A non-peptide NK₁-receptor antagonist, RP 67580, inhibits neurogenic inflammation postsynaptically

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1 The non-peptide neurokinin NK₁-receptor antagonist, RP 67580 (3aR, 7aR), a perhydroisoindolone derivative, powerfully reduced plasma extravasation in rat hind paw skin induced by local application of xylene (ID₅₀ = 0.03 mg kg⁻¹, i.v.) or capsaicin (ID₅₀ = 0.06 mg kg⁻¹, i.v.), or by i.v. injection of exogenous substance P (SP) or septide ([pGlu⁶,Pro⁹]SP(6–11)) (ID₅₀ = 0.04–0.05 mg kg⁻¹, i.v.). RP 67580 (1 mg kg⁻¹, i.v.) also abolished capsaicin-induced nasal fluid hypersecretion (by 82 ± 5%). These effects were found to be stereospecific, the enantiomer, RP 68651 (3aS, 7aS), being inactive at 1 mg kg⁻¹, i.v.

2 In rats neonatally treated with capsaicin (50 mg kg⁻¹, s.c.), plasma extravasation induced by SP was significantly increased (by 43 ± 7%). RP 67580 (1 mg kg⁻¹, i.v.) completely inhibited the SP-induced plasma extravasation in capsaicin neonatally treated animals, as it did in control animals. This result suggests that RP 67580 acts at the postsynaptic level for the inhibition of plasma extravasation.

3 Opioid receptor agonists, μ -(morphine) and κ -(PD-117302) at 10 mg kg⁻¹, s.c., in contrast to NK₁-receptor antagonists, did not inhibit plasma extravasation induced by exogenous SP. They were, however, partially effective against plasma extravasation induced by electrical nerve stimulation (74 ± 4% and 48 ± 9% inhibition at 10 mg kg⁻¹, s.c. of morphine and PD-117302, respectively, compared to 90 ± 3% inhibition obtained with RP 67580, 3 mg kg⁻¹, s.c.). These results indicate the presynaptic action of opioid receptor agonists, in contrast to the postsynaptic action of NK₁-receptor antagonists for the inhibition of plasma extravasation.

4 Ligature of the saphenous nerve distal to the point of electrical stimulation, local application of lignocaine to the saphenous nerve, neonatal capsaicin pretreatment, and colchicine at very low doses (120 μ g kg⁻¹ day⁻¹ given for 3 days) were found to prevent plasma extravasation elicited by electrical nerve stimulation.

5 The foregoing results demonstrate that the non-peptide NK₁-receptor antagonist, RP67580, is a potent inhibitor of plasma extravasation induced in skin by NK₁-receptor agonists, by local application of chemical irritants (capsaicin or xylene) or by electrical nerve stimulation. Moreover, opioid receptor agonists and colchicine inhibit plasma extravasation induced by electrical nerve stimulation but not that elicited by exogenous SP. Therefore, it is possible to inhibit neurogenic inflammation either at the presynaptic level with opioid receptor agonists and colchicine, or at the postsynaptic level with NK₁-receptor antagonists, and that the new non-peptide NK₁-receptor antagonists may have a great potential for alleviation of inflammation in various pathological syndromes in man.

Keywords: Non-peptide NK₁-receptor antagonists; opioid receptor agonists; colchicine; neurogenic inflammation; NK₁-receptors; substance P

Introduction

Neurogenic inflammation is a complex process involving vasodilatation, plasma protein extravasation and oedema. It can be elicited by antidromic stimulation of the unmyelinated C-fibres of sensory nerves or by topical application of chemical irritants, such as capsaicin, mustard oil and xylene (Jancso *et al.*, 1967; Chahl & Ladd, 1976; Lembeck & Holzer, 1979; Lynn & Shakhaneh, 1988). If rats were treated neonatally with high doses of capsaicin, a substance that selectively destroys C-fibres (Nagy *et al.*, 1983), neurogenic inflammation cannot subsequently be elicited by stimulation (Gamse *et al.*, 1980). Therefore, C-fibres are directly involved in the inflammatory process. In 1953, Lembeck suggested that substance P (SP) is the primary afferent neurotransmitter of inflammation. Subsequent studies demonstrated the accuracy of this proposal: SP along with neurokinin A (Dalsgaard *et al.*, 1985) was found in the C-fibres of sensory neurones (Hökfelt *et al.*, 1975), indicating that it may play a role in neurogenic inflammation. Moreover, the application of SP to the skin, both in rodents and man, elicits local oedema and plasma extravasation, thus mimicking the vascular responses to antidromic nerve

stimulation (Hägermark *et al.*, 1978; Gamse *et al.*, 1980; Foreman & Jordan, 1984; Foreman, 1987; 1988; Pedersen-Bjergaard *et al.*, 1991). The afferent sensory neurones containing SP were found to control vascular permeability in numerous tissues other than the skin, such as the air passages, nasal mucosa, meninges, eyes, urinary bladder and joints (Moskowitz *et al.*, 1979; Bill *et al.*, 1979; Saria *et al.*, 1983; Lundberg *et al.*, 1983; 1984; Pernow, 1985; Helme *et al.*, 1985; Lam & Ferrell, 1991).

There is good evidence that SP induces neurogenic inflammation via NK₁-receptors, whereas NK₂- and NK₃-receptors, for which neurokinin A and neurokinin B, respectively, are considered to be the preferential agonists (Regoli *et al.*, 1987), do not seem to be involved (Andrews *et al.*, 1989; Abelli *et al.*, 1991). Therefore, if SP is the main mediator of neurogenic inflammation, selective and potent NK₁-receptor antagonists potentially represent a new therapeutic approach to various pathologies. Recently, RP 67580, a perhydroisoindolone derivative, and CP-96,345, a quinuclidine derivative, were reported to be non-peptide NK₁-receptor antagonists that both act competitively on NK₁-receptors (Garret *et al.*, 1991; Snider *et al.*, 1991). Moreover, RP 67580 (3aR, 7aR) and CP-96,345 were reported to be antinociceptive agents in rodents (Garret *et al.*, 1991; Yamamoto &

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Yaksh, 1991; Moussaoui *et al.*, 1992a). We previously showed that RP 67580, but not its enantiomer RP 68651 (3aS, 7aS), inhibits plasma extravasation induced in rat skin by electrical stimulation of the saphenous nerve (Garret *et al.*, 1991), and induced by NK₁-receptor agonists in rat urinary bladder (Moussaoui *et al.*, 1992a). We thought these NK₁-receptor antagonists could be useful in extending our understanding of the role of SP and NK₁-receptors in physiological and pathological processes. In the present work, we examined the importance of NK₁-receptors in plasma extravasation in the rat paw and on nasal mucus production in the air ways.

Methods

Male Wistar rats (200–300 g, Charles River Laboratories, St Aubin lès Elbeuf, France) were used in all experiments except in the test of xylene-induced plasma extravasation. They were anaesthetized with sodium pentobarbitone (40 mg kg⁻¹, i.p.). In the experiments on xylene-induced plasma extravasation, male Sprague-Dawley hairless rats (220–260 g, Iffa Credo, L'Arbresle, France) were used and anaesthetized with urethane (1.10 g kg⁻¹, i.p.). The administration time for each drug was decided on the basis of preliminary results of pilot experiments (see below).

Plasma extravasation induced by electrical nerve stimulation (neurogenic inflammation)

Saphenous nerves were stimulated electrically (25 V, 2 ms, 10 Hz, duration 15 min) according to the procedure of Lembeck & Holzer (1979). Plasma extravasation was visualized with Evans blue as previously described (Garret *et al.*, 1991). The inhibitory effect of drugs was calculated by comparison with the effect of vehicle, and ID₅₀ was determined by regression analysis (graphic method).

(±)-CP-96,345 (racemate) was given i.v. 5 min before the saphenous nerve was stimulated. RP 67580 (3aR, 7aR) and opioid receptor agonists were administered s.c. 30 min before electrical nerve stimulation. Lignocaine (20 µl of a 2% solution) was applied directly to the saphenous nerve 2 min before electrical stimulation. Colchicine (120 µg kg⁻¹ day⁻¹) was administered i.p. and Evans blue extravasation, induced by electrical nerve stimulation, was evaluated in rats (treated for 1, 2 or 3 consecutive days, 4 h) after the last administration of colchicine. Capsaicin (50 mg kg⁻¹) or its vehicle (100 µl of saline containing 10% ethanol and 10% Tween 20) was given s.c. to 2-day-old rats, and 2 months later, the male rats (200–250 g) were used for neurogenic inflammation studies. In additional experiments, the saphenous nerve was ligated; its proximal part was stimulated and plasma extravasation was determined as previously described.

Plasma extravasation induced by intravenous injection of peptide agonists

Plasma extravasation was induced in hind paw skin by NK₁-receptor agonists, exogenous SP or septide (1 µg kg⁻¹, i.v.), and was determined by the method described by Saria *et al.* (1983). A simultaneous injection of NK₁-receptor agonist (1 µg kg⁻¹, i.v.) and Evans blue (30 mg kg⁻¹, i.v.) was given 5 min after i.v. injection of RP 67580 or RP68651; 30 min after s.c. injection of RP 67580, morphine or PD-117 302; or 4 h after the last administration of colchicine. Colchicine and capsaicin pretreatments were performed as described above.

Plasma extravasation induced by topical application of capsaicin or xylene to skin

Capsaicin was applied topically to the shaven skin of one hind paw, with a paper patch that had been soaked with 50 µl of a 7.5 mg ml⁻¹ capsaicin solution in olive oil. The

corresponding area of the other hind paw, used as a control, was covered with a patch soaked with olive oil alone. Test drug and Evans blue were administered i.v. 5 min before capsaicin was applied. Thirty minutes later, the animals were killed and the skin under the patches was removed and weighed and its dye concentration was assayed. In the test of xylene-induced plasma extravasation, a mixture of meta-xylene/ethanol (1:1) was applied directly to the skin of one hind paw, and vehicle of xylene (10 µl of a mixture of 0.9%

Figure 1 Effects of RP 67580 on plasma extravasation in hind paw skin of rat induced by electrical stimulation of the saphenous nerve (a), by intravenous injection of substance P (b), and by topical application of xylene (c). In (a) RP 67580 or vehicle (control) was administered s.c. 30 min before the saphenous nerve was stimulated, and Evans blue extravasation in the hind paw skin area innervated by this nerve was determined. In (b) Evans blue extravasation in hind paw skin of rat was determined 5 min after simultaneous i.v. injection of RP 67580 and substance P (1 µg kg⁻¹, i.v.). In (c) RP 67580 and Evans blue were administered i.v., 5 min before the topical application of xylene or vehicle. Xylene or vehicle was applied to a hind paw skin area for 30 min before the Evans blue extravasation in this same skin area was determined. Data (mean ± s.e.mean, n = 6) are expressed as a percentage of the value obtained with the control (mean value of animals treated with vehicle of RP 67580). *P < 0.05; **P < 0.01; ***P < 0.001 (Student's *t* test). Open column: controls; hatched columns: RP 67580-treated.

NaCl solution/ethanol, 1:1) was applied to the skin of the other hind paw of hairless rats. The dye concentration in the skin was assayed as described above.

Nasal fluid hypersecretion

Hypersecretion of nasal fluid was induced according to the method described by Petersson *et al.* (1989). Dry strips of filter paper were weighed and inserted into the nasal cavities for 5 min. Upon removal, the strips were reweighed and the weight gain (i.e. the basal nasal secretion) was calculated (see below). Another pair of paper strips was prepared (one strip loaded with 25 µl of a 0.2% capsaicin solution in ethanol and the other one, with ethanol alone) and introduced into the nasal cavities for 5 min. Capsaicin-stimulated nasal secretion was considered to be that remaining after what was measured in the vehicle-treated contralateral nasal cavity had been subtracted. RP 67580 or its enantiomer, RP 68651, was administered i.v. after determination of basal nasal secretion and 5 min before capsaicin or vehicle application.

Materials

RP 67580 (3aR, 7aR) (7,7-diphenyl-2-(1-imino-2-(2-methoxyphenylethyl) perhydroisoindol-4-one), RP 68651 (3aS, 7aS) (7,7-diphenyl-2-(1-imino-2-(2-methoxyphenylethyl)perhydroisoindol-4-one), (±)-CP-96,345 (2RS, 3RS) (*cis*-2-(diphenylmethyl)-*N*-(2-methoxyphenyl)-methyl-1-azabicyclo(2.2.2)octan-3-amine) and PD-117302 (±)-(*trans*-*N*-methyl-*N*-(2-(1-pyrrolidinyl)-cyclohexyl)-benzo-(b)-thiophene-4-acetamide were obtained from the Department of Chemistry, Rhône-Poulenc Rorer (France). Morphine was purchased from Francopia (France). Naloxone, colchicine and capsaicin were purchased from Sigma (France). SP and septide ([*p* Glu⁶,Pro⁹]-SP(6–11)) which is a selective NK₁-receptor agonist (Laufer *et al.*, 1986) were purchased from Bachem (Switzerland). Lignocaine was from Laboratoires Roger Bellon (France).

Results

Plasma extravasation induced by electrical nerve stimulation (neurogenic inflammation)

Electrical stimulation of the rat saphenous nerve induced an intense Evans blue extravasation ($2.49 \pm 0.24 \mu\text{g}$ per 100 mg of tissue) in the hind paw skin innervated by this nerve (compared to the basal Evans blue extravasation which was equal to $0.07 \pm 0.003 \mu\text{g}$ per 100 mg of tissue). Used by i.v. route, (±)-CP-96,345 potentially reduced plasma extravasation ($\text{ID}_{50} = 0.6 \text{ mg kg}^{-1}$, i.v.), with a similar potency to RP 67580 ($\text{ID}_{50} = 0.15 \text{ mg kg}^{-1}$, i.v. as we previously found: Garret *et al.*, 1991). Given subcutaneously, RP 67580 was able to inhibit plasma extravasation in a dose-dependent manner ($\text{ID}_{50} = 0.3 \text{ mg kg}^{-1}$, s.c.) (Figure 1a).

Both μ - (morphine) and κ -(PD-117302) opioid receptor agonists inhibited neurogenic inflammation (Table 1). However, in contrast to the NK₁-receptor antagonist, RP 67580, neither of these opioid receptor agonists reduced the neurogenic inflammation by more than 80%, even when they were used at high doses of 10 mg kg^{-1} , s.c. (Table 1). The opioid receptor antagonist naloxone (10 mg kg^{-1} , s.c.) was without effect on neurogenic inflammation. However, it reversed the effect of the opioid agonists used here, but not that of RP 67580 (not shown).

Plasma extravasation was totally blocked by a saphenous nerve ligation distal to the site of stimulation, but was not blocked by a ligation proximal to this site. Local lignocaine application to the nerve before its stimulation also blocked the extravasation (Table 1). Colchicine treatment and neonatal capsaicin treatment also strongly reduced neurogenic plasma extravasation (Table 1).

Plasma extravasation induced by exogenous peptides

The intravenous injection ($1 \mu\text{g kg}^{-1}$, i.v.) of SP or septide produced an intense plasma extravasation in hind paw skin (3.03 ± 0.15 and $3.10 \pm 0.34 \mu\text{g}$ per 100 mg of tissue, respectively). The basal Evans blue extravasation was $0.08 \pm 0.01 \mu\text{g}$ per 100 mg of tissue.

Table 1 Effects of RP 67580, opioid receptor agonists, nerve ligature, lignocaine local application, and capsaicin and colchicine pretreatments on plasma extravasation induced by electrical nerve stimulation or by exogenous substance P

		Plasma extravasation elicited by:	
		Electrical nerve stimulation	Exogenous substance P
		(% of control)	(% of control)
RP 67580	1 mg kg^{-1} , s.c.	$41 \pm 8^{***}$	$9 \pm 3^{***}$
Morphine	1 mg kg^{-1} , s.c.	$69 \pm 4^{**}$	—
	10 mg kg^{-1} , s.c.	$26 \pm 4^{***}$	105 ± 6
PD 117302	1 mg kg^{-1} , s.c.	$64 \pm 7^{**}$	—
	10 mg kg^{-1} , s.c.	$52 \pm 9^{***}$	97 ± 8
Ligature of saphenous nerve		$8 \pm 7^{***}$	—
Local application of lignocaine to saphenous nerve		$7 \pm 1^{***}$	—
Neonatal capsaicin treatment (50 mg kg^{-1} , s.c.)		$5 \pm 1^{***}$	$143 \pm 7^{***}$
Colchicine	$120 \mu\text{g kg}^{-1} \text{ day}^{-1}$, i.p. for:		
	- 1 day	97 ± 26	—
	- 2 days	$62 \pm 14^*$	—
	- 3 days	$24 \pm 5^{***}$	89 ± 5

Data (mean $\pm$ s.e.mean of 6 values) are expressed as a percentage of the control value (mean value of non-treated animals). RP 67580 and opioid receptor agonists were administered s.c. 30 min before the saphenous nerve was stimulated or the intravenous injection of substance P. The saphenous nerve was ligated at the part distal to the stimulation. Lignocaine ($20 \mu\text{l}$ of a 2% solution) was directly applied to the saphenous nerve before the electrical stimulation. Capsaicin (50 mg kg^{-1}) was administered s.c. to two-day-old rats, and 2 months later, the rats were used for plasma extravasation studies. Colchicine ($120 \mu\text{g kg}^{-1} \text{ day}^{-1}$) was given i.p. for 1, 2 or 3 consecutive days. In all cases, Evans blue extravasation in hind paw skin innervated by the saphenous nerve was measured as described in the Methods section. Data (mean $\pm$ s.e.mean, $n = 6$) are expressed as a percentage of the control value (mean value of non-treated animals).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Student's *t* test).

RP 67580 reduced, in a dose-dependent manner, septide- or SP-induced plasma extravasation in the skin ($ID_{50} = 0.04$ and 0.05 mg kg^{-1} , i.v., respectively) (see Figure 1b for results obtained with SP). Given by s.c. route, RP 67580 was also able to reduce vascular permeability (Table 1). The effect of RP 67580 was stereospecific since its enantiomer, RP 68651 (1 mg kg^{-1} , i.v.), did not affect septide- and SP-induced plasma extravasation.

Neither opioid agonists nor colchicine affected plasma extravasation when it had been induced by SP, but they did inhibit it when it had been evoked by electrical nerve stimulation (see above and Table 1).

Neonatal capsaicin treatment led to an increase of SP-induced plasma extravasation (by $43 \pm 7.26\%$) (Table 1). RP 67580 at 1 mg kg^{-1} , i.v., blocked SP-induced plasma extravasation in capsaicin-treated animals ($96 \pm 1.5\%$ inhibition).

Capsaicin- or xylene-induced plasma extravasation

Local application of capsaicin or xylene to a cutaneous area of hind paw elicited an intense Evans blue extravasation ($5.64 \pm 0.61 \mu\text{g}$ per 100 mg of wet skin and $8.85 \pm 2.00 \mu\text{g}$ in the whole stained skin area, respectively), compared to the basal Evans blue extravasation induced by vehicle of capsaicin or xylene (0.10 ± 0.06 or $0.15 \pm 0.12 \mu\text{g}$, respectively). This chemically induced plasma extravasation was powerfully inhibited by RP 67580 ($ID_{50} = 0.06$ and 0.03 mg kg^{-1} , i.v. for capsaicin and xylene, respectively; see Figure 1c for xylene-induced plasma extravasation), while it was not affected by the enantiomer, RP 68651, used at 1 mg kg^{-1} , i.v.

Nasal fluid hypersecretion

The topical application of capsaicin to nasal cavities increased nasal fluid secretion from $4.51 \pm 0.36 \text{ mg}$ (vehicle-treated) to $7.81 \pm 0.77 \text{ mg}$ (capsaicin-treated). RP 67580 (1 mg kg^{-1} , i.v.) inhibited the nasal hypersecretion induced by capsaicin by 82% (Figure 2).

Figure 2 Effect of RP 67580 on capsaicin-induced nasal fluid hypersecretion. Nasal hypersecretion was induced by inserting into nasal cavities strips of filter paper soaked in a capsaicin solution ($25 \mu\text{l}$, 0.2% in ethanol). RP 67580 was given 5 min before capsaicin or vehicle application. Data (mean $\pm$ s.e.mean, $n = 6$) are expressed as a percentage of the control value (mean value of animals treated with vehicle of RP 67580). *** $P < 0.001$ (Student's t test). Open column: controls; hatched column: RP 67580-treated.

Discussion

The major findings in the present study are that the NK_1 -receptor antagonist, RP 67580 at low doses ($0.3 \pm 1 \text{ mg kg}^{-1}$), inhibits plasma extravasation induced in skin either by NK_1 -receptor agonists (SP and septide) or by chemical irritants (xylene or capsaicin applied topically). In addition, RP 67580 blocks capsaicin-elicited nasal fluid hypersecretion in the rat. Opioid receptor agonists and colchicine inhibit plasma extravasation induced by electrical nerve stimulation, but not that elicited by exogenous SP. All these results clearly indicate that NK_1 -receptor antagonists act at the post-synaptic level while opioid receptor agonists and colchicine act at the presynaptic site to inhibit neurogenic vascular permeability.

RP 67580, used in our experiments as an antagonist of NK_1 -receptors, has been described as highly selective for this receptor type (Garret *et al.*, 1991). Indeed, RP 67580 was found to act potently and competitively at NK_1 -receptors ($K_i = 2.9\text{--}3.3 \text{ nM}$), without interaction with NK_2 - and NK_3 -receptors and had no affinity for a variety of peptide receptors, nor with numerous other receptors of classical neurotransmitters, as shown in binding assays (Fardin *et al.*, 1992) or in *in vitro* functional studies (Carruette *et al.*, 1992). In contrast, $(\pm)$ -CP-96,345, a second non-peptide NK_1 -receptor antagonist, was recently reported to interact with calcium channels (Guard & Watling, 1992) and to present cardiovascular effects (Donnerer *et al.*, 1992) unrelated to actions on NK_1 -receptors. The fact that RP 67580 is highly selective for NK_1 -receptors without interaction with other receptors indicates that the inhibition of plasma extravasation we obtained when using this compound is due to selective blockade of NK_1 -receptors. Moreover, the effect of RP 67580 was found to be stereospecific, since its enantiomer (RP 68651) at a high dose of 1 mg kg^{-1} , i.v. was without effect on plasma extravasation induced by NK_1 -receptor agonists, capsaicin and xylene.

$(\pm)$ -CP-96,345 was slightly less active than RP 67580 in our experiments, because we used it as racemate (its inactive enantiomer was not available) while RP 67580 is the active (3aR, 7aR) enantiomer, RP 68651 (3aS, 7aS) being the inactive enantiomer. The difference in potency in inhibiting plasma extravasation between RP 67580 and $(\pm)$ -CP-96,345 found in the present work could also be explained by the lower affinity of $(\pm)$ -CP-96,345 than of RP 67580 for rat NK_1 -receptors as compared to those of other species such as rabbit and guinea-pig (Beresford *et al.*, 1991; Fardin *et al.*, 1992). Indeed, CP-96,345 was found to be 15 times less potent than RP 67580 for the inhibition of [^3H]-SP binding in subcortical structures of brain and in submaxillary gland of rat (Fardin *et al.*, 1992).

Our results, in accordance with other reports (Jessel & Iversen, 1977; Morton & Chahl, 1980; Barthó & Szolcsányi, 1981; Brodin *et al.*, 1983), confirm that the μ -opioid receptor agonist, morphine, inhibits neurogenic plasma extravasation at presynaptic level. Recently, κ -agonists have received particular attention owing to their potentially analgesic properties. Indeed, systemic administration of non-peptide opioid receptor agonists, such as PD-117302, induce antinociception (Leighton *et al.*, 1987). We showed that this κ -agonist, like morphine, was effective in inhibiting plasma extravasation induced by electrical nerve stimulation, but not that induced by exogenous SP (Table 1), suggesting that κ -agonists act through receptors located presynaptically on afferent fibres to inhibit tachykinin release. In our experiments, the blockade of neurogenic inflammation by both μ - and κ -agonists was not complete even when these substances were used at high doses. The reason for this is not clear: other substances, such as histamine, may increase tachykinin release when nerves are electrically stimulated (see Tani *et al.*, 1990 for review).

Furthermore, we have shown that colchicine inhibited plasma extravasation induced by electrical stimulation, but not that induced by exogenous SP (Table 1). This is of

interest because colchicine is very effective in various pathological inflammatory states in man, such as acute gouty attacks and familial Mediterranean fever (Famey, 1988; Zemer *et al.*, 1991). It is possible that SP contributes to the pain and inflammation that accompany these pathologies and that colchicine, by inhibiting axonal transport at the presynaptic level, indirectly reduces tachykinin release. Previous studies have shown SP to be present in C-fibres of nerves supplying small dermal vessels and sweat glands as well as in free nerve endings in the epidermis (Hökfelt *et al.*, 1975; Hartschuh *et al.*, 1983; Moussaoui *et al.*, 1992b). We demonstrated that neonatal capsaicin treatment leads to an increase of SP-induced plasma extravasation (see above). This suggests the existence of an up-regulation of NK₁-receptors in capsaicin-treated animals due to the chronic lack of tachykinin release from nerve terminals. However, other explanations such as effects of capsaicin on a second messenger system or on blood flow cannot be excluded. Our results also suggest that NK₁-receptors are probably not

located on terminals of C-fibres, since the destroying of these fibres by neonatal capsaicin treatment leads to an increase of SP-induced plasma extravasation.

Our results showing that RP 67580 is a potent inhibitor of plasma extravasation are in accordance with those of Lembeck *et al.* (1992) who demonstrated that CP-96,345, the other non-peptide NK₁-receptor antagonist, is also a potent blocker of plasma extravasation induced in rat by SP, mustard oil or electrical nerve stimulation.

In conclusion, non-peptide NK₁-receptor antagonists, such as RP 67580, inhibit vascular permeability in the skin and the nasal mucosa, and consequently, they could be of great potential for the treatment of dermatosis and rhinitis. They also could represent a new therapeutic approach to various other pathologies in which neurogenic inflammation often accompanies pain.

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