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NK-1 receptor mediation of neurogenic plasma extravasation in rat skin

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1 Plasma extravasation was induced by electrical nerve stimulation and by perfusion of tachykinins over a vacuum-induced blister base on rat footpad.

2 Stimulation of the sciatic nerve (18 V, 15 Hz, 0.5 ms) for 20 min produced a significant increase in the protein content of the perfusate. The response in capsaicin pretreated rats was only 4% of the control response. This indicates that the electrically-induced plasma extravasation response was mediated by capsaicin-sensitive sensory fibres.

3 Exogenous perfusion of the mammalian tachykinins substance P, neurokinin A and neurokinin B and the non-mammalian tachykinins physalaemin, kassinin and eledoisin was used to determine the tachykinin receptor type mediating the plasma extravasation response. Dose-response curves of the tachykinins (10^{-9} M–10^{-4} M) gave a rank order of potency of substance P = physalaemin > eledoisin ≥ kassinin > neurokinin B = neurokinin A.

4 In addition, specific agonists of neurokinin receptors were perfused. Perfusion of [Glp^6, D-Pro^9]SP_{6-11} and [Glp^6, L-Pro^9]SP_{6-11} demonstrated that the L-Pro isomer was much more potent than the D-Pro isomer.

5 The rank order of potency and the greater potency of [Glp^6, L-Pro^9]SP_{6-11} over its D-isomer indicate an NK-1 neurokinin receptor mediates plasma extravasation in rat footpad skin.

Introduction

Neurogenic inflammation is defined as vasodilatation and plasma extravasation that occurs following electrical nerve stimulation (Jancso *et al.*, 1967). This response is believed to be mediated by peptides such as the tachykinins, which are released from primary afferent nerve terminals (Lembeck & Holzer, 1979). Electrical nerve stimulation releases substance P (SP), the first identified tachykinin, and induces plasma extravasation in a thermal injury blister model (White & Helme, 1985). We have previously used a vacuum-induced blister model of inflammation to demonstrate that neurogenic plasma extravasation can be induced by mechanical, chemical and thermal stimulation (Andrews & Helme, 1986; Helme *et al.*, 1986). In the current study we demonstrate that plasma extravasation can also be induced by electrical nerve stimulation in the vacuum blister model, that this plasma extravasation is neurogenic in nature and that mediation of the response is likely to occur at a specific tachykinin receptor.

Lee *et al.* (1982) were among the first to recognize the nature of multiple receptors for SP, namely, the SP-P receptor where all the tachykinins were essentially equipotent and the SP-E receptor where eledoison (Ele) and kassinin (Kas) were more potent than SP and physalaemin (Phy). The SP-E receptor was subsequently found to be probably the combined effects of two receptors. One of these, the SP-N receptor, was identified in guinea-pig ileum when the effects of SP were inhibited by desensitization (Laufer *et al.*, 1985) and the other receptor, SP-K, was observed after binding studies on rat brain (Buck *et al.*, 1984). Using rank order of potency and currently defined terminology, the receptors can be differentiated into three subtypes as follows:

NK-1: SP > Phy > NKA > NKB > Ele > Kas,

NK-2: NKA > NKB > Kas > Ele > SP > Phy

and

NK-3: NKB ≫ Ele > Kas > NKA = Phy > SP (Lee *et al.*, 1986).

Many of the functional studies with neurokinin receptors have been performed on smooth muscle preparations *in vitro* (Regoli *et al.*, 1984; Mizrahi *et*

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al., 1985) and few have investigated neurogenic plasma extravasation *in vivo*. NKA and SP were almost equipotent in producing plasma extravasation in rat bladder and ureters (Maggi *et al.*, 1986) whereas Gamse & Saria (1985), who investigated Evans blue extravasation to intradermal injection of the tachykinins in abdominal skin of rats, obtained a rank order of potency NKB > SP > NKA. On the dorsal skin of the rat, intradermal injection of the mammalian tachykinins gave similar results, namely NKB > NKA > SP (Couture & Kerouac, 1987) suggesting NK-3 receptor mediation of the response. On the other hand, intradermally injected tachykinins in human skin induce wheal responses with the rank order of potency SP > NKA > NKB (Devillier *et al.*, 1986). These authors also demonstrated that in rat dorsal skin, the rank order for inducing Evans blue extravasation was SP = NKA > NKB which suggests NK-1 mediation.

In the present study, tachykinins (both mammalian and non-mammalian) as well as SP_{1-9}, SP_{5-11}, and the newly described specific agonists D- and L-Pro isomers of [Glp^6, Pro^9]SP_{6-11} (Lee *et al.*, 1986) were perfused over the blister base on rat footpad to determine which neurokinin receptor mediates neurogenic plasma extravasation from dermal vessels. Preliminary data were presented at the Substance P and Neurokinins '86 meeting (Andrews & Helme, 1987).

Methods

Male Sprague-Dawley rats (200–320 g) were used; some had been pretreated as neonates with capsaicin (50 mg kg^{-1}) (Jancso *et al.*, 1977). For all experiments, animals were anaesthetized (sodium pentobarbitone, 65 mg kg^{-1}, i.p.), a femoral vein catheter was inserted (for fluid load [1 ml 50 g^{-1}] and maintenance of anaesthesia), and the footpad placed over a plate heated to 40°C with an aperture opening to a vacuum pump. With a pressure of −40 kPa a blister was induced in 30 min involving separation of epidermis from dermis (Helme *et al.*, 1985). After blister induction, the blister epidermis was removed, replaced by a perfusion chamber (approximately 1 cm^3 capacity) attached to a peristaltic pump (LKB, Sweden) and the chamber perfused with Ringer solution at 4 ml h^{-1}, thus superfusing the blister base. The perfusion chamber was maintained at 37°C with a heating element in the roof of the chamber. Perfusates were collected into an equal volume of cold 2 M acetic acid and stored at −20°C before they were analysed for plasma extravasation by a simple protein assay (Bradford, 1976).

Experiments with electrical nerve stimulation were performed on normal and capsaicin pretreated rats. The sciatic nerve was dissected free, cut proximally and suspended over bipolar platinum electrodes in a paraffin oil bath formed by raising the skin flaps of the wound. The nerve was stimulated at 18 V, 15 Hz, 0.5 ms pulse duration (an intensity sufficient to stimulate 'C' fibres, Yaksh *et al.*, 1980) or 4 V, 15 Hz, 0.5 ms (insufficient to stimulate 'C' fibres maximally) for 20 min. Perfusates were collected from the stimulated footpad and the opposite footpad control. Results are expressed as the mean ± s.e.mean of the increased protein content of the stimulated footpad perfusate over the control footpad perfusate.

Experiments with tachykinin perfusion were performed for two 30 min periods, an initial control period with perfusion of Ringer solution followed by perfusion with a tachykinin solution in both normal and capsaicin pretreated animals. SP, NKA, NKB, Phy, Kas, Ele, SP_{1-9} and SP_{5-11} (Peninsula Labs., San Carlos, U.S.A.) were dissolved in Ringer solution and stored at −20°C until used. NKB was also dissolved in sulpholane (BDH) 1:30 with Ringer solution. The D- and L-Pro isomers of [Glp^6, Pro^9]SP_{6-11} (kindly donated by Dr L.L. Iversen, Merck Sharp and Dohme, U.K.) were dissolved in 10% dimethylsulphoxide (DMSO) and diluted to 1% for perfusion. Plasma extravasation induced by the tachykinins is expressed as the mean ± s.e.mean of the increased amount of protein in the tachykinin perfusion perfusate of each animal compared to its control period. The mean protein concentration of all the control periods was 32.4 $\mu g\,ml^{-1}$ ± 1.46, $n = 178$. The pD_2 s for the peptides were calculated by linear regression analysis (Hays, 1973).

Results

Plasma extravasation induced by electrical nerve stimulation

One way analysis of variance (Kirk, 1968) revealed that there were significant differences in the plasma extravasation (increased protein content above control) induced by 18 V (23.6 $\mu g\,ml^{-1}$ ± 6.7; $n = 8$) and 4 V stimulation (7.6 $\mu g\,ml^{-1}$ ± 2.6; $n = 9$) and 18 V stimulation in capsaicin pretreated animals (0.9 $\mu g\,ml^{-1}$ ± 1.2; $n = 8$) ($F_{2,24} = 7.7665$, $P = 0.0028$). Using the Student-Newman-Keuls multiple range test each of the three groups was significantly different from the others at the $P < 0.05$ level.

Plasma extravasation induced by exogenous tachykinins

Exogenous SP induced plasma extravasation at a threshold of 10^{-8} M and produced maximal plasma

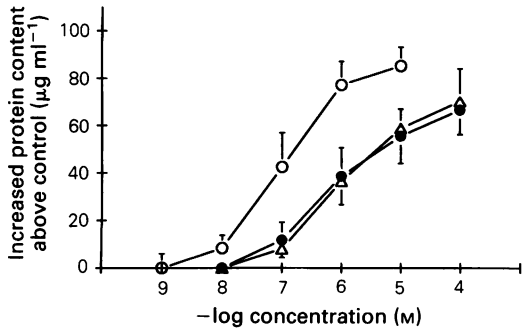

Figure 1 The dose-response curves for plasma extravasation induced by perfusion of the mammalian tachykinins (substance P (SP) ○; neurokinin A (NKA) ●; NKB △). Points are the mean results from 4 animals (s.e.mean shown by vertical bars) except for; SP 8(6) 7(6) 6(10); NKA 6(6) 5(6) 4(10); NKB 4(6). Number of animals in parentheses.

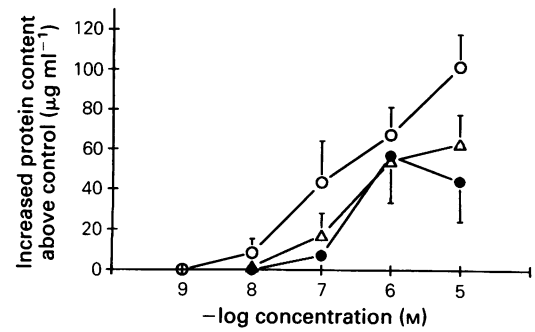

Figure 3 The dose-response curves for plasma extravasation induced by perfusion of the non-mammalian tachykinins (physalaemin ○; kassinin ●; eledoisin △). Points are the mean from 4 animals; vertical bars show s.e.mean.

extravasation ($85\,\mu g\,ml^{-1} \pm 8.5$) at 10^{-5} M (Figure 1). N- and C-terminal analogues of SP were also tested. SP_{1-9} at 10^{-5} M was inactive in inducing plasma extravasation. SP_{5-11} at the same concentration induced plasma extravasation although it was less active than SP ($61.5\,\mu g\,ml^{-1} \pm 6.5$ and $85.5\,\mu g\,ml^{-1} \pm 12.0$ respectively) (Figure 2).

SP (10^{-7} M) was also perfused in rats that had been pretreated with capsaicin. There was no difference between the normal and capsaicin pretreated animals in their response to SP (normal: $73.3\,\mu g\,ml^{-1} \pm 15.45$; capsaicin: $82.0\,\mu g\,ml^{-1} \pm 7.43$; $n = 6$ in both groups).

NKA and NKB were equipotent and more than an order of magnitude less potent than SP. Because NKB is not fully soluble in Ringer solution it was also dissolved in sulpholane in an attempt to increase its solubility, however the ability of NKB (10^{-6} M) to induce plasma extravasation was not altered ($36.5\,\mu g\,ml^{-1} \pm 7.0$ and $48.0\,\mu g\,ml^{-1} \pm 12.0$; $n = 4$ in both groups).

The non-mammalian tachykinins induced plasma extravasation at similar potencies to their mammalian homologues (Figure 3). Phy was the most potent and Kas and Ele were equipotent and about an order of magnitude less potent than Phy. Table 1 is a list of the pD_2s for the peptides obtained from their dose-response curves.

Plasma extravasation induced by the D *and* L*-Pro isomers of* [*Glp*^{6}, *Pro*^{9}]*SP*$_{6-11}$

The L-Pro^9 isomer of [Glp^6, Pro^9]SP_{6-11} induced plasma extravasation into the perfusate with a potency about an order of magnitude less than SP. The D-Pro^9 isomer was much less active with plasma

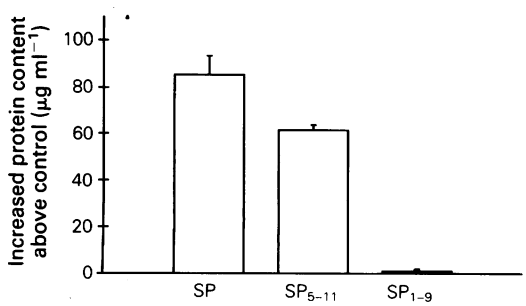

Figure 2 The plasma extravasation response induced by perfusion of substance P (SP), its C-terminal analogue (SP_{5-11}) and its N-terminal analogue (SP_{1-9}), each at a concentration of 10^{-5} M. Columns are the mean from 4 animals; vertical bars show s.e.mean.

Table 1 pD_2s for the tachykinins in inducing a plasma extravasation response in the blister base

Peptide	*pD_2 (95% confidence limits)*
SP	6.93 (7.40 – 6.45)
NKA	5.57 (6.92 – 4.22)
NKB	5.60 (6.55 – 4.65)
Phy	6.90 (7.70 – 6.10)
Ele	6.39 (7.32 – 5.46)
Kas	6.25 (8.79 – 3.71)

SP, substance P; NKA and NKB, neurokinin A and B; Phy, physalaemin; Ele, eledoisin; Kas, kassinin.

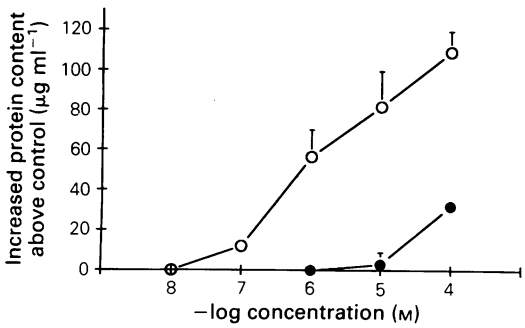

Figure 4 The dose-response curves for plasma extravasation induced by [Glp^6, L-Pro^9]SP$_{6-11}$ (○) and [Glp^6, D-Pro^9]SP$_{6-11}$ (●). Points are the mean from 4 animals; vertical bars show s.e.mean.

extravasation activity only towards the mM concentration range (Figure 4).

Discussion

Neurogenic plasma extravasation in the vacuum blister model

In the past, neurogenic inflammation in the skin was induced by noxious chemical stimulation with mustard oil and xylene (Jancso *et al.*, 1967) and the plasma extravasation response measured as accumulated Evans blue dye in the skin. Neurogenic plasma extravasation has also been quantified following electrical nerve stimulation (Jancso *et al.*, 1967; Lembeck & Holzer, 1979; Morton & Chahl, 1980). In animals that had been treated as neonates with capsaicin, a neurotoxin that selectively destroys unmyelinated nerves in the primary afferent nerve pathway, electrically induced plasma extravasation does not occur thus implicating involvement of small diameter 'C' fibres of the primary afferent nerve in the response.

In the present study neurogenic plasma extravasation was quantified for the first time in an 'on-line' system using the vacuum induced blister model. The neurogenic nature of the plasma extravasation was evident from the lack of a response in capsaicin pretreated animals when the sciatic nerve was stimulated at 18 V, 15 Hz, 0.5 ms, a stimulation intensity that did produce significant plasma extravasation in normal animals and from the small plasma extravasation response when the sciatic nerve of normal animals was stimulated at 4 V, 15 Hz, 0.5 ms. Stimulation at 4 V has been shown to stimulate only A_α and A_β fibres (Yaksh *et al.*, 1980) and no plasma extravasation was observed at this stimulus intensity. However, it is possible that stimulation at 4 V is still able to excite some 'C' fibres and thus account for the small but significant increase in plasma extravasation that was observed.

The vacuum blister model has previously been shown to be appropriate for studying neurogenic inflammation to physiological noxious stimuli (Andrews & Helme, 1986; Helme *et al.*, 1986) and now we have shown that non-physiological electrical nerve stimulation also induces neurogenic plasma extravasation in this model. This neurogenic response is mediated through capsaicin-sensitive primary afferent neurones that have been shown to contain a number of peptides including the tachykinins (Jancso *et al.*, 1981). It is likely that these substances mediate their responses through different receptors. We have therefore attempted to determine which neurokinin receptor is responsible for neurogenic plasma extravasation in rat footpad skin.

Tachykinin-induced plasma extravasation

In the current study the tachykinins, both mammalian and non-mammalian, induced plasma extravasation in a dose-dependent manner. The tachykinins were active in the range of 2 pmol to 200 nmol peptide perfused. This amount of peptide is comparable to studies where plasma extravasation in skin was induced by intra-arterial infusion (Lembeck & Holzer, 1979) and intra-dermal injection (Foreman *et al.*, 1983; Gamse & Saria, 1985; Devillier *et al.*, 1986; Couture & Kerouac, 1987) but is more potent than intravenously administered SP (Saria *et al.*, 1983).

SP was also perfused over the blister base of animals that had been pretreated with capsaicin as neonates. In these animals the plasma extravasation response was no different from that of normal animals. This indicates that the tachykinin-induced plasma extravasation response occurs independently of the primary afferent nerve pathway as had earlier been shown with the wheal response to SP in capsaicin-desensitized human skin (Anand *et al.*, 1983; Foreman *et al.*, 1983) and with local administration to the rat nasal mucosa (Lundblad *et al.*, 1983) and rat tracheal mucosa (Lundberg & Saria, 1983).

The SP-induced plasma extravasation was shown to be mediated by an interaction of the C-terminal of the peptide with its receptor. An N-terminal analogue of SP had no plasma extravasation inducing activity. This verifies previous work in human skin where an N-terminal analogue was inactive in inducing wheal (Foreman *et al.*, 1983). The common C-terminal of the tachykinins would suggest that they

should all induce a plasma extravasation response and this was found to be the case.

The rank order of potency of the tachykinins in inducing plasma extravasation was SP ≥ Phy > Ele ≥ Kas > NKB ≥ NKA. Previously, binding studies have demonstrated rank orders of potency of agonists to inhibit radiolabelled-SP binding in brain tissues of:

SP ≥ Phy ≫ NKA = Kas = Ele > NKB (Buck & Burcher, 1986)

SP > Phy > NKA ≥ NKB ≥ Ele ≥ Kas (Lee *et al.*, 1986)

SP > Phy > NKA > Ele > Kas > NKB (Quirion, 1985).

These orders of potency have been used to classify receptors of the NK-1 type where SP is the most potent agonist. On this basis, it could be suggested that plasma extravasation in rat footpad skin is mediated by an NK-1 receptor.

Studies *in vitro* have demonstrated release of histamine from mast cells by SP and its N-terminal analogues (Devillier *et al.*, 1985) but not with NKA or NKB (Devillier *et al.*, 1986). Thus it is possible that the greater potency of SP over NKA and NKB is due to mast cell-derived histamine. However, the C-terminal dependence of the plasma extravasation in this study suggests that histamine is not involved in the response and antihistamine treatment did not affect the plasma extravasation induced by SP in this model (Andrews & Helme, 1987).

More recent studies from our laboratory have examined SP-induced plasma extravasation in more detail with 10 min perfusate collections (Khalil & Helme, 1989). It was observed that there is histamine-independent plasma extravasation early in the stimulatory period and histamine is involved only in the later stages. Thus a significant proportion of the SP-induced plasma extravasation measured in the current study is histamine-independent and the potency difference between SP, NKA and NKB is due to neurokinin receptor activation. To date, the effect of other mast cell mediators on the response has not been examined in this model.

The order of potency observed in the present study contrasts with two previously published studies (Gamse & Saria, 1985; Couture & Kerouac, 1987) which demonstrated an order of potency NKB > SP > NKA to intradermal injections in rat skin suggesting NK-3 receptor-mediation of the response. Another study (Devillier *et al.*, 1986) observed a rank order of potency of SP = NKA > NKB in rat skin and SP > NKA > NKB in human skin which is more equivalent to the results of the current study.

Solubility was not found to be a factor affecting the different potencies of NKB. A 1:1 solution of sulpholane in water dissolved NKB for *in vitro* experiments (D'Orléans-Juste *et al.*, 1985) however sulpholane induced plasma extravasation from the blister base at this concentration. A 1:30 solution had no activity of its own and, while increasing the solubility of NKB, had no effect on plasma extravasation. NKB dissolved in 12.5 N acetic acid or 0.1% trifluoroacetic acid has the same effect as NKB dissolved in water (Couture & Kerouac, 1987).

Use of selective agonists to differentiate receptors

In the current study, the receptor type mediating plasma extravasation was investigated further using specific neurokinin receptor agonists, the D-Pro and L-Pro isomers of [Glp^6, Pro^9]SP$_{6-11}$ (Lee *et al.*, 1986) which have different D-Pro/L-Pro ratios of their EC_{50}s on the different neurokinin receptors. The ratios on NK-1, NK-2 and NK-3 receptors are about 100, 0.001 and 0.1 respectively. The [Glp^6, Pro^9]SP$_{6-11}$ analogues were used to ascertain which neurokinin receptor mediated inositol phospholipid hydrolysis in hamster bladder (Bristow *et al.*, 1987) and with smooth muscle contraction of the guinea-pig ileum, rat vas deferens and rat portal vein (Iversen *et al.*, 1987).

In the current study the D-Pro/L-Pro ratio of [Glp^6, Pro^9]SP$_{6-11}$ could not be determined as it was not possible to produce a full dose-response curve for the D-Pro isomer due to the high concentrations of the peptide that would have been required. However, the L-Pro isomer was significantly more potent than the D-Pro isomer in inducing plasma extravasation in rat skin. This is highly suggestive that an NK-1 type neurokinin receptor mediates the response in rat footpad vessels. As there are no selective tachykinin antagonists available this is the most conclusive evidence that can be produced to distinguish receptor types. The Gamse & Saria (1985) and Couture & Kerouac (1987) studies did not use selective agonists and thus lack this confirmatory evidence. However, the reason for the different findings remain unproven.

It should also be noted that the major critical difference in receptor differentiation between the studies is the greater potency of NKB over SP. Although NKB has been measured in the spinal cord of rats, its concentrations in the spinal roots and dorsal root ganglia are 35–40 times less than SP (Ogawa *et al.*, 1985). NKB has not yet been demonstrated in nerve fibres in the periphery and consequently it would seem to be unlikely that it has a major role in the peripheral function of the primary afferent nerves. Taken together with the present results, which are suggestive of an NK-1-mediated

response, the involvement of an NK-3 receptor in the plasma extravasation response in rat footpad skin is therefore doubtful.

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References

ANAND, P., BLOOM, S.R. & McGREGOR, G.P. (1983). Topical capsaicin inhibits axon reflex vasodilation caused by somatostatin and vasoactive intestinal polypeptide in human skin. *Br. J. Pharmacol.*, **78**, 665–669.

ANDREWS, P.V. & HELME, R.D. (1986). Neurogenic plasma extravasation in response to mechanical, chemical and thermal stimuli. *Clin. Exp. Neurol.*, **23**, 95–100.

ANDREWS, P.V. & HELME, R.D. (1987). Tachykinin-induced plasma extravasation in rat skin is mediated through the SP-P receptor. In *Substance P and Neurokinins*. ed. Henry, J.L., Couture, R., Cuello, A.C., Pelletier, G., Quirion, R. & Regoli, D. pp. 203–204. New York: Springer-Verlag.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

BRISTOW, D.R., CURTIS, N.R., SUMAN-CHAUHAN, K.J., WATLING, K.J. & WILLIAMS, B.J. (1987). Effects of tachykinins on inositol phospholipid hydrolysis in slices of hamster urinary bladder. *Br. J. Pharmacol.*, **90**, 211–217.

BUCK, S.H. & BURCHER, E. (1986). The tachykinins: a family of peptides with a brood of receptors. *Trends Pharmacol.*, **7**, 65–68.

BUCK, S.H., BURCHER, E., SHULTS, C.W., LOVENBERG, W. & O'DONOHUE, T.L. (1984). Novel pharmacology of substance K-binding sites: A third type of tachykinin receptor. *Science*, **226**, 987–989.

COUTURE, R. & KEROUAC, R. (1987). Plasma protein extravasation induced by mammalian tachykinins in rat skin: influence of anaesthetic agents and an acetylcholine antagonist. *Br. J. Pharmacol.*, **91**, 265–273.

DEVILLIER, P., REGOLI, D., ASSEREF, A., DESCOURS, B., MARSAC, J. & RENOUX, M. (1986). Histamine release and local responses of rat and human skin to substance P and other mammalian tachykinins. *Pharmacology*, **32**, 340–347.

DEVILLIER, P., RENOUX, M., GIROUD, J.-P. & REGOLI, D. (1985). Peptides and histamine release from rat peritoneal mast cells. *Eur. J. Pharmacol.*, **117**, 89–96.

D'ORLEANS-JUSTE, P., DION, S., DRAPEAU, G. & REGOLI, D. (1985). Different receptors are involved in the endothelium-mediated relaxation and the smooth muscle contraction of the rabbit pulmonary artery in response to substance P and related neurokinins. *Eur. J. Pharmacol.*, **125**, 37–44.

FOREMAN, J.C., JORDAN, C.C., OEHME, P. & RENNER, H. (1983). Structure-activity relationships for some substance P-related peptides that cause wheal and flare reactions in human skin. *J. Physiol.*, **335**, 449–465.

GAMSE, R. & SARIA, A. (1985). Potentiation of tachykinin-induced plasma extravasation by calcitonin gene-related peptide. *Eur. J. Pharmacol.*, **114**, 61–66.

HAYS, W.L. (1973). *Statistics for the Social Sciences*. Second Edition. London: Holt, Rinehart and Winston.

HELME, R.D., ANDREWS, P.V. & WATSON, B.A. (1986). Neurogenic inflammation caused by wool fabric in the rat; possible mediation by substance P. *Neurosci. Lett.*, **66**, 333–337.

HELME, R.D., WHITE, D.M. & ANDREWS, P.V. (1985). Neurogenic inflammation in skin blisters. *Exp. Brain Res.*, **59**, 382–387.

IVERSEN, L.L., FOSTER, A.C., WATLING, K.J., McKNIGHT, A.T., WILLIAMS, B.J. & LEE, C.M. (1987). Multiple receptors and binding sites for tachykinins. In *Substance P and Neurokinins*. ed. Henry, J.L., Couture, R., Cuello, A.C., Pelletier, G., Quirion, R. & Regoli, D. pp. 40–43. New York: Springer-Verlag.

JANCSO, G., HOKFELT, T., LUNDBERG, J.M., KIRALY, E., HALASZ, N., NILSSON, G., TERENIUS, L., REHFELD, J., STEINBUSCH, H., VERHOFSTAD, A., ELDE, R., SAID, S. & BROWN, M. (1981). Immunohistochemical studies on the effect of capsaicin on spinal and medullary peptides and monoamine neurons using antisera to substance P, gastrin/CCK, somatostatin, VIP, enkephalin, neurotensin, and 5-hydroxytryptamine. *J. Neurocytol.*, **10**, 963–980.

JANCSO, G., KIRALY, E. & JANCSO-GABOR, A. (1977). Pharmacologically induced selective degeneration of chemosensitive primary sensory neurons. *Nature*, **270**, 741–743.

JANCSO, N., JANCSO-GABOR, A. & SZOLCSANYI, J. (1967). Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. *Br. J. Pharmacol. Chemother.*, **31**, 138–151.

KHALIL, Z. & HELME, R.D. (1989). Sequence of events in substance P-mediated plasma extravasation in rat skin. *Brain Res.* (in press).

KIRK, E.R. (1968). *Experimental Design: Procedures for the Behavioural Sciences*. Belmont, CA: Brookes/Cole Publishing Co.

LAUFER, R., WORMSER, U., FRIEDMAN, Z.Y., GILON, C., CHOREV, M. & SELINGER, Z. (1985). Neurokinin B is a preferred agonist for a neuronal substance P receptor and its action is antagonized by enkephalin. *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 7444–7448.

LEE, C.-M., IVERSEN, L.L., HANLEY, M.R. & SANDBERG, B.E.B. (1982). The possible existence of multiple receptors for substance P. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **318**, 281–287.

LEE, C.-M., CAMPBELL, N.J., WILLIAMS, B.J. & IVERSEN, L.L. (1986). Multiple tachykinin binding sites in peripheral tissues and in brain. *Eur. J. Pharmacol.*, **130**, 209–217.

LEMBECK, F. & HOLZER, P. (1979). Substance P as neurogenic mediator of antidromic vasodilatation and neurogenic plasma extravasation. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **310**, 175–183.

LUNDBERG, J.M. & SARIA, A. (1983). Capsaicin induced desensitization of the airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature*, **302**, 251–253.

LUNDBLAD, L., SARIA, A., LUNDBERG, J.M. & ANGGARD,

A. (1983). Increased vascular permeability in rat nasal mucosa induced by substance P and stimulation of capsaicin-sensitive neurons. *Acta Oto-laryngol.*, **96**, 479–484.

MAGGI, C.A., SANTICIOLI, P., GIULIANI, S., REGOLI, D. & MELI, A. (1986). Activation of micturition reflex by substance P and substance K: Indirect evidence for the existence of multiple receptors in the rat urinary bladder. *J. Pharmacol. Exp. Ther.*, **238**, 259–266.

MIZRAHI, J., DION, S., D'ORLEANS-JUSTE, P., ESCHER, E., DRAPEAU, G. & REGOLI, D. (1985). Tachykinin receptors in smooth muscles: a study with agonists (substance P, neurokinin A) and antagonists. *Eur. J. Pharmacol.*, **118**, 25–36.

MORTON, C.R. & CHAHL, L.A. (1980). Pharmacology of the neurogenic oedema response to electrical stimulation of the saphenous nerve in the rat. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **314**, 271–276.

OGAWA, T., KANAZAWA, I. & KIMURA, S. (1985). Regional distribution of substance P, neurokinin α and neurokinin β in rat spinal cord, nerve roots and dorsal root ganglion, and the effects of dorsal root section or spinal transection. *Brain Res.*, **359**, 152–157.

QUIRION, R. (1985). Multiple tachykinin receptors. *Trends Neurosci.*, **8**, 183–185.

REGOLI, D., ESCHER, E., DRAPEAU, G., D'ORLEANS-JUSTE, P. & MIZRAHI, J. (1984). Receptors for substance P. III. Classification by competitive antagonists. *Eur. J. Pharmacol.*, **97**, 179–189.

SARIA, A., LUNDBERG, J.M., SKOFITSCH, G. & LEMBECK, F. (1983). Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, serotonin, histamine and by antigen challenge. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **324**, 212–218.

WHITE, D.M. & HELME, R.D. (1985). Release of substance P from peripheral nerve terminals following electrical stimulation of the sciatic nerve. *Brain Res.*, **336**, 27–31.

YAKSH, T.L., JESSELL, T.M., GAMSE, R., MUDGE, A.W. & LEEMAN, S.E. (1980). Intrathecal morphine inhibits substance P release from mammalian spinal cord *in vivo*. *Nature*, **286**, 155–157.

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