, spantide did not block the actions of NKA and eledoisin. On the guinea-pig ileum preparation, the contractions elicited by NKA were blocked in a noncompetitive manner. These findings indicated that spantide has a high affinity for SP receptors but not for NKA and eledoisin receptors. However, it has recently been found in experiments on the isolated spinal cord-tail preparation of the newborn rat that spantide depressed the actions of not only SP but also NKA (Nussbaumer *et al.*, 1989). Therefore, a hypothesis emerges that an antagonistic action of spantide for tachykinin receptors existing in peripheral organs may differ from that existing in the central nervous system. In the present study, however, involvement of NKA in the changes in CBF observed on antidromic stimulation cannot be completely neglected because it is known that NKA coexists with SP in primary afferent neurones (Maggio & Hunter, 1984; Dalsgaard *et al.*, 1985) and is synthesized via the same precursor (Nawa *et al.*, 1983). According to Ogawa *et al.* (1985), the amount of NKA in the dorsal roots and the dorsal root ganglion in the rat spinal cord was about one-third of that of SP. In our perfusion experiment, moreover, an increase in NKA release evoked by antidromic stimulation of the sectioned sciatic nerve was not observed. These observations including our results suggest that SP released from the peripheral endings of capsaicin-sensitive small-diameter, afferent fibres present in the sciatic nerve is mainly involved in the changes in CBF observed following electrical stimulation of the sectioned sciatic nerve. This suggestion is also supported by the fact that the responses evoked by electrical stimulation were mimicked by infusion of SP into the side of measurement via an arterial cannula inserted into the contralateral common iliac artery; this is, i.a. infusion of SP induced by biphasic flow response with an initial transient decrease, followed by an increase. D'Orleans-Juste *et al.* (1985) demonstrated that arterial relaxation *in vitro* was endothelium-dependent. According to Furchgott (1983), moreover, SP is a potent vasodilator whose action depends on the presence of endothelial cells. If these findings also apply to skin blood vessels, SP should be administered directly to its receptor by intra-arterial infusion, whereas SP released by antidromic stimulation should have to diffuse to its receptor sites. Concerning the participation of SP in microcirculatory haemodynamics, Galligan *et al.* (1990) reported that with an optical method in preparations of guinea-pig submucosal plexus *in vitro*, intrinsic intestinal neurones containing SP but not calcitonin gene-

related peptide (CGRP) and vasoactive intestinal polypeptide supply submucosal arterioles after sympathetic efferents and sensory afferents are removed, and stimulation of these neurones releases SP to produce arteriolar dilatation.

The reason for the initial phase of reduction of CBF observed on antidromic stimulation or i.a. infusion of SP is unknown. If this reduction reflects vasoconstriction, SP may initially induce contraction of smooth muscle by activating SP receptors located on smooth muscle cells (Mastrangelo *et al.*, 1983) or by promoting the release of an endothelium-derived contracting factor such as endothelin (De May & Vanhoutte, 1982; Yanagisawa *et al.*, 1988) through activating SP receptors on the endothelium. In this connection, it is noteworthy that SP receptors were found on the endothelium of dog renal arteries (Stephenson *et al.*, 1987; Stephenson & Summers, 1987). Another possibility is that the initial decrease in CBF, could occur in compensation for rapid transient vasodilatation induced by SP released locally around the skin microvessels. Intra-arterial infusion of spantide induced decrease in the basal CBF compared with that in the saline-treated group (Figure 4), so in the normal basal state, the small-diameter afferent fibres probably maintain some vasodilator tone through spontaneous release of SP from peripheral nerve terminals.

There is evidence that SP can release histamine from mast cells (Johnson & Erdos, 1973; Erjavee *et al.*, 1981) and it has been suggested that mast cell histamine plays a part in vasodilatation following electrical stimulation of cutaneous nerves (Lembeck & Holzer, 1979). However, in this study, vasodilatation induced by antidromic stimulation was not influenced by mepyramine plus cimetidine, indicating that the vascular response was not mediated by the release of histamine from mast cells. This is similar to findings in the dental pulp of cats, where antihistamines did not influence vasodilatation produced by inferior alveolar nerve stimulation (Gazelius & Olgart 1980). It is difficult to explain the discrepancy between our results and the observations of others indicating a role of histamine. Perhaps the link between the SP system and the release of histamine from mast cells is less essential for vasodilatation induced by antidromic stimulation in the instep of the hind paw of rats than in other tissue, but more research is needed on this problem.

In the present study, we did not exclude the possibility of participation of other peptides present in capsaicin-sensitive, small-diameter afferent fibres and autonomic neurones, and other endogenous inflammatory substances. CGRP, for instance, has been shown to be localized with SP in many sensory neurones and tissues including skin (Gibbins *et al.*, 1985; Wallengren *et al.*, 1987), and this peptide is a potent vasodilator in several species such as rats (Gamse & Saria, 1985) and man (Wallengren & Håkanson, 1987). Bradykinin, a chemical mediator of inflammation, may also act on the peripheral sensory nerve endings to elicit the release of SP (White & Zimmermann, 1988).

In conclusion, our study provides further evidence that endogenous SP and its receptors are mainly responsible for the vasodilator response induced by sciatic nerve stimulation, and suggests that SP-containing nerves, capsaicin-sensitive, small-diameter afferent fibres, tonically regulate vascular tone in cutaneous microvessels.

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