Br. J. Pharmac. (1987), **91**, 265–273

Plasma protein extravasation induced by mammalian tachykinins in rat skin: influence of anaesthetic agents and an acetylcholine antagonist

[1]Réjean Couture & René Kérouac

Département de Physiologie, CRSN, Faculté de Médecine, Université de Montréal, C.P. 6208, Succursale A, Montréal, Québec, Canada, H3C 3T8

1 The effect of mammalian tachykinins on plasma protein extravasation was assessed in the rat dorsal skin. Substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) increased vascular permeability in a dose-related manner with a threshold dose of about 0.07 pmol in sodium pentobarbitone-anaesthetized animals.

2 Plasma protein extravasation induced by the tachykinins was 100–500 times less in magnitude in animals anaesthetized with urethane.

3 Plasma protein extravasation induced by SP (66 pmol) was significantly reduced (63%; $P < 0.001$) by atropine (a muscarinic inhibitor) while that induced by NKA or NKB was unaffected by the inhibitor suggesting that a cholinergic component might only be involved in the vascular permeability elicited by SP.

4 The rank order of potency for the tachykinins on plasma protein extravasation was: NKB > SP > NKA (in absence of atropine) and NKB > NKA > SP (in presence of atropine), suggesting that this vascular response is mediated by a SP-E-receptor type.

5 The amplitudes of the plasma protein extravasation induced by NKB and its hydrophilic analogue [Arg^0]NKB were similar, indicating that the lipophilic features of the native peptide cannot account for its potent biological activity.

6 Plasma protein extravasation was enhanced by the SP analogue [D-Pro^4, Lys^6, D-Trp^{7,9,10}, Phe^{11}] SP (4–11), thus showing the limitation of such SP analogues (antagonists) for characterizing the tachykinin receptors involved in vascular permeability.

Introduction

The tachykinin peptide family is characterized by the common amino acid sequence -Phe-X-Gly-Leu-Met-NH_2 at the C terminus, where X is either an aliphatic (Val, Ile) or an aromatic (Phe, Tyr) residue (Harmar, 1984). For a long time, substance P (SP) was the only tachykinin identified in mammalian tissues. Recently, two decapeptides, neurokinin A (NKA) and neurokinin B (NKB) were also identified in mammalian nervous tissues (Maggio *et al.*, 1983; Kimura *et al.*, 1983; Kangawa *et al.*, 1983). These tachykinins show variation in relative potencies in several pharmacological assays such as blood pressure (Hua *et al.*, 1984; Hancock & Hoover, 1985; Maggi *et al.*, 1985; Holzer-Petsche *et al.*, 1985), plasma extravasation (Hua *et al.*, 1984; Gamse & Saria, 1985) and contraction or relaxation of isolated smooth muscle preparations (Regoli, 1985). A common precursor for SP and NKA has been indentified in bovine striatum (Nawa *et al.*, 1983) and the mRNA coding for these peptides has been found in sensory neurones (Nawa *et al.*, 1984). Using incorporated radiolabelled amino acids, co-synthesis of NKA and SP in rat sensory ganglia has been demonstrated (Harmar & Keen, 1984). SP and NKA were found to be distributed in a very similar manner with fairly constant molar ratios in the rat spinal cord, while the distribution of NKB was different from the other tachykinins. It appears that SP and NKA could be co-localized in certain primary afferent neurones, while NKB is localized in interneurones or ascending pathways rather than in primary afferents originating from the dorsal root ganglia (Ogawa *et al.*, 1985). SP coexists additionally with calcitonin gene-related peptide (CGRP) in cell bodies of the trigeminal ganglion and the spinal dorsal

[1] Correspondence.

root ganglia (Skofitsch & Jacobowitz, 1985). CGRP was ineffective in causing plasma protein extravasation when given alone. However, this peptide potentiated that induced by SP and NKA (Gamse & Saria, 1985).

The role of SP, released from the peripheral terminals of sensory C-fibres in the skin, has been studied in detail and there is convincing evidence that it is involved in the mediation of antidromic vasodilatation and plasma protein extravasation (Jessel, 1983; Pernow, 1983; Chahl & Szolcsanyi, 1983; Lembeck, 1985; for reviews). It is likely, however, that the neurogenic vascular responses that are now attributed to SP may, in fact, be mediated by other tachykinins and potentiated by CGRP.

The present study was undertaken to quantitate the vascular permeability or plasma protein extravasation elicited by subcutaneous injections of mammalian tachykinins (SP, NKA and NKB) in the dorsal skin of the rat. A SP analogue [D-Pro^4, Lys^6, D-Trp^{7,9,10}, Phe^{11}]SP (4–11), which has been found to be a SP antagonist showing some selectivity for the SP-E-receptor type on peripheral isolated organs (Regoli *et al.*, 1985), was used in an attempt to characterize the tachykinin receptor type mediating plasma protein extravasation. The involvement of a cholinergic component in trigeminal antidromic vasodilatation and neurogenic plasma extravasation has been suggested (Couture & Cuello, 1984; Couture *et al.*, 1985b, c). In the present study, we have explored further the possible effects of atropine on plasma protein extravasation induced by the three tachykinins. In addition, it has been found that neurogenic plasma extravasation is less marked in animals anaesthetized with urethane than in animals anaesthetized with sodium pentobarbitone (Couture *et al.* 1985b). Urethane also depresses cutaneous vascular permeability responses to histamine, histamine-releasing agents and 5-hydroxytryptamine (O'Duffy & Chahl, 1980; Chahl, 1983). These findings are compatible with the involvement of histamine and 5-hydroxytryptamine in neurogenic plasma extravasation (Lembeck, 1983; Couture & Cuello, 1984). We were therefore also interested in comparing the effects of the two anaesthetics, urethane and sodium pentobarbitone on cutaneous plasma extravasation produced by mammalian tachykinins.

Methods

Experimental conditions

Male Wistar rats (250–300 g) purchased from Charles River, St-Constant, Québec, were anaesthetized with sodium pentobarbitone (65 mg kg^{-1}, i.p.) or with urethane (1.4 g kg^{-1}, i.p.). All experiments were done at room temperature. Animals were tracheotomized to facilitate respiration. The right jugular vein and the left carotid artery were cannulated using polyethylene tubing (PE-50) filled with physiological saline containing heparin (100 u ml^{-1}) and used for drug injections and for direct blood pressure recording. Arterial blood pressure and heart rate were recorded using a Statham pressure transducer (P23ID) and a cardiac tachometer (model 7P4) (triggered by the arterial blood pressure pulses) coupled to a Grass polygraph (model 79).

Plasma protein extravasation

The assay method for evaluating changes in vascular permeability was based on the leakage of plasma protein-bound Evans Blue dye into the skin. Evans Blue was injected intravenously (35 mg kg^{-1}) 5 min before the tachykinins which were dissolved in Krebs solution (composition in g l^{-1}: NaCl 6.90, KCl 0.35, $CaCl_2$ 0.28, KH_2PO_4 0.16, $MgSO_4$ 0.15, dextrose 2.00 and $NaHCO_3$ 2.10). The tachykinins were injected subcutaneously in 100 µl volumes into four areas of the shaved rat dorsal skin in random order. Krebs solution was also injected in one additional site as control. This pattern of injection was repeated in 4–6 rats for each experiment. After 30 min extravasation period, rats were killed by decapitation and the dorsal skin removed and the blue areas around the injection sites punched out (15 mm diameter). In addition, a piece of skin was taken from an untreated portion of the dorsal skin to determine the content of Evans Blue within cutaneous vessels. Tissues were weighed and placed in 8 ml of formamide before incubation at 60°C for 18 h, then the tissues were homogenized and re-incubated for another 6 h. After incubation, samples were centrifuged and the Evans Blue content in the supernatants measured in a spectrophotometer at 620 nm. Plasma protein extravasation (PPE) was assessed by measuring the amount of Evans Blue in the skin of tachykinin-treated areas as compared with the skin of Krebs control areas.

Drugs and solutions

Atropine sulphate, heparin, formamide and Evans Blue dye were purchased from Sigma, sodium nitroprusside and trifluoroacetic acid (TFA) from Fisher Scientific Co. Substance P, neurokinin A and neurokinin B were purchased from Institut Armand-Frappier, Laval, Canada. [Arg°] neurokinin B and [D-Pro^4, Lys^6, D-Trp^{7,9,10}, Phe^{11}]SP (4–11) were supplied by D. Regoli of the Sherbrooke University Medical School.

Evans Blue dye, heparin, atropine sulphate and nitroprusside were dissolved in physiological saline (0.9% w/v NaCl solution). Concentrated solutions (1 mg ml^{-1}) of SP, [D-Pro^4, Lys^6, D-Trp^{7,9,10}. Phe^{11}]

SP(4–11), [Arg⁶] NKB and NKA were prepared in distilled water. NKB and also NKA for comparison, were dissolved in acetic acid (12.5 N) or in trifluoroacetic acid (TFA; 0.1%), the pH was subsequently adjusted to 7.2 with sodium hydroxide (10 N) and Krebs solution was added to obtain solutions of 1 mg ml^{-1}. The concentrated solutions of peptides were divided in aliquots of 100 µl each and stored at −20°C until used. Daily dilutions of peptides were made in Krebs solution. Concentrations of peptides are given in mol of the salt.

Statistical analysis

The results are expressed as means ± s.e. of % increase in plasma protein extravasation (PPE) as compared to controls or in ng of Evans Blue mg^{-1} wet weight tissue (ng mg^{-1} w.t.). The mean number of determinations refer to the numbers of rats (each rat value is the mean of 4 injection sites). Results were evaluated by means of Student's t test for unpaired samples. Probability values (P) smaller than 0.05 were considered to be significant

Results

Effect of anaesthetic used on the plasma protein extravasation induced by tachykinins

Baseline mean arterial pressure (MAP) and heart rate (HR) were 89.5 ± 5.0 mm Hg and 385 ± 12 beats min^{-1} ($n = 21$) in urethane-anaesthetized rats and 128.0 ± 4.0 mmHg and 392 ± 11 beats min^{-1} (n = 40) in pentobarbitone-anaesthetized animals. The difference in the MAP between the two groups of animals was statistically significant ($P < 0.001$). Subcutaneous (s.c.) injections of tachykinins into the dorsal skin at concentrations varying from 0.1 to 100 pmol failed to alter MAP and HR. However, higher concentrations of SP (1 and 10 nmol) caused a reduction of the MAP (−24 ± 4 mmHg, $n = 9$; $P < 0.01$) lasting between 15 to 30 min and a transient increase in HR (+ 22 ± 3 beats min^{-1}, $n = 9$; $P < 0.01$) lasting between 4 to 7 min. The latter cardiovascular responses were accompanied by an important increase of PPE in the skin of the hind paws (552% increase at 1 nmol and 636% increase at 10 nmol) and of the ears (200% increase at 1 nmol and 456% increase at 10 nmol) when compared to control levels ($n = 5$; $P < 0.001$). Therefore, these effects of SP at high concentrations are presumably the result of systemic SP absorption. The amount of non-specific Evans Blue in the tissue (e.g. that within cutaneous vessels) was assessed in a dorsal skin areas which did not receive any injection. The values were 20.0 ± 0.6 ng mg^{-1} w.t., $n = 7$ and 20.0 ± 1.0 ng mg^{-1} w.t., $n = 13$, respectively in urethane- and pentobarbitone-anaesthetized animals. The injection of Krebs solution into the dorsal skin failed to produce any significant PPE (in urethane-anaesthetized animals: 21.4 ± 0.6 ng mg^{-1} w.t., $n = 7$; in pentobarbitone anaesthetized animals: 22.0 ± 0.6 ng mg^{-1} w.t., $n = 13$).

The effects of increasing concentrations of tachykinins injected s.c. into the dorsal skin in urethane- and sodium pentobarbitone-anesthetized rats are presented in Figure 1. Threshold concentrations producing PPE were around 0.06–0.08 pmol for SP, NKB and NKA per injection site in sodium pentobarbitone-anaesthetized animals. At 10 pmol, the amplitude of the PPE was not significantly different between NKB (138.3 ± 22.3% increase, $n = 5$) and SP (81.0 ± 17.0% increase, $n = 5$) but was between NKB and NKA (69.5 ± 12.5% increase, $n = 5$; $P < 0.05$). In addition, at 1 pmol a significant difference was observed only between NKB (70.0 ± 13% increase, $n = 5$) and NKA (32.9 ± 5.8% increase, $n = 5$; $P < 0.05$). The data showed that there was no significant difference between the abilities of SP and NKA to enhance PPE at any concentrations tested in pentobarbitone-anaesthetized rats (Figure 1).

In urethane-anaesthetized rats, the PPE (both threshold and amplitude) induced by SP, NKA and NKB was tremendously reduced (Figure 1). Threshold concentrations for the three peptides were shifted to the right by two and three log units. At a concentration of 0.1 nmol, the PPE induced by SP was 8 fold higher in magnitude in animals anaesthetized with sodium pentobarbitone (121.8 ± 16.8% increase, $n = 7$) than in animals anaesthetized with urethane (15.2 ± 3.2% increase, $n = 4$, $P < 0.001$). Similar results were obtained with NKB (in sodium pentobarbitone animals: 142.7 ± 18.5% increase, $n = 5$; urethane animals: 42.5 ± 5.8% increase, $n = 4$; $P < 0.005$) and NKA (in sodium pentobarbitone animals: 113.7 ± 12.7% increase, $n = 5$; urethane animals: 13.4 ± 3.1% increase, $n = 4$; $P < 0.001$). In urethane-anaesthetized rats, a significant difference in the amplitude of the PPE between NKB and the two other tachykinins was observed. This difference was more striking at 0.1 nmol where the effect on PPE was as follows NKB (42.5 ± 5.8% increase, $n = 4$), SP (15.2 ± 3.2% increase, $n = 4$; $P < 0.01$) and NKA (13.4 ± 3.1% increase, $n = 4$; $P < 0.02$). When SP was given at higher concentrations than 1 nmol, no significant different in PPE was observed between sodium pentobarbitone- and urethane-anaesthetized animals (146.4 ± 6.4% increase, $n = 5$ and 120.8 ± 20.4, $n = 5$, respectively).

An intravenous infusion of nitroprusside (0.98 µg min^{-1}) was used to lower MAP to 82.5 ± 6.2 mmHg in pentobarbitone-anaesthetized rats. The values for PPE obtained with NKA (0.8 nmol) were not significantly different from those obtained following an

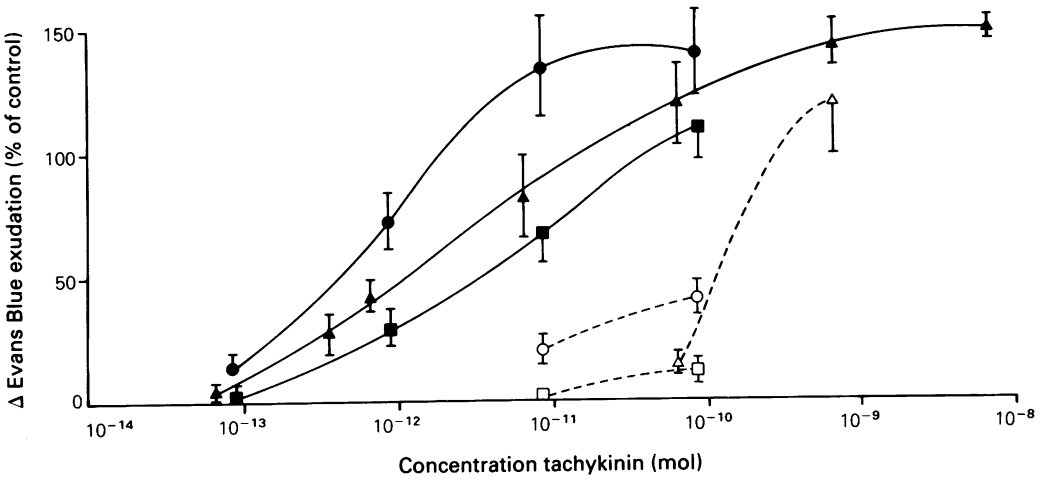

Figure 1 Plasma protein extravasation increases induced in the dorsal skin following subcutaneous injections of substance P (▲, △), neurokinin A (■, □) and neurokinin B (●, ○) in rats anaesthetized with sodium pentobarbitone (solid lines, closed symbols) with urethane (broken lines, open symbols). Ordinate scale: variations in Evans Blue exudation expressed as % of control. Abscissa scale: concentration of tachykinins in mol per injection site. Each point represents the mean and vertical lines s.e.mean from 4–6 rats.

intravenous infusion of saline (infusion of nitroprusside: 160.9 ± 15.2% increase, $n = 4$; infusion of saline: 165.1 ± 13.0% increase, $n = 4$). Thus, the reduction of PPE in urethane-anaesthetized rats does not seem to be attributable to a decrease in MAP.

In the experiments described below, all animals were anaesthetized with sodium pentobarbitone.

Effect of an acetylcholine antagonist and a substance P analogue on plasma protein extravasation induced by tachykinins

Atropine (3 mg kg^{-1}, i.v., 20 min earlier) cause a slight and transient hypotension without affecting HR. The basal values of PPE elicited by s.c. injection of Krebs solution remained unaffected by atropine (untreated rats: 22 ± 2.0 ng mg^{-1} w.t.; $n = 13$; atropine-treated rats: 21.7 ± 0.5 ng mg^{-1} w.t.; $n = 7$). The amount of Evans Blue measured in skin which did not receive any injection also remained unaltered (20.0 ± 1.0, $n = 13$ and 20.6 ± 0.6 ng mg^{-1} w.t.; $n = 7$, respectively). The PPE induced by SP (66 pmol) was reduced by 63% with atropine ($P < 0.001$, $n = 6$). On the other hand, atropine failed to modify PPE induced by a smaller concentration of SP (0.66 pmol) and by two different concentrations of NKB (0.78 and 7.8 pmol) and NKA (0.8 and 8.0 pmol) (Figure 2). The SP analogue [D-Pro^4, Lys^6, D-Trp^{7,9,10}, Phe^{11}] SP (4–11) failed to alter MAP and HR up to 7.8 nmol following subcutaneous injection. However, this analogue enhanced PPE: 28.6 ± 5.8% increase, $n = 4$; 32.6 ± 6.4% increase, $n = 4$; 57.2 ± 7.1% increase, $n = 5$ at 78.0 pmol, 0.78 nmol and 7.8 nmol, respectively.

Effects of a hydrosoluble analogue of neurokinin B and comparison of solubilisation medium on the activity of tachykinins on plasma protein extravasation

The magnitude of the plasma protein extravasation induced by [Arg°] NKB, the NKB molecule soluble in water and that induced by NKB dissolved in acetic acid or in TFA was not significantly different at 7.8 pmol and 0.78 pmol. Similarly, no significant difference on PPE was observed between NKA (80 and 0.8 pmol) dissolved in water, in acetic acid or in TFA (Figure 3).

Discussion

In the present study, the three tachykinins SP, NKA and NKB were found to increase plasma protein extravasation in a dose-dependent manner when injected locally into the rat dorsal skin. Urethane anaesthesia depressed markedly basal mean arterial pressure and the plasma protein extravasation induced by three tachykinins in a non-specific manner. The mechanism of the depressant effect of urethane on plasma extravasation is not known. However, it does not appear to be due to a reduction of hydrostatic pressure within the venule lumen as a consequence of lowered arterial blood pressure, since the reduction of MAP to the same degree as that observed with urethane, following an infusion of nitroprusside, failed to reduce

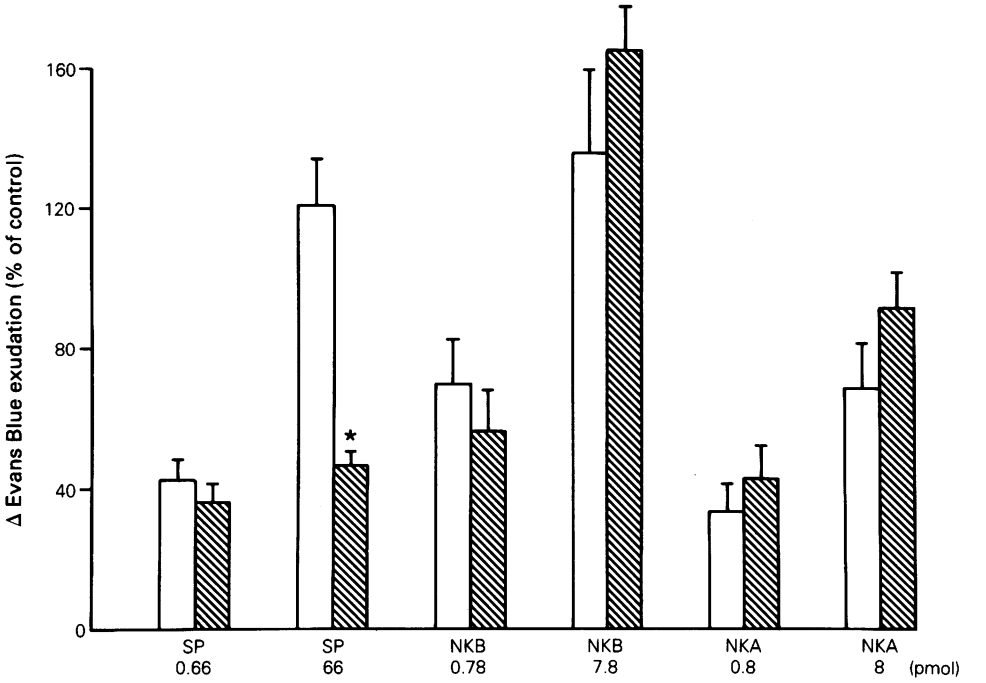

Figure 2 Effect of atropine on plasma protein extravasation induced in the dorsal skin following subcutaneous injections of substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) in rats anaesthetized with sodium pentobarbitone. Ordinate scale: variation in Evans Blue exudation expressed a % of control in untreated (open columns) and atropine-treated (hatched columns) animals. Values represent the means and vertical lines s.e.mean from 4–6 rats. $*P < 0.001$, statistically significant difference in Evans Blue exudation between untreated and atropine-treated animals, calculated using Student's t test for unpaired samples on means ± s.e.mean from 6 rats. Atropine (3 mg kg^{-1}) was given intravenously 15 min before the injection of Evans Blue.

PPE elicited by NKA. The inhibition of tachykinin extravasation in rats anaesthetized with urethane may be attributed to a reduced availability of both intracellular and extracellular calcium (Altura & Weinberg, 1979). In fact, urethane depresses cutaneous vascular permeability responses to histamine, 5-hydroxytryptamine and mast-cell releasing agents (O'Duffy & Chahl, 1980; Chahl, 1983). More recently, it has been shown that neurogenic plasma extravasation was 34% less in magnitude in animals anaesthetized with urethane than in animals anaesthetized with sodium pentobarbitone (Couture *et al.*, 1985b). Urethane, in contrast to pentobarbitone, is known to diminish the pressor effect of noradrenaline and angiotensin (Volicer & Loew, 1971; Bunag & Mullenix, 1972; Brezenoff, 1973) as well as the sensitivity of small vessels to different vasoconstrictor agents (Miller & Wiegman, 1977; Altura & Weinberg, 1979). Furthermore, urethane has a direct depressive effect on histamine-induced contractions of guinea-pig tracheal smooth muscle both *in vivo* and *in vitro* (Maggi *et al.*, 1982) and reduces cardiovascular responses that are mediated by the stimulation of α_2-adrenoceptors located on the resistance blood vessels, cardiac postganglionic sympathetic nerves and in brain centres proposed to control cardiovascular functions (Armstrong *et al.*, 1982).

As the plasma protein extravasation induced by tachykinins in urethane-anaesthetized animals can be more easily measured at concentrations higher than 0.1 nmol, caution must be taken in interpreting those results since cardiovascular effects and systemic plasma protein extravasation were observed with 1 nmol of SP. These findings stress the importance of using sodium pentobarbitone as the anaesthetic, when measuring plasma extravasation in response to concentrations of tachykinins that do not produce any changes in the cardiovascular functions. Taken together, the present results support the hypothesis that urethane anaesthesia decreases the sensitivity of the microvasculature to tachykinins. It is suggested that this anaesthetic should be avoided, particularly in

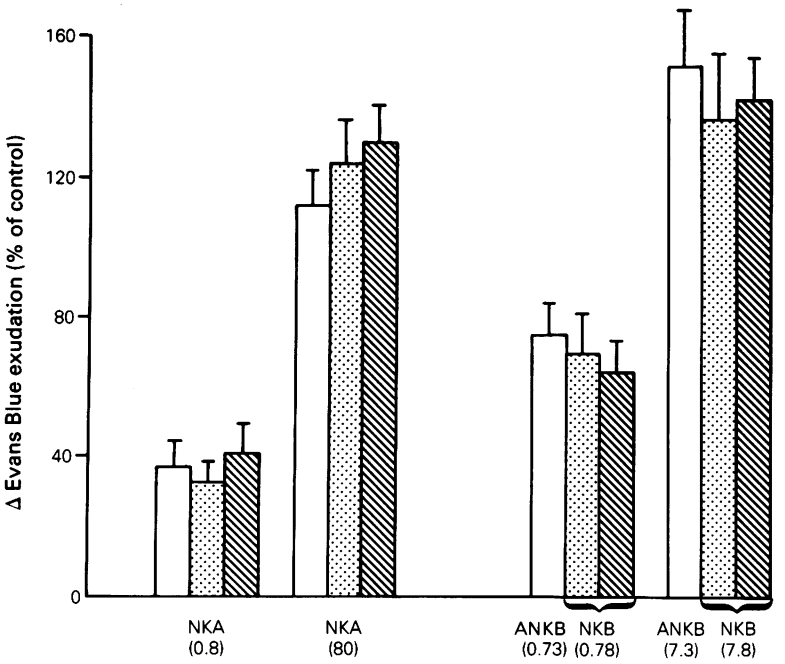

Figure 3 Effect of the solvent used to dissolve the peptide on plasma extravasation induced in the dorsal skin following subcutaneous injections of neurokinin A (NKA), neurokinin B (NKB) and [Arg°] NKB (ANKB) in rats anaesthetized with sodium pentobarbitone. Ordinate scale: variation in Evans Blue exudation expressed as % of control for peptides dissolved in distilled water (open columns), in 12.5 N acetic acid (stippled columns) and in 0.1% trifluoroacetic acid (hatched columns). Values represent the means and vertical lines s.e.means from 4–6 rats. Numbers in parentheses represent doses in pmol.

studies designed to assess the vascular permeability induced by tachykinins and possibly by other agents.

The rank order of potency for tachykinins on plasma protein extravasation in sodium pentobarbitone- and in urethane-anaesthetized rats was: NKB > SP > NKA and confirms the results obtained by other workers (Gamse & Saria, 1985). This order of potency cannot be attributed to the high lipophilic features of NKB, compared to the hydrophilic features of SP and NKA, as no significant difference in biological activity was found on plasma extravasation between [Arg°]NKB (an analogue of NKB soluble in water) and NKB, or between the three solutions of NKA obtained by dissolving the peptide in water or in two different acid solvents.

Atropine reduced by 63% the plasma protein extravasation induced by SP (66 pmol) without affecting that observed with lower concentrations of SP (0.66 pmol) or with NKA and NKB at the concentrations tested in pentobarbitone-anaesthetized rats. The rank order of potency for tachykinins on plasma protein extravasation in atropine-treated animals was: NKB > NKA > SP. These data suggest that part of the SP-induced plasma protein extravasation is mediated by some cholinergic mechanism. It has been shown that atropine reduces antidromic vasodilatation and neurogenic plasma extravasation (Couture & Cuello, 1984; Couture *et al.*, 1985b). Atropine has also been found to abolish the residual antidromic vasodilatation of the hind leg in capsaicin-pretreated rats (Lembeck & Holzer, 1979). The atropine-sensitive component of the plasma extravasation, observed following antidromic stimulation of trigeminal sensory branches in the rat, was abolished by chronic bilateral extirpation of the superior cervical ganglia. This suggests that sympathetic postganglionic cholinergic fibres may participate in the increase of vascular permeability upon electrical stimulation of the trigeminal nerve and therefore, should be present in the skin (Couture *et al.*, 1985c). In addition, electrolytic lesions of the Gasserian ganglia failed to modify levels of choline acetyltransferase activity in the substantia gelatinosa and tractus spinalis of the trigeminal nerve, indicating that a major sensory cholinergic component is unlikely in this system (Couture *et al.*, 1985a).

It is worth noting that acetylcholine is a potent histamine releasing agent on isolated mast cells, an effect mediated by muscarinic receptors (Blandina *et al.*, 1980). A link between cholinergic systems and those involving SP and histamine relase from mast cells, as already proposed by Couture & Cuello (1983), could enhance plasma extravasation induced by SP. It is well known that SP induces the release of histamine from mast cells (Johnson & Erdös, 1973; Kitada *et al.*, 1980; Erjavec *et al.*, 1981; Foreman & Jordan, 1981; Foreman *et al.*, 1982; 1983; Fewtrell *et al.*, 1982; Devillier *et al.*, 1985; 1986). However, NKA and NKB were unable to induce release of histamine from rat peritoneal mast cells (Devillier *et al.*, 1985; 1986) and NKA failed to increase the release of histamine from rat perfused hindquarter (Holzer-Petsche *et al.*, 1985). It is interesting to note that NKA and NKB also failed to generate plasma extravasation by activating an atropine-sensitive mechanism in the rat skin. Taken together, these findings suggest that NKA and NKB enhanced vascular permeability through a direct action on capillary-venular permeability, while acetylcholine and histamine might participate in the increase of vascular permeability in response to SP.

There is a strong body of evidence for the existence of multiple tachykinin receptors in mammalian tissues (Quirion & Dam, 1985; Regoli, 1985; for reviews). On the basis of the rank order of potency of tachykinins (NKB > NKA > SP) it is suggested that an SP-E receptor type (according to the initial classification of Lee *et al.*, 1982) is involved in the increase of vascular permeability induced by tachykinins in rat skin. An attempt to characterize further the receptor type mediating tachykinin plasma extravasation has been carried out by using the SP analogue [D-Pro^4, Lys^6, D-Trp^{7,9,10}, Phe^{11}]SP (4–11) which shows selectivity for SP-E-receptors in some pharmacological preparations (Regoli *et al.*, 1985). However, this analogue was found to increase the plasma protein extravasation, possibly by promoting the release of histamine and acetylcholine. Indeed, SP analogues containing D-tryptophan are potent at inducing histamine release from rat mast cells (Foreman *et al.*, 1982; Fewtrell *et al.*, 1982; Sydbom, 1982; Devillier *et al.*, 1985) and are also more potent than SP in producing wheal and flare responses in human skin (Foreman *et al.*, 1982; 1983). In addition, these SP analogues possess considerable inherent stimulatory activity when tested for both [^{3}H]-acetylcholine release and myotropic activity in the guinea-pig ileum (Hawcock *et al.*, 1982; Featherstone *et al.*, 1986). Therefore, the use of such compounds to characterize receptors for tachykinins in our model appears to be unsatisfactory.

In conclusion, this is the first time a comparison between two anaesthetic agents on the plasma extravasation induced by three mammalian tachykinins in the rat skin has been performed. The results indicate the involvement of a cholinergic component in the vascular permeability elicited only by SP and stress the possibility that other tachykinins (e.g. NKA) also present in the primary sensory afferents (Ogawa *et al.*, 1985) can play a role in neurogenic inflammation.

This work was supported by grants to R.C. from the Medical Research Council of Canada, The Kidney Foundation of Canada, The Quebec Heart Foundation and the University of Montreal (CAFIR). R.C. is a 'chercheur-boursier du Fonds de la Recherche en Santé du Québec'. We thank Ms Suzanne Burke for the secretarial assistance and Mr Giovanni Baptista Filosi for the illustrations and photographs.

Note added in Proof

At a satellite symposium on 'Substance P and Neurokinins' held in Montréal, Canada in July 1986 as part of the XXX International Congress of Physiological Sciences, a majority of participants agreed to rename SP-P, SP-E and SP-N tachykinin receptors NK-1, NK-2 and NK-3 receptors respectively. Therefore the present paper suggests that plasma protein extravasation induced by tachykinins in rat skin is mediated by a NK-3 receptor type.

References

ALTURA, B.M. & WEINBERG, J. (1979). Urethane and contraction of vascular smooth muscle. *Br. J. Pharmac.*, **67**, 255–263.

ARMSTRONG, J.M., LEFEVRE-BORG, F., SCATTON, B. & CAVERO, I. (1982). Urethane inhibits cardiovascular responses mediated by the stimulation of alpha-2 adrenoceptors in the rat. *J. Pharmac. exp. Ther.*, **223**, 524–535.

BLANDINA, P., FANTOZZI, R., MANNAIONI, P.F. & MASINI, E. (1980). Characteristics of histamine release evoked by acetylcholine in isolated rat mast cells. *J. Physiol.*, **301**, 281–293.

BREZENOFF, H.E. (1973). Cardiovascular responses to noradrenaline in rat before and after administration of various anaesthetics. *Br. J. Pharmac.*, **49**, 565–572.

BUNAG, R. & MULLENIX, P. (1972). Augmentation of drug-induced blood pressure increases in rats by amobarbital. *Br. J. Pharmac.*, **46**, 511–513.

CHAHL, L.A. (1983). Influence of season and anaesthetic on cutaneous vascular permeability responses to histamine, serotonin and mast cell-releasing agents in rats. *Clin. exp. Pharmac. Physiol.*, **10**, 415–420.

CHAHL, L.A. & SZOLCSANYI, J. (1983). Antidromic

vasodilatation and neurogenic inflammation. *Neurosci. Letts.*, **42,** 219–225.

COUTURE, R. & CUELLO, A.C. (1983). Substance P antagonists and the antidromic stimulation of peripheral sensory branches of the trigeminal nerve. In *Substance P* ed. Skrabanek P. & Powell, D. pp. 192–193. Dublin: Boole Press.

COUTURE, R., CONSOLAZIONE, A. & CUELLO, A.C. (1985a). Choline acetyltransferase activity in the rat trigeminal system. *J. Neurochem.*, **45,** 1027–1029.

COUTURE, R. & CUELLO, A.C. (1984). Studies on the trigeminal antidromic vasodilatation and plasma extravasation in the rat. *J. Physiol.*, **346,** 273–285.

COUTURE, R., CUELLO, A.C. & HENRY, J.L. (1985b). Trigeminal antidromic vasodilatation and plasma extravasation in the rat: effects of acetylcholine antagonists and cholinesterase inhibitors. *Br. J. Pharmac.*, **84,** 637–643.

COUTURE, R., CUELLO, A.C. & HENRY, J.L. (1985c). Trigeminal antidromic vasodilatation and plasma extravasation in the rat: effects of sensory, autonomic and motor denervation. *Brain Res.*, **346,** 108–114.

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

DEVILLIER, P., REGOLI, D., ASSEROF, 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.

ERJAVEC, F., LEMBECK, F., FLORJANC-IRMAN, T., SKOFITSCH, G., DONNERER, J., SARIA, A. & HOLZER, P. (1981). Release of histamine by substance P. *Naunyn-Schmiedebergs Arch. Pharmac.*, **317,** 67–70.

FEATHERSTONE, R.L., FOSBRAEY, P. & MORTON, I.K.M. (1986). A comparison of the effects of three substance P antagonists on tachykinin-stimulated [^{3}H]-acetylcholine release in the guinea-pig ileum. *Br. J. Pharmac.*, **87,** 73–77.

FEWTRELL, C.M.S., FOREMAN, J.C., JORDAN, C.C. OEHME, P., RENNER, H. & STEWART, J.M. (1982). The effect of substance P on histamine and 5-hydroxytryptamine release in the rat. *J. Physiol.*, **330,** 393–412.

FOREMAN, J.C. & JORDAN, C.C. (1981). Comparison of the activities of some peptides in human skin and as histamine releasing agents. *Br. J. Pharmac.*, **73,** 207P.

FOREMAN, J.C., JORDAN, C.C. & PIOTROWSKI, W. (1982). Interaction of neurotensin with the substance P receptor mediating histamine release from rat mast cells and the flare in human skin. *Br. J. Pharmac.*, **77,** 531–539.

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 takykinin-induced plasma protein extravasation by calcitonin gene-related peptide. *Eur. J. Pharmac.*, **114,** 61–66.

HANCOCK, J.C. & HOOVER, D.B. (1985). Effect of substance P and other tachykinins on arterial pressure in guinea-pigs. *J. autonom. Pharmac.*, **5,** 25–30.

HARMAR, A.J. (1984). Three tachykinins in mammalian brain. *Trends Neurosci.*, **7,** 58–60.

HARMAR, A.J. & KEEN, P. (1984). Rat sensory ganglia incorporate radiolabelled amino acids into substance K (neurokinin α) in vitro. *Neurosci. Letts.*, **51,** 387–391.

HAWCOCK, A.R., HAYES, A.G. & TYERS, M.B. (1982). Agonist effect of [D-Pro^2, D-Phe^7, D-Trp^9]-Substance P – Evidence for different receptors. *Eur. J. Pharmac.*, **80,** 135–138.

HOLZER-PETSCHE, U., SCHIMEK, E., AMANN, R. & LEMBECK, F. (1985). In vivo and in vitro actions of mammalian tachykinins. *Naunyn-Schmiedebergs Arch. Pharmac.*, **330,** 130–135.

HUA, X., LUNDBERG, J.M., THEODORSSON-NORHEIM, E. & BRODIN, E. (1984). Comparison of cardiovascular and bronchoconstrictor effects of substance P, substance K and other tachykinins. *Nauyn-Schmiedebergs Arch. Pharmac.*, **328,** 196–201.

JESSELL, T.M. (1983). Substance P in the Nervous System. In *Handbook of Psychopharmac.*, vol. 16 ed. Iversen, L.L., Iversen, S.D. & Snyder, S.H. pp. 1–105. New York: Plenum Press.

JOHNSON, A.R. & ERDÖS, E.G. (1973). Release of histamine from mast cells by vasoactive peptides. *Proc. Soc. exp. Biol. Med.*, **142,** 1252–1256.

KANGAWA, K., MINAMINO, N., FUKUDA, A. & MATSUO, H. (1983). Neuromedin K: a novel of mammalian tachykinin identified in porcine spinal cord. *Biochem, biophys, Res. Commun.*, **114,** 533–540.

KIMURA, S., OKADA, M., SUGITA, Y., KANAGAWA, I. & MUNEKATA, E. (1983). Novel neuropeptides, neurokinin α and β isolated from porcine spinal cord. *Proc. Jap. Acad.*, **59,** 101–104.

KITADA, C., ASHIDA, Y., MAKI, Y., FUJIMO, M., HIRAI, Y., YASUHARA, T., NAKAJIMA, T., TAKEYAMA, M., KAYAMA, K. & YAJIMA, H. (1980). Synthesis of granuliberin-R and various fragment peptides and their histamine releasing activities. *Chem. Pharm. Bull., Tokyo*, **28,** 887–892.

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. Pharmac.*, **318,** 281–287.

LEMBECK, F. (1983). Mediators of vasodilatation in the skin. *Br. J. Dermatol.*, **109,** (Suppl. 25) 1–9.

LEMBECK, F. (1985). Substance P and sensory neurones. In *Substance P-Metabolism and Biological Actions*, ed. Jordan, C.C. & Oehme, P. pp. 137–151. London: Taylor and Francis.

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

MAGGI, C.A., SANTICIOLI, P., EVANGELISTA, S. & MELI, A. (1982). The effect of urethane on histamine-induced contraction of guinea-pig tracheal smooth muscle. *Experientia*, **38,** 1474–1476.

MAGGI, C.A., GIULIANI, S., SANTICIOLI, P., REGOLI, D. & MELI, A. (1985). Comparison of the effects of substance P and substance K on blood pressure, salivation and urinary bladder motility in urethane-anaesthetized rats. *Eur. J. Pharmac.*, **113,** 291–294.

MAGGIO, J.E., SANDBERG, B.E.B., BRADLEY, C.V., IVERSON, L.L., SANTIKARN, S., WILLIAMS, D.H., HUNTER, J.C. & HANLEY, M.R. (1983). Substance K: a novel tachykinin in mammalian spinal cord. In *Substance P*, ed.

Skrabanek, P. & Powell, D. pp. 20–21. Dublin: Boole Press.

MILLER, F., & WIEGMAN, D.L. (1977). Anesthesia-induced alteration of small vessel responses to norepinephrine. *Eur. J. Pharmac.*, **44**, 331–337.

NAWA, H., HIROSE, T., TAKASHIMA, H. INAYAMA, S. & NAKANISHI, S. (1983). Nucleotide sequenes of cloned cDNAs for two types of bovine substance P precursor. *Nature*, **306**, 32–36.

NAWA, H., KOLANI, H. & NAKANISHI, S. (1984). Tissue-specific generation of two preprotachykinins mRNAs from one gene by alternative RNA slicing. *Nature*, **312**, 729–734.

O'DUFFY, G. & CHAHL, L.A. (1980). The influence of laboratory anaesthetics on the increased cutaneous vascular permeability responses to histamine and 5-hydroxytryptamine in rats. *J. Pharmac. Methods*, **4**, 231–235.

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 ganglia, and the effects of dorsal root section or spinal transection. *Brain Res.*, **359**, 152–157.

PERNOW, B. (1983). Substance P. *Pharmac. Rev.*, **35**, 85–141.

QUIRION, R. & DAM, T.V. (1985). Multiple tachykinin receptors. In *Substance P-Metabolism and Biological Actions*, ed. Jordan, C.C. & Oehme, P. pp. 45–64. London: Taylor & Francis.

REGOLI, D. (1985). Peptide antagonists. *Trends pharmac Sci.*, **62**, 481–484.

REGOLI, D., MIZRAHI, J., D'ORLÉANS-JUSTE, P., DION, S., DRAPEAU, G. & ESCHER, E. (1985). Substance P antagonists showing some selectivity for different receptor types. *Eur. J. Pharmac.*, **109**, 121–125.

SKOFITSCH, G. & JACOBOWITZ, D.M. (1985). Calcitonin-gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. *Peptides*, **6**, 747–754.

SYDBOM, A. (1982). Histamine release from rat mast cells by neurotensin and other peptides. *Agents & Actions*, **12**, 91–93.

VOLICER, L. & LOEW, C.G. (1971). The effect of urethane anaesthesia on the cardiovascular action of angiotensin II. *Pharmacology*, **6**, 193–201.

(Received May 13, 1986.
Revised January 8, 1987.
Accepted February 6, 1987.)