Br. J. Pharmac. (1983), **78**, 665–669

Topical capsaicin pretreatment inhibits axon reflex vasodilatation caused by somatostatin and vasoactive intestinal polypeptide in human skin

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1 Wheal and flare reactions are described following intradermal injections of somatostatin, vasoactive intestinal polypeptide, substance P and histamine in normal human forearm skin. Bombesin failed to produce a significant wheal and flare.

2 Pretreatment of skin with capsaicin in all cases dramatically inhibited the flare but not the wheal. This result is in accord with the hypothesis that capsaicin blocks the effector side of the axon reflex, perhaps by depleting nerve terminals of vasodilatory peptide(s).

Introduction

The axon reflex flare (vasodilatation) follows intradermal injection of histamine (Lewis, 1927) and substance P (SP) (Hagermark, Hokfelt & Pernow, 1978) in human skin, and is inhibited by topical capsaicin pretreatment (Carpenter & Lynn, 1981; Bernstein, Swift, Soltani & Lorincz, 1981). It has been postulated that axon reflex flare is produced by release of SP from nociceptive sensory afferent terminals in the skin (Burnstock, 1977; Henry, 1977), and it has been demonstrated that SP is released from the central terminals of such nociceptive fibres (Nicoll, Schenker & Leeman, 1980). Topical capsaicin produces local desensitization of skin to chemogenic noxious agents (Jansco, 1960), possibly by depleting SP in nerve terminals (see Carpenter & Lynn, 1981). Parenteral capsaicin reduces both neurogenic plasma extravasation (Jansco, 1960; Arvier, Chahl & Ladd, 1977; Gamse, Holzer & Lembeck, 1980) and SP levels in rat skin (Gamse *et al.*, 1980; Hayes & Tyers, 1980). SP-related peptides which fail to produce a flare when injected into human skin also fail to release histamine from rat mast cells *in vitro* (Foreman & Jordan, 1982).

However, SP may not be the exclusive mediator of axon reflex vasodilatation. Capsaicin has been shown to deplete rat spinal cord of a substance which produces cutaneous oedema and which is not SP (Chahl & Manley, 1980). SP, somatostatin, vasoactive intestinal polypeptide (VIP) and bombesin have been located in rat dorsal cord (see Hokfelt, Johansson, Ljungdahl, Lundberg & Schultzberg,1980; Moody, Thoa, O'Donohue & Jacobowitz, 1981) and in human dorsal spinal cord (Cuello, Polak & Pearse, 1976; unpublished data). Both SP and somatostatin applied topically to the spinal cord produce behavioural responses in rats suggestive of nociception (Seybold, Hylden & Wilcox, 1982), and have been located, as has VIP, in primary sensory neurones (Hokfelt, Elde, Johansson, Luft, Nilsson & Arimura, 1976; Lundberg, Hokfelt, Nilsson, Terenius, Rehfeld, Elde & Said, 1978).

In view of the demonstration of somatostatin, SP, VIP and bombesin in significant quantities in mammalian skin, and of SP- and VIP-containing nerves surrounding cutaneous blood vessels (O'Shaughnessy, McGregor, Ghatei, Blank, Springall, Gu, Polak & Bloom, 1983), this study compared their ability to produce wheal and flare reactions in human skin.

Methods

The subjects were 4 volunteers (aged 18–30 years) from the Department of Medicine, Hammersmith Hospital, who gave their informed consent. The study was approved by the local Ethics Committee.

Each subject applied an aqueous cream preparation of capsaicin (1%) to the flexor area (18 cm × 5 cm) of one forearm. To the corresponding area of the other forearm, aqueous cream was applied as control. Successive applications of capsaicin produced decreasing erythema and burning sensa-

tions, and on average after 8 applications over 3 days no local reaction occurred.

Wheal and flare reactions on control and capsaicin pretreated skin were produced by intradermal injections with a 0.4 mm diameter needle and a volume of 0.03 ml in all cases. The following drugs were used: histamine acid phosphate injection B.P. (1 mg/ml), synthetic SP (Peninsula), pure porcine VIP (gift from Professor V. Mutt), synthetic somatostatin (Bachem), synthetic bombesin (Peninsula) and capsaicin (Sigma, prepared by Mr Roger Thompson, Staff Pharmacist, Hammersmith Hospital). SP, VIP and bombesin were stored at −20°C in freeze-dried aliquots prepared in a solution (FDS) consisting of lactose (140 mmol/l), bovine serum albumin (40 μmol/l), L-cysteine hydrochloride (6 mmol/l) and aprotinin (8×10^5 KIU/l), necessary to prevent gross peptide loss by adsorption and degradation. For each set of experiments, the peptides and FDS (as controls) were reconstituted with sterile saline (0.9 w/v NaCl solution) immediately before injection. The pH of both peptide and FDS solutions was between 6.5 and 7.0. For histamine and somatostatin, sterile saline was used as control.

All experiments were performed between 14 h 00 min and 16 h 00 min at an ambient temperature between 18°C and 25°C. The wheal and flares were measured when maximal by marking their borders on the skin with ink, and transferring the marks onto tracing paper, which was then placed on graph paper. The areas were calculated by counting the mm squares within the margins on the graph paper.

Areas were calculated from tracings taken 2, 4, 6 and 8 min after each injection, and the maximum area selected (for flares this was usually either 2 or 4 min and for wheals 6 or 8 min after injection).

Results

Injections of SP, somatostatin and VIP produced wheal and flare responses in normal skin in all subjects (Table 1); wheal and flare responses to peptide injections were significantly greater than their corresponding control injections ($P < 0.05$, paired Student's t test). Injections of bombesin produced small responses which were not significantly larger than the control FDS solution ($P > 0.05$). Histamine (1 mg/ml, 0.03 ml) produced a significant wheal ($1.1 \pm 0.2\,cm^2$; $P < 0.01$) and flare ($17.6 \pm 4.2\,cm^2$; $P < 0.01$).

Responses were maximal 2–3 min after injection, and faded over 5–30 min. The area, intensity and duration of the flare generally decreased in the order SP > somatostatin > VIP when the same dose was injected (see Figure 1), but this was not invariably so for an individual subject. Histamine (0.03 ml, 1 mg/ml) produced the longest and darkest flare. All subjects reported itch with histamine, 2 with SP and 1 with somatostatin.

Capsaicin pretreatment significantly reduced or abolished all flares and itch (see Figure 2), but wheals were not affected. Full recovery of flares that were abolished by capsaicin pretreatment took 3–4 weeks.

Discussion

Intradermal injections of SP, somatostatin and VIP in comparable doses produce wheal and flare responses in normal skin; the flares but not wheals are inhibited by capsaicin pretreatment. The results are compatible with the hypothesis that all these peptides may release histamine from mast cells at the injection

Table 1 Wheal (W) and Flare (F) in normal skin; area in cm^2

Substance		10 pmol	30 pmol	60 pmol	*Control*
SP	W	0.9 ± 0.2 (<0.025)	1.0 ± 0.1 (<0.001)	1.3 ± 0.3 (<0.01)	0.3 ± 0.03
	F	10.9 ± 1.6 (<0.001)	13.1 ± 2.8 (<0.01)	18.0 ± 4.1 (<0.01)	0.75 ± 0.10
Somatostatin	W	0.7 ± 0.1 (<0.01)	0.8 ± 0.1 (<0.01)	1.0 ± 0.1 (<0.001)	0.25 ± 0.02
	F	6.7 ± 0.8 (<0.001)	11.2 ± 1.2 (<0.001)	14.5 ± 2.5 (<0.01)	0.3 ± 0.02
VIP	W	0.8 ± 0.1 (<0.01)	0.9 ± 0.1 (<0.01)	1.0 ± 0.2 (<0.02)	0.3 ± 0.03
	F	4.5 ± 1.5 (<0.05)	7.1 ± 1.9 (<0.025)	12.5 ± 3.5 (<0.025)	0.75 ± 0.10
Bombesin	W	Not done	0.4 ± 0.3 (NS)	0.5 ± 0.3 (NS)	0.30 ± 0.03
	F		1.2 ± 0.50 (NS)	1.1 ± 0.6 (NS)	0.75 ± 0.10

Values are ± s.e.mean. $n = 4$. Figures in parentheses are P values for paired Student's t test, comparing response to each dose with that to its control solution. NS = not significant ($P > 0.05$).

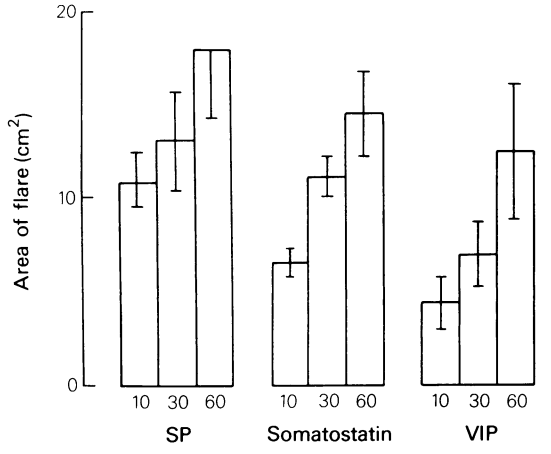

Figure 1 Flare areas in normal skin in cm^2 (mean values) to increasing (10, 30, 60 pmol) doses of substance P, somatostatin and vasoactive intestinal polypeptide (VIP). $n = 4$. Vertical lines show s.e.mean.

site, and that histamine in turn activates sensory nerve terminals to cause axon reflex vasodilatation following release of one or more peptides (Burnstock, 1977; Hagermark *et al.*, 1978). Other studies support this hypothesis. SP and somatostatin release histamine from mast cells *in vitro* (Johnson & Erdos, 1973; Theoharides, Betchaku & Douglas, 1981). Antihistamines reduce flare and itch induced by histamine and SP; local histamine depletion by compound 48/80 abolishes wheal and flare caused by subsequent injection of SP and compound 48/80 but

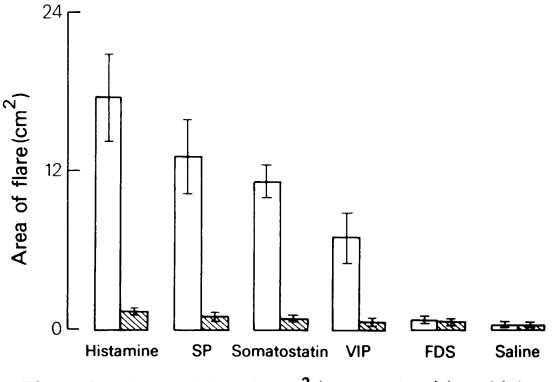

Figure 2 Areas of flare in cm^2 (mean values) in vehicle pretreated (open columns) and capsaicin pretreated (hatched columns) skin. All peptides: 30 pmol; histamine 1 mg/ml, 0.03 ml; $n = 4$; vertical lines show s.e.mean. For each substance, differences between responses in capsaicin and control pretreated skin using paired Student's t test: Histamine $P < 0.02$; SP $P < 0.01$; somatostatin $P < 0.001$; vasoactive intestinal polypeptide (VIP) $P < 0.025$; FDS and saline not significant ($P > 0.05$).

not histamine (Hagermark *et al.*, 1978). In contrast, VIP-like immunoreactivity has been reported in mast cells of rat and mouse lung and intestine, and its release by compound 48/80 from rat peritoneal mast cells (Cutz, Chan, Track, Goth & Said, 1978). However, this may not be true VIP as the VIP-like immunoreactivity has not been characterized.

The physiological significance of wheals and flares caused by the peptides in these experiments is uncertain. The results must be interpreted with caution because the doses of peptides injected in this and other studies are high compared to the levels actually measured in human skin (unpublished data). Neurotensin can induce wheal and flare in human skin (Foreman & Jordan, 1982), but it has not yet been detected in the skin.

Capsaicin may deplete sensory nerve terminals of peptide(s), thus abolishing the axon reflex. It may also damage the nerve terminals (Szolcsanyi, Jansco-Gabor & Joo, 1975), or terminal receptors. The wheal is unaffected by capsaicin pretreatment, and is presumably caused by the direct action of released histamine and possibly of the peptides on capillary permeability at the site of injection. Thus capsaicin pretreated skin responds like denervated human skin to intradermal histamine (Lewis, 1927; Celander & Folkow, 1953; Bonney, 1954) and to SP, VIP and somatostatin (unpublished data on denervated skin) in that the flare response is markedly reduced. It is not known, at present, which peptide is released from sensory nerve terminals to cause the flare, or if there is more than one such peptide. If two or more peptides are released, they may have different roles or interact in different ways: for example, neurotensin may antagonize SP-induced flare in human skin (Foreman & Jordan, 1982).

The slow recovery of the flare response after capsaicin pretreatment suggests another possible action of capsaicin: it may inhibit repletion of the nerve terminals by vasodilatory neuropeptides. If capsaicin is applied to a nerve trunk, two weeks later neurogenic or chemogenic inflammatory responses are absent in the territory of that nerve (Jansco, Kiraly & Jansco-Gabor, 1980) and its neuropeptides are depleted (Ainsworth, Hall, Wall, Allt, Lynn Mackenzie, Gibson & Polak, 1981), although there is no significant change in the size of the nerve compound action potential (Wall & Fitzgerald, 1981). One may speculate that there is a constant physiological (possibly trophic) release of peptides from cutaneous sensory nerve terminals, leading in turn to histamine release, and that this process is enhanced by skin (or neurogenic) stimuli. The terminals are being constantly repleted by axonal transport from the site of peptide synthesis in the cell body (Harmar & Keen, 1982). Capsaicin may act in two ways. It may act acutely on nerve terminals to enhance the

release of neuropeptides causing depletion, and it may also act chronically on axons to inhibit the repletion of the terminals by neuropeptides. In support of the idea of constant physiological release of neuropeptides from nerve terminals in the skin is the finding that following capsaicin treatment of newborn rats, which leads to irreversible degeneration of unmyelinated afferent nerve fibres, there is a permanent increase in skin concentrations of histamine (Holzer, Saria, Skofitsch & Lembeck, 1981).

In conclusion, somatostatin and VIP may exert inflammatory effects in skin, as may SP. The results presented are in accord with the hypothesis that capsaicin inhibits axon reflex flares by blocking the effector side of the axon reflex, perhaps by depleting nerve terminals of vasodilatory peptide(s).

P.A. is an Action Research Training Fellow. We thank Karen Herron for secretarial assistance, and Mr Roger Thompson and colleagues of Hammersmith Hospital Pharmacy for their enthusiastic co-operation.

References

AINSWORTH, A., HALL, P., WALL, P.D., ALLT, G., LYNN MACKENZIE, M., GIBSON, S. & POLAK, J.M. (1981). Effects of capsaicin applied locally to adult peripheral nerve II. *Pain*, **11**, 379–388.

ARVIER, P.T., CHAHL, L.A. & LADD, R.J. (1977). Modification by capsaicin and compound 48/80 of dye leakage induced by irritants in the rat. *Br. J. Pharmac.*, **59**, 61–68.

BERNSTEIN, J.E., SWIFT, R.M., SOLTANI, K. & LORINCZ, A.L. (1981). Inhibition of axon reflex vasodilatation by topically applied capsaicin. *J. Invest. Dermatol.*, **76**, 394–395.

BONNEY, G. (1954). The value of axon responses in determining the site of lesion in traction injuries of the brachial plexus. *Brain*, **77**, 588–609.

BURNSTOCK, G. (1977). Autonomic neuroeffector junctions – reflex vasodilatation of the skin. *J. Invest. Dermatol.*, **69**, 47–57.

CARPENTER, S.E. & LYNN, B. (1981). Vascular and sensory responses of human skin to mild injury after topical treatment with capsaicin. *Br. J. Pharmac.*, **73**, 755–758.

CELANDER, O. & FOLKOW, B. (1953). The nature and distribution of afferent fibres provided with the axon reflex arrangement. *Acta. physiol. scand.*, **29**, 359–370.

CHAHL, L.A. & MANLEY, S.W. (1980). Inflammatory peptide in spinal cord: evidence that the mediator of antidromic vasodilatation is not substance P. *Neurosci. Lett.*, **18**, 99–103.

CUELLO, A.C., POLAK, J. & PEARSE, A.G.E. (1976). Substance P: a naturally occurring transmitter in human spinal cord. *Lancet*, **ii**, 1054–1056.

CUTZ, E., CHAN, W., TRACK, N.S., GOTH, A. & SAID, S.I. (1978). Release of vasoactive intestinal polypeptide in mast cells by histamine liberators. *Nature*, **275**, 661–662.

FOREMAN, J.C. & JORDAN, C.C. (1982). Antagonism by neurotensin of the substance P induced flare in human skin and its relationship to histamine release. *J. Physiol.*, **328**, 58–59P.

GAMSE, R., HOLZER, P. & LEMBECK, F. (1980). Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. *Br. J. Pharmac.*, **68**, 207–213.

HAGERMARK, O., HOKFELT, T. & PERNOW, B. (1978). Flare and itch induced by substance P in human skin. *J. Invest. Dermatol.*, **71**, 233–235.

HARMER, A.J. & KEEN, P. (1982). Peptide biosynthesis in sensory ganglia. In *Neuropeptides: Basic and Clinical Aspects*, ed. Fink, G. & Whalley, L.J. Edinburgh: Churchill Livinstone.

HAYES, A.G. & TYERS, M.B. (1980). Capsaicin depletes substance P from dorsal horn and skin and discriminates heat from chemical and pressure nociceptive stimuli in the conscious rat. *J. Physiol.*, **300**, 25P.

HENRY, J.L. (1977). Substance P and pain: a possible relation in afferent transmission. In *Substance P*, ed. von Euler, U.S. & Pernow, B. pp. 232–240. New York: Raven Press.

HOKFELT, T., ELDE, R., JOHANSSON, O., LUFT, R., NILSSON, G. & ARIMURA, A. (1976). Immunohistochemical evidence for separate populations of somatostatin-containing and substance P-containing primary afferent neurons in the rat. *Neurosci.*, **1**, 131–136.

HOKFELT, T., JOHANSSON, O., LJUNGDAHL, A., LUNDBERG, J.M. & SCHULTZBERG, M. (1980). Peptidergic neurones. *Nature*, **284**, 515–521.

HOLZER, P., SARIA, A., SKOFITSCH, G. & LEMBECK, F. (1981). Increase in tissue concentrations of histamine and 5-hydroxytryptamine following capsaicin treatment of newborn rats. *Life Sci.*, **28**, 1099–1105.

JANSCO, N. (1960). Role of nerve terminals in the mechanism of inflammatory reaction. *Bull. Millard Fillmore Hosp., Buffalo, New York*, **7**, 53–77.

JANSCO, G., KIRALY, E. & JANSCO-GABOR, A. (1980). Direct evidence for an axonal site of action of capsaicin. *Naunyn Schmiedebergs Arch. Pharmac.*, **313**, 91–94.

JOHNSON, A.R. & ERDOS, E.G. (1973). Release of histamine from mast cells by vasoactive peptides. *Proc. Soc. exp. Biol. Med.*, **142**, 1253–1256.

LEWIS, T. (1927). *Blood Vessels of the Human Skin and their Responses.* London: Shaw.

LUNDBERG, J.M., HOKFELT, T., NILSSON, G., TERENIUS, L., REHFELD, J., ELDE, R. & SAID, S. (1978). Peptide neurons in the vagus, splanchnic and sciatic nerves. *Acta. physiol. scand.*, **104**, 499–501.

MOODY, T.W., THOA, N.B., O'DONOHUE, T.L. & JACOBOWITZ, D.M. (1981). Bombesin-like peptides in rat spinal cord: biochemical characterization, localization and mechanism of release. *Life Sci.* **29**, 2273–2279.

NICOLL, R.A., SCHENKER, C. & LEEMAN, S.E. (1980). Substance P as a transmitter candidate. *A. Rev. Neurosci.*, **3**, 227–268.

O'SHAUGHNESSY, D.J., McGREGOR, G.P., GHATEI, M.A., BLANK, M.A., SPRINGALL, D.R., GU, J., POLAK, J.M. & BLOOM, S.R. (1983). Distribution of bombesin, somatostatin, substance P and vasoactive intestinal polypeptide in feline and porcine skin. *Life Sci.*, (in press).

SEYBOLD, V.S., HYLDEN, J.L.K. & WILCOX, G.L. (1982). Intrathecal substance P and somatostatin in rats: behaviours indicative of sensation. *Peptides*, **3**, 49–52.

SZOLCSANYI, J., JANSCO-GABOR, A. & JOO, F. (1975). Functional and fine structural characteristics of the sensory neuron blocking effect of capsaicin. *Naunyn Schmiedebergs Arch. Pharmac.*, **287**, 157–169.

THEOHARIDES, T.C., BETCHAKU, T. & DOUGLAS, W.W. (1981). Somatostatin-induced histamine secretion in mast cells. Characterization of the effect. *Eur. J. Pharmac.*, **69**, 127–137.

WALL, P.D. & FITZGERALD, M. (1981). Effects of capsaicin applied locally to adult peripheral nerve I. *Pain*, **11**, 363–377.

(*Received August 30, 1982.*
Revised November 26, 1982.)