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In vitro and in vivo evidence for a tachykinin NK_1 receptor antagonist effect of vapreotide, an analgesic cyclic analog of somatostatin

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Abstract

Vapreotide, a long-acting somatostatin analog, possesses an analgesic effect. The purpose of this work was to determine a tachykinergic involvement. Vapreotide reduced substance P-induced biting and scratching in mice. This inhibitory effect of substance P action was confirmed by experiments performed on the bronchial apparatus of guinea-pigs known to possess tachykinin NK_1 and NK_2 receptors. (i) Vapreotide reduced the substance P-induced plasmatic exudation. (ii) It inhibited selectively the tachykinin-dependent second contractile phase induced by electrical field stimulation of isolated bronchi. (iii) It shifted to the right the concentration-effect curve of substance P-induced contraction of isolated main bronchi. The peptide displaced [^{3}H]substance P ($IC_{50} = 3.3 \pm 1.8 \times 10^{-7}$ M) from guinea-pig bronchial tachykinin NK_1 sites. The displacement of [^{125}I]neurokinin A, a specific tachykinin NK_2 receptor ligand, needed higher concentrations ($IC_{50} = 4.5 \pm 0.6 \times 10^{-6}$ M). It is concluded that vapreotide possesses an antagonist activity on guinea-pig tachykinin NK_1 receptors; the involvement in its analgesic action is discussed.

Keywords: Vapreotide; Somatostatin analog; Substance P; Tachykinin NK_1 receptor; Tachykinin NK_2 receptor; Airway; Binding

1. Introduction

An analgesic effect of somatostatin has been demonstrated in animals (Rezek et al., 1978) and also in humans after intravenous (Sicuteri et al., 1984), intrathecal (Chrubasik et al., 1984) or epidural (Chrubasik et al., 1985; Meynadier et al., 1985; Gennari et al., 1987) injection. However, the rapid degradation of somatostatin (plasma elimination half-life: 3 min (Kutz et al., 1986)) limits its therapeutic utility. Octreotide, its best known analog (Bauer et al., 1992), elicits the same response after subcutaneous (Williams et al., 1987; Wolfe and Cathey, 1990), intramuscular (Pincus et al., 1989), intravenous (Lamberts et al., 1985; Williams et al., 1986), intrathecal (Penn et al., 1990, 1992) or topical (Ellis, 1990) administration.

Vapreotide (RC-160) (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2), an analog of somatostatin synthesized by Cai et al. (1986), was shown to produce a very long-lasting central opioid-mediated antinociceptive effect in rodents (Eschalier et al., 1991; Bétoin et al., 1994a,b) after subcutaneous injection. Preliminary clinical case reports have confirmed this activity (Aumaître et al., 1991). Bétoin et al. (1994a) showed that the naloxone-reversible antinociceptive effect of vapreotide indirectly involved an opioidergic mechanism suggesting that other mechanisms might be involved.

The aim of this work was to examine the mechanism of this action, particularly for a tachykinergic involvement. First, we tested the effect of vapreotide on the intrathecal substance P-induced behavioral manifestations in mice (Hylden and Wilcox, 1981). Behaviours elicited by intrathecally administered substance P are inhibited by agents with spinal antinociceptive activity, such as opiates and noradrenergic agents (Hylden and

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Wilcox, 1983). Secondly, studies were performed on the bronchial smooth muscle, which is known to possess numerous tachykinin receptors (NK_1, NK_2) (Frossard and Advenier, 1991). And finally, the binding of vapreotide to tachykinin NK_1 receptors of rat brain and guinea-pig bronchi and to tachykinin NK_2 receptors of rat duodenum was determined.

2. Materials and methods

2.1. Behavioral studies

Animals

Male Swiss Webster mice (17–25 g) (Biolab, White Bear Lake, MN, USA) were used. There were ten mice per cage, who had free access to food and water and were acclimatized to the laboratory for at least 24 h before the experiments. Each series of experiments was carried out between 10:00 a.m. and 12:00 noon.

Injection procedure and protocol

Intrathecal injections of substance P or vapreotide were carried out in unanaesthetized mice at L-5, L-6 intervertebral space using 30-gauge 0.5-inch disposable needles with plastic Luer hubs (B-D Yale) mated to a 50-μl Hamilton syringe according to the method of Hylden and Wilcox (1980). Each needle was used for not more than six injections. All agents injected were diluted in physiological saline-acetic acid vehicle (Hall and Stewart, 1983) for intrathecal injection of 5-μl volumes over a 20–30 s period.

Each animal received a first intrathecal injection of vapreotide (8 nmol/mouse) or vehicle, then 5 min later, a second intrathecal injection of substance P (10 ng/mouse) or vehicle. Each animal was treated with one of the following four regimens: vehicle + vehicle, vapreotide + vehicle, vehicle + substance P, vapreotide + substance P. $n = 8$ in each group.

Behavioral measures

After substance P injection, mice were placed in a two-litre glass beaker and observed continuously for 1 min. The number of caudally directed licks and scratches together with the number of episodes of reciprocal hind limb scratching were counted by the same observer to maintain consistency.

2.2. Airway studies

Measurement of airway microvascular leakage

Vascular permeability was quantified by the extravasation of Evans blue dye (Evans et al., 1988; Rogers et al., 1989; Udaka et al., 1970). Tricoloured guinea-pigs weighing 250–350 g were anaesthetized with urethane (1.25 g/kg intraperitoneally). A jugular vein was cannulated for drug injections. At time 0, thiorphan (0.1 mg/kg i.v.) was injected, followed, 30 min later, by Evans blue dye (30 mg/kg i.v.). Vapreotide (8–800 nmol/kg i.v.) or saline (1 ml/kg) was injected 20 min before Evans blue dye. One minute after Evans blue, saline (1 ml/kg), substance P (3 μg/kg i.v.) or histamine (30 μg/kg i.v.) were injected; 5 min later the thorax was opened and a blunt-ended, 13-gauge needle passed through a left ventriculotomy into the aorta. The ventricles were cross-clamped and blood was expelled through an incision in the right atrium at 80 mm Hg pressure with about 100 ml saline, in order to remove the intravascular dye from the circulation until the perfusate was clear. The lungs were then removed. The connective tissues, vasculature, and parenchyma were gently straped, and the airways were divided into four components: lower part of trachea, main bronchi and proximal (the proximal 3 mm portion) and distal intrapulmonary airways (Rogers et al., 1989; Udaka et al., 1970). The tissues were blotted dry, placed in preweighed tubes and reweighed, and their dye content was extracted in formamide at 37° C for 18–24 h. Dye concentration was quantified from light absorbance at 620 nm (DCP spectrophotometer, Vial, Dieren, Netherlands) and its tissue content (ng dye/mg wet weight tissue) was calculated from a standard curve of dye concentrations in the 0.5–10 μg/ml range.

All animals ($n = 6$) were pretreated with thiorphan to block substance P metabolism (Ichinose and Barnes, 1990) and potentiate its effect. Time to remove tissues was chosen in agreement with Rogers et al. (1990) and Qian et al. (1993) who have shown that substance P and neurokinin A-induced leakage was maximal in all airways after 5 min and that these effects were specifically inhibited by CP-96,345, a tachykinin NK_1 receptor antagonist and SR 48968, a tachykinin NK_2 receptor antagonist, respectively.

Electrical field stimulation

Preparation of bronchial smooth muscle. Main bronchi rings were obtained from tricoloured guinea-pigs of either sex (250–350 g) anaesthetized with urethane (1.25 g/kg i.p.). The rings were equilibrated under an initial tension of 2.0 g in Krebs solution at 37° C gassed with 95% O_2-5% CO_2. After 1 h of equilibration, resting tension was between 1.5 and 2 g. Under these conditions, responses to agonists were reproducible over several hours. Tension was measured isometrically with Pioden Strain gauges (UF-1) and amplifiers (Dei Lierre Electronique, France) and displayed on recorders (Linseis L6512, France). The composition of the Krebs solution was (mM): NaCl 118.0; Kcl 5.4; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0 and glucose 11.7.

Transmural stimulation. Each organ bath was fitted with two platinum plate electrodes (1 cm^2) placed alongside the tissue (10 mm apart) for transmural electrical field stimulation (biphasic pulse with 1 ms, constant current of 320 mA for 10 s) (Szolcsanyi and Bartho, 1982; Undem et al., 1990; Martin et al., 1992). These stimulus parameters caused an optimal reproducible biphasic contraction which consisted of a fast contraction followed by a sustained contractile response, both abolished by tetrodotoxin. The first, fast, component is inhibited by atropine and results from stimulation of cholinergic nerves. The late and prolonged second phase is nonadrenergic noncholinergic (NANC) in nature, and is abolished or strongly reduced by antagonists of tachykinin NK_2 receptors, such as SR 48968 and MEN 10,207, and partially reduced by CP-96,345 (Lundberg et al., 1983; Maggi et al., 1991; Martin et al., 1992).

Protocol. After a 1 h resting period, bronchial rings were contracted to maximal tension with acetylcholine (3×10^{-3} M) and then allowed to equilibrate for 60 min while they were washed with Krebs solution every 15 min. The resting tension was adjusted to 1.5–2.0 g. In all experiments, propranolol (10^{-6} M) was added to the buffer solution at the start of the experiment to avoid the influence of adrenergic nerve stimulation, and indomethacin (10^{-6} M) to avoid indirect effects of prostaglandins on the neuronal responses (Linden et al., 1991; Maggi et al., 1991; Undem et al., 1990).

After tension had returned to the baseline tone, the preparation was stimulated every 10–15 min increasing the frequency (0.5, 1, 4, 8, 16 and 32 Hz), for 1 ms, and 320 mA for 10 s using a stimulator (Dei Lierre Electronique, France) where the voltage output was adjusted to give a constant current of 320 mA and a biphasic rectangular pulse of alternating polarity. These procedures were repeated in the absence or presence of vapreotide (10^{-7} to 10^{-4} M) which was administered 30 min before transmural stimulation. The results were expressed as percentages of the maximal contraction induced by acetylcholine (3×10^{-3} M), $n = 5$.

Effect of vapreotide on cumulative concentration-response curves for acetylcholine, [Nle^{10}]neurokinin A-(4–10) and substance P

A similar preparation of isolated bronchi was used. After obtaining a stable baseline tone cumulative doses of acetylcholine, [Nle^{10}]neurokinin A-(4–10) or substance P was added without any pretreatment or 30 min after vapreotide administration (10^{-7} to 10^{-5} M). Concentration-response curves were obtained by addition of these compounds every 5–10 min until a plateau response was attained. The results were expressed as percentages of the maximal concentration induced by acetylcholine (3×10^{-3} M), $n = 6$. We have previously shown that, under similar conditions, neurokinin A concentration-response curves were shifted to the right by SR 48968 whereas concentration-response curves of substance P were shifted to the right by CP-96,345 (Martin et al., 1992).

Drugs

The drugs used were: acetylcholine (Pharmacie Centrale des Hôpitaux, Paris, France); vapreotide (Debiopharm, Lausanne, Switzerland); indomethacin (Merck); propranolol, thiorphan, histamine, substance P (Sigma, St. Louis, MO, USA); [Nle^{10}]neurokinin A-(4–10) (Cambridge Research Biochemicals, UK). All drugs were dissolved in distilled water and then diluted in Krebs solution, except for indomethacin which was dissolved in ethanol and then diluted in Krebs solution. The maximal amount of ethanol used (0.4%) did not alter the response to acetylcholine.

Statistical analysis of results

Data are expressed as means ± S.E.M. Results were analysed by a Student's t-test. The significance level was $P < 0.05$.

2.3. Binding studies

Tritiated substance P (40–49 Ci/mmol) and iodinated neurokinin A (around 2000 Ci/mmol) were purchased from Amersham (France). All peptides were obtained from Bachem (Switzerland) except vapreotide, which was ordered from Debiopharm. Stock solutions of peptides were stored at −80° C in aliquots sufficient for a single experiment. Tachykinin NK_1 receptor binding studies were performed in rat brain and guinea-pig homogenates as previously reported (Fardin and Garret, 1991; Floch et al., 1994, respectively). Tachykinin NK_2 receptor binding assays were done with rat duodenum homogenates as described by Garret et al. (1991) except that [^{125}I]neurokinin A was used as the ligand instead of tritiated neurokinin A.

Preparation of tissues

For the tachykinin NK_1 receptor binding assays, whole brain (minus cerebral cortices and cerebellum) from male Sprague-Dawley rats (250–300 g, Charles River, France) and bronchi from male albino Dunklin-Hartley guinea-pigs (300–400 g, Charles River, France) were removed rapidly after decapitation of the animals and homogenized, using an Ultra-Turrax, in 15 volumes of ice-cold 50 mM tricine buffer (pH 7.4 at 20° C) containing glucose (1 g/l) and $MgCl_2$ (10 mM) (buffer 1). The homogenates were then centrifuged twice at $50\,000 \times g$ for 10 min with intermediate rehomogenization of the pellets in fresh buffer after the first centrifugation. The final pellets were resuspended in 5 vol-

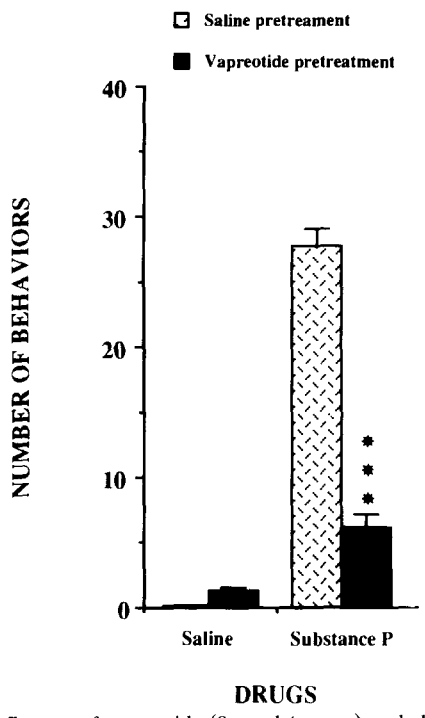

Fig. 1. Influence of vapreotide (8 nmol/mouse) on behavioral manifestations induced in mice by intrathecally injected substance P (10 ng/mouse). Pretreatment was performed, by intrathecal injection (5 μl/mouse), 5 min before injection of substance P or saline. The concentration of injected solution was 1.6×10^{-3} M. $n = 8$ in each group. $^{***}P < 0.01$ versus saline + substance P-treated group.

umes (w/v) of buffer, aliquoted and kept at −80° C until required.

For the tachykinin NK_2 receptor binding assays, duodenums were rapidly removed after decapitation of the rats which were used after an overnight fast. Tissues were rinsed and the mucosa scratched off. The homogenates were then treated in the same way as for the tachykinin NK_1 receptor binding assays, except that a Tris-HCl buffer (50 mM, pH 7.4 at 20° C) containing 0.05% bacitracin was used.

Binding assays

The peptides were freshly diluted from the stock solutions into ice-cold buffer containing bovine serum albumin 0.06%. They were tested for their ability to displace the binding of 0.3 nM [^{3}H]substance P (final concentration) according to the procedure of Lee et al. (1986) (slightly modified) or the binding of 0.1 nM [^{125}I]neurokinin A. For the tachykinin NK_1 receptor binding assays, the binding interaction was initiated by the addition of homogenates (1 ml) to 0.1 ml of the solution containing the ligand and 0.1 ml of binding buffer either alone (determination of total binding) or containing the competing drug (eight concentrations), in a final volume of 1.2 ml (final concentration of protein: 0.3 mg per ml) in buffer 1 containing bovine serum albumin (0.02%), bacitracin (40 mg/l), leupeptin (4 mg/l) and bestatin (5 mg/l). Kelatorphan (1 μM) was added to the buffer for assays in guinea-pig bronchi. Non-specific binding was defined in the presence of 1 μM substance P. For the tachykinin NK_2 receptor binding assays, aliquots (0.5 ml) of rat duodenum homogenates (final concentration of protein: 0.2 mg per ml) were added to 0.05 ml of the binding buffer either alone (for total binding) or containing the competing drug (eight concentrations) (final volume 0.6 ml). In these assays, the binding buffer was a Tris-HCl buffer (50 mM, pH 7.4 at 20° C) supplemented with bovine serum albumin (0.02%), $MnCl_2$ (3 mM), bacitracin (40 mg/l), leupeptin (4 mg/l) and hymostatin (4 mg/l). Non-specific binding was defined in the presence of 1 μM neurokinin A. Incubations were performed at 25° C for 20 min ([^{3}H]substance P) or 60 min ([^{125}I]neurokinin A). The binding interaction was terminated by rapid filtration (Skatron cell harvester) over Whatman GF/B glass fibre filters presoaked with 0.3% polyethyleneimine. Filters were immediately rinsed with ice-cold buffer and the radioactivity trapped on the filters was measured by liquid scintillometry in 5 ml of Ready-solv scintillant (Beckman). Individual displacement curves were analysed with the iterative curve-fitting program EBDA, using a non-linear regression procedure. Results are the means ± S.E.M. of at least 3 independent determinations, each one performed in duplicate.

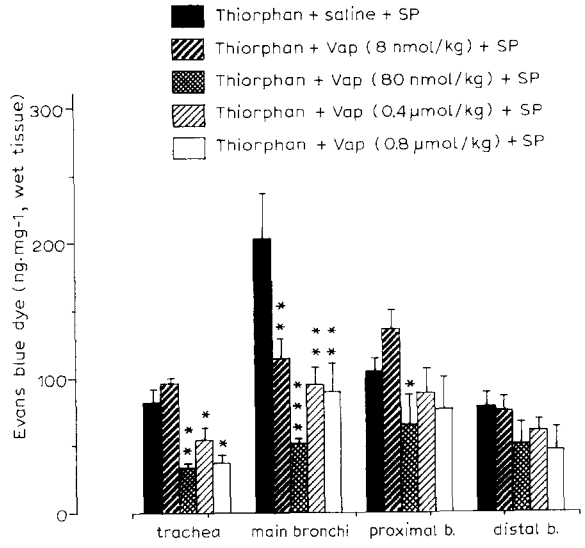

Fig. 2. Influence of vapreotide on substance P (3 μg/kg i.v.)-induced plasma exudation in guinea-pig airways. Columns represent the response to substance P (injected 1 min after Evans blue dye injection) after saline (1 ml/kg) or vapreotide (8–800 nmol/kg i.v.) injected 20 min before Evans blue dye injection. Concentrations of injected solutions were 8–800 μM. Experiments were performed in the presence of thiorphan (0.1 mg/kg i.v.). Means ± S.E.M. of six animals are shown. Significant differences from the saline + substance P-treated group were determined: $^{*}P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.001$.

3. Results

3.1. Behavioral studies

Animals treated only with saline did not develop any behavioral manifestations of biting and scratching while animals co-treated with vapreotide + saline or substance P + saline presented such behaviours (1.3 ± 0.2 and 27.6 ± 4.0 behaviours, respectively). Intrathecally administered vapreotide significantly inhibited substance P-induced behaviour (−78%) (Fig. 1).

3.2. Airway studies

Influence of vapreotide on the effects of substance P or histamine on vascular permeability

The substance P-induced increase in vascular permeability in trachea and main bronchi was partially but significantly inhibited by vapreotide at all the doses used (except for the 8 nmol/kg for trachea) (Fig. 2). Only one dose (80 nmol/kg) induced a significant effect in proximal bronchi; no significant effect was observed in distal bronchi. The same doses of vapreotide induced a weak decrease in vascular permeability of animals untreated by substance P (results not shown). Finally, histamine-induced increase in Evans blue extravasation was not modified by vapreotide (results not shown).

Effect of vapreotide on the biphasic contraction induced by electrical field stimulation of isolated guinea-pig main bronchi

Vapreotide did not influence significantly the first phase contraction (Fig. 3A) but dramatically reduced the second one for concentrations of 10^{-5} and 10^{-4} M (Fig. 3B). Lower concentrations were ineffective.

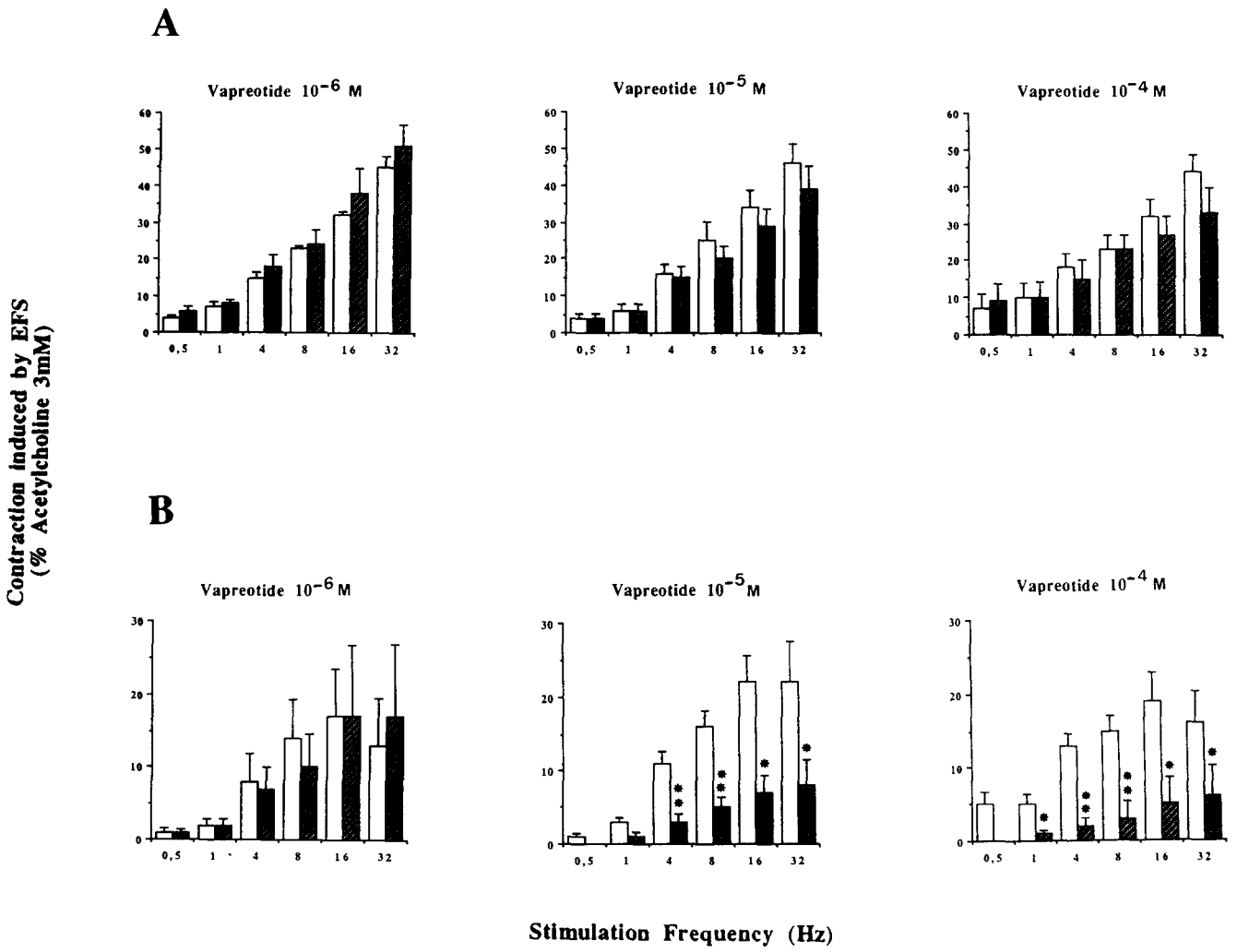

Fig. 3. Effect of vapreotide (10^{-6} to 10^{-4} M) effects on the first (A) and the second (B) contraction induced by electrical field stimulation (0.5, 1, 4, 8, 16 and 32, 1 ms, 320 mA for 10 s) of isolated main bronchi. The first contractile phase results from stimulation of cholinergic nerves, the second phase is NANC in nature. Columns (open: saline; hatched: vapreotide-treated group) represent contractions expressed as percent of the maximal contraction induced by acetylcholine (3 mM). Experiments were performed in the presence of propranolol (10^{-6} M) plus indomethacin (10^{-6} M). Means ± S.E.M. of five animals are shown. Significant differences from the saline-treated group were determined: $^{*}P < 0.05$, $^{**}P < 0.01$.

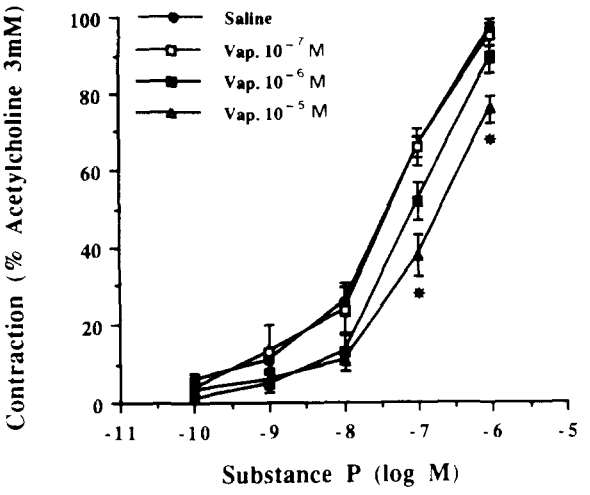

Fig. 4. Influence of vapreotide on substance P concentration-response curves on guinea-pig isolated main bronchi. Means ± S.E.M. of six animals are shown. Significant differences from the saline-treated group were determined: $^{*}P < 0.05$.

Effects of vapreotide on the concentration-response curves for acetylcholine, [Nle^{10}]neurokinin A-(4–10) and substance P in isolated guinea-pig main bronchi

Vapreotide concentration-dependently shifted the curves obtained with substance P slightly but significantly at 10^{-5} M to the right (Fig. 4). For low concentrations of substance P (e.g. 10^{-8} M, which gave 20% of contraction, i.e. the same level of contraction as that induced by electrical field stimulation), the decrease in the effect was approx. 50%. The concentration-response curves for [Nle^{10}]neurokinin A-(4–10) and acetylcholine were not modified (results not shown).

3.3. Binding studies

Increasing concentrations of vapreotide inhibited [^{3}H]substance P binding to rat brain membranes and guinea-pig bronchi. Half-maximum inhibitory concentrations (IC_{50}) were $2.1 \pm 1.5 \times 10^{-6}$ M and $3.3 \pm 1.8 \times 10^{-7}$ M, respectively. Concentrations of the peptide needed to inhibit [^{125}I]neurokinin A binding to rat duodenum were higher: $IC_{50} = 4.5 \pm 0.6 \times 10^{-6}$ M.

4. Discussion

All the results obtained in in vivo studies lead to the same conclusion: vapreotide reduces the effects of substance P. First, this reduction was demonstrated by its inhibition of the motor behaviour of substance P induced at the spinal level. The decrease in the score of this reaction, considered as the result of an excitation of spinal nociceptive neurons (Hylden and Wilcox, 1981) would suggest an antinociceptive potential of the peptide. However, this conclusion must be modulated by two data: Huang (1992) thinks that this behavioral motor component was not a nociceptive reaction and secondly vapreotide itself weakly induced biting and scratching when it was intrathecally injected. This last result is in line with the effect of somatostatin known to induce the same behaviour (Lembeck et al., 1981; Seybold et al., 1982; Fasmer and Post, 1983). Finally, whatever may be the true significance of biting and scratching behaviour, these results show that vapreotide is able to inhibit the effect of substance P.

This ability to reduce the effect of substance P was confirmed by the results obtained in guinea-pigs: vapreotide reduced the substance P-induced extravasation of Evans blue dye in trachea and main bronchi. It is important to note that the action of vapreotide was dose-dependent and plateaued for doses from 80 nmol/kg to 0.8 μmol/kg, a phenomenon which is in line with results obtained in pain tests (Bétoin et al., 1994a). Comparison of these results with others, obtained with agonists or antagonists of tachykinin NK_1 receptors, might suggest a tachykinin NK_1-dependent mechanism for vapreotide. Substance P (an agonist of tachykinin NK_1 receptors) has been demonstrated to be more potent than other tachykinins (agonists of tachykinin NK_2 receptors) in increasing bronchus vascular permeability (substance P > neurokinin A = neurokinin B) (Rogers et al., 1988). Tachykinin NK_1 receptor antagonists inhibit the neurogenic plasmatic exudation in the airways (Delay-Goyet and Lundberg, 1991; Qian et al., 1993 and Garret et al., 1991 for CP-96,345 and RP-67580, respectively). Like capsaicin (which induces neuronal depletion of substance P), CP-96,345 inhibits the increase of vascular permeability due to bradykinin (Qian et al., 1993).

This hypothesis that vapreotide acts on the tachykinin NK_1 receptor was confirmed by all the other results obtained after in vitro studies. Vapreotide inhibited selectively the second contractile phase induced by electrical field stimulation of isolated bronchi. This second phase has been shown to be due to tachykinin release with a mixed involvement of substance P and neurokinin A while the first phase involves a cholinergic component (Barnes, 1992; Martin et al., 1992). This effect of vapreotide was qualitatively similar to that obtained with a lower concentration of the tachykinin NK_1 receptor antagonist, CP-96,345 (Martin et al., 1992). This observed similarity would suggest a tachykinin NK_1 antagonist activity for vapreotide, though its effect could also have been obtained by blocking tachykinin NK_2 receptors. The possibility of an interaction with tachykinin NK_2 receptors was excluded by the inability of vapreotide to induce any change in the [Nle^{10}]neurokinin A-(4–10)-induced contraction of guinea-pig isolated bronchi, a result in line with the demonstrated low ability of vapreotide to displace a tachykinin NK_2 receptor lig-

and. In contrast, both binding and in vitro studies performed with guinea-pig bronchi demonstrated an interaction of vapreotide with tachykinin NK_1 receptors. Vapreotide displaced [^{3}H]substance P with an IC_{50} of $3.3 \pm 1.8 \times 10^{-7}$ M (on guinea-pig bronchi), higher than that obtained with CP-96,345 (3.4 ± 0.8 nM, Snider et al., 1991). The peptide exerts an antagonist effect on these receptors as demonstrated by the concentration-dependent inhibition of substance P-induced contraction of isolated main bronchi. Interestingly, the first concentration which induced a significant inhibition (10^{-5} M) was the same as the first active concentration during the second contractile phase induced by the electrical field stimulation of isolated bronchi. Moreover, this value, like the doses effective in in vivo studies (corresponding to concentrations from 8×10^{-6} to 8×10^{-4} M of the injected solutions), was compatible with the obtained IC_{50} value. Thus, it can be concluded that the effects observed in guinea-pigs are due to an antagonist activity of vapreotide on tachykinin NK_1 receptors.

Taking into account that tachykinin NK_1 receptors are involved in nociceptive transmission at the dorsal horn of the spinal cord (Sandberg and Iversen, 1982; Besson and Chaouch, 1987) and that vapreotide was shown both to cross the blood-brain barrier (Banks et al., 1990) and to possess a central antinociceptive effect (Bétoin et al., 1994a), it can be postulated that this last property of the peptide might, at least in part, be due to its antagonist activity on tachykinin NK_1 receptors. This mechanism might explain the inhibition by vapreotide of the substance P-induced motor behavior at the spinal level in mice. However, high concentrations of vapreotide were needed to inhibit [^{3}H]substance P binding to rat brain membranes ($IC_{50} = 2.1 \pm 1.5 \times 10^{-6}$ M) suggesting a low affinity which does not seem to be able to account for an antinociceptive action based on a direct antagonist effect of the peptide on tachykinin NK_1 receptors in healthy rats or mice (the tachykinin NK_1 receptors are similar in the two species (Beresford et al., 1991; Fardin et al., 1992)). However, in humans, who possess the same tachykinin NK_1 receptors as guinea-pigs (Beresford et al., 1991; Fardin et al., 1992, 1993; Gorbulev et al., 1992), a better affinity of vapreotide for these receptors can be expected and the antagonist effect of the peptide might participate in its analgesic activity (Aumaître et al., 1991).

It is interesting to note that the antinociceptive effect of vapreotide was suppressed by naloxone (Bétoin et al., 1994a,b) which suggests that this peptide could stimulate inhibiting controls of pain and inhibit, directly or indirectly, substance P-dependent pronociceptive mechanisms. Such a profile suggests a possible therapeutic interest in various pain syndromes. Its prolonged duration of action (Eschalier et al., 1991; Bétoin et al., 1994a,b) and the suspected increased involvement of central substance P-mediated transmission of nociception in hyperalgesic diabetic animals (Courteix et al., 1993) would suggest a particular potential in the treatment of chronic neuropathic pain. Furthermore, the results obtained here on airways might be of interest taking into account that some inflammatory processes with plasmatic exudation are involved in airway pathologies (Chung et al., 1990; Pearson, 1991).

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