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In vitro and in vivo pharmacology of S 16474, a novel dual tachykinin NK_1 and NK_2 receptor antagonist

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Abstract

Since tachykinins released from lung sensory nerve endings are thought to play a role in inflammatory diseases of airways via NK_1 and NK_2 receptors, dual tachykinin NK_1 and NK_2 receptor antagonists may have a great therapeutic potential. In vitro, the cyclopeptide S 16474 (cyclo-[Abo-Asp(D-Trp(Suc0Na)-Phe-N-(Me)Bzl)]) bound to both human tachykinin NK_1 and NK_2 receptors expressed in two lines of transfected Chinese hamster ovary cells (IC_{50} values 85 nM and 129 nM, respectively), while showing a poor affinity for the rat tachykinin NK_1 receptor. S 16474 inhibited the contractions induced by substance P in isolated rabbit vena cava (pA_2 7.0) and by neurokinin A in rabbit pulmonary artery (pA_2 5.6). In vivo in anaesthetized guinea-pigs, S 16474 was found to dose dependently inhibit the bronchoconstrictions induced by intravenously administered substance P, neurokinin A and capsaicin. Plasma extravasation evoked in bronchi by endogenously released tachykinins under vagus nerve stimulation was abolished by S 16474 (10 μmol/kg i.v.). These results demonstrate clearly that S 16474 is a tachykinin receptor antagonist exhibiting, in vitro and in vivo, a dual inhibitory effect on NK_1 and NK_2 receptors.

Keywords: Bronchoconstriction; Airway neurogenic inflammation; Substance P; Neurokinin A; Capsaicin

1. Introduction

The release of tachykinins from lung sensory nerve endings identified as C-fibers has been implicated in non-adrenergic non-cholinergic (NANC) bronchoconstriction, neurogenic mucosal plasma extravasation and mucus hypersecretion in the airways (Lundberg et al., 1983; Barnes et al., 1991). Since these effects may be important in inflammatory diseases of airways such as asthma, the discovery of tachykinin receptors antagonists is a widely pursued research objective.

Receptors for the mammalian tachykinins, substance P, neurokinin A and neurokinin B, have been classified on pharmacological criteria into three subtypes (Regoli et al., 1987), termed NK_1, NK_2 and NK_3, which have been subsequently cloned and sequenced (Masu et al., 1987; Yokota et al., 1989; Hershey and Krause, 1990; Shigemoto et al., 1990). Although substance P, neurokinin A and neurokinin B share a common carboxy-terminal sequence (FXGLM-NH2) and bind to all three receptor subtypes, substance P binds most strongly to the tachykinin NK_1 receptors, whereas neurokinin A and neurokinin B preferentially bind to tachykinin NK_2 and NK_3 receptors, respectively. Since NANC bronchoconstriction is mostly mediated by tachykinin NK_2 receptors (Advenier et al., 1987) and neurogenic plasma extravasation and mucus hypersecretion are induced by activation of tachykinin NK_1 receptors (Rogers et al., 1988), dual antagonists for both NK_1 and NK_2 receptors may be of interest.

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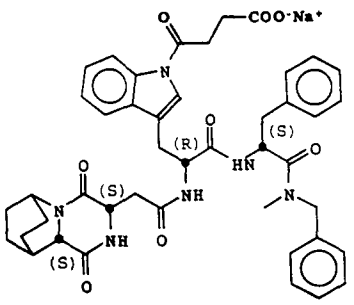

Fig. 1. Structural formula of S 16474 (cyclo-[Abo-Asp(D-Trp (Suc0Na)-Phe-N-(Me)Bzl)]). Abo = 2-azabicyclo[2.2.2]octane-3(*S*)-carboxylic acid.

In an attempt to design new tachykinin receptors antagonists, we developed series of low-molecular-weight peptides derived from the *C*-terminal sequence of substance P (Kucharczyk et al., 1993). The lead compound, S 16474 (cyclo-[Abo-Asp(D-Trp(Suc0Na)-Phe-N-(Me)Bzl)] with Abo = 2-azabicyclo[2.2.2]octane-3(*S*)-carboxylic acid), is a partially cyclized, stable and water-soluble pseudo-tetrapeptide (Fig. 1) displaying mixed tachykinin NK_1 and NK_2 receptors antagonistic activity.

In this report, we describe the in vitro and in vivo pharmacological characteristics of this new dual antagonist.

2. Materials and methods

2.1. Binding assays

Radioligand binding assays were performed with stably transfected Chinese hamster ovary (CHO) cells which expressed either recombinant rat tachykinin NK_1 (CHO-rNK_1R) or NK_2 (CHO-rNK_2R) receptors generated from Sprague-Dawley rat cerebella and urinary bladder respectively, or human tachykinin NK_1 (CHO-hNK_1R) or NK_2 (CHO-hNK_2R) or NK_3 (CHO-hNK_3R) receptors, as described by Takeda et al. (1991, 1992) and Guard et al. (1990). The cells were incubated for 2 h (to equilibrium) at 4°C with [^{125}I]Tyr^1-substance P (0.1 nM) or ^{125}I-His-neurokinin A (0.1 nM) to label tachykinin NK_1 and NK_2 sites, respectively and for 1 h at 22°C with [^{3}H]senktide (5 nM) to label NK_3 sites. Non-specific binding was defined by addition of 1 μM (NK_1 and NK_2 sites) or 10 μM (NK_3 sites) tachykinin. CHO-rNK_1R and CHO-hNK_1R cells contained high affinity binding sites with K_d of 1.5 and 1.7 nM, respectively. With CHO-rNK_2R and CHO-hNK_2R cells K_d values of 2.1 and 1.7 nM were observed, while CHO-hNK_3R cells showed binding sites with K_d of 2.8 nM. The potency of defined concentrations (3-fold dilutions) of S 16474 to displace labeled tachykinins from their binding sites was assessed with a filtration assay in independent experiments. Binding data were processed as IC_{50} values.

2.2. Bioassays

Rabbits (Albino, New-Zealand 1.5–2.0 kg) were sacrificed by stunning and exsanguination. Rabbit vena cava (for tachykinin NK_1 receptor preparation) and rabbit pulmonary artery without endothelium (for tachykinin NK_2 receptor preparation) were rapidly dissected away, prepared as strips according to Nantel et al. (1990) and D'Orleans-Juste et al. (1985), and suspended in Krebs solution and gassed with 95% O_2 and 5% CO_2 at 37°C. Tissues were stretched to a passive tension of 0.5 g (vena cava) and 1 g (pulmonary artery) and allowed to stabilize for a period of 60–90 min before measuring the contractile effect of the standard agonists acetyl-[Arg^6,Sar^9,Met(O$_2$)^{11}]substance P-(6–11) for the tachykinin NK_1 sites of the vein and [β-Ala^8]neurokinin A-(4–10) for the tachykinin NK_2 sites of the artery. Contractions were measured isometrically with Grass transducers (FT03C) and recorded on a Grass polygraph (model 7D). Drugs to be assayed were added to the bath 10 min before inducing response to cumulative concentrations of the appropriate agonist. Experiments were performed in parallel in rings from the same tissue. Only one agonist concentration-response curve was performed on a single ring. Apparent affinities (pA_2) and competitive nature of the antagonist were evaluated by Schild plot method.

2.3. Mast cell degranulation

2.3.1. Rat peritoneal mast cells

Male Sprague-Dawley rats (350–400 g, Charles River) were sacrified by CO_2 asphyxiation and mast cells were obtained by rinsing the peritoneal cavity as described by Johnson and Moran (1966). Pooled washing cells were centrifuged at $400 \times g$ for 10 min at 4°C and resuspended in buffer to a density of 2×10^4 cells/ml. After a 10 min incubation at 37°C, S 16474 was added to the cell suspension and incubation was carried on for 10 min in order to allow histamine release. The reaction was then quenched by placing the tubes in ice. The cell and supernatant fractions were separated by centrifugation ($700 \times g$ for 10 min at 4°C) and assayed fluorometrically for histamine after condensation with *o*-phthalaldehyde. The amount of histamine released from mast cells into the supernatant was expressed as a percentage of the total histamine content.

2.3.2. Human lung mast cells

Human lung parenchyma, obtained from patients subjected to surgery for various diseases, was chopped with scissors into small pieces (8 mm^3). Lung fragments

(400 mg) were incubated with 4 ml of Tyrode buffer (pH 7.8) for 10 min at 37°C under a 95% O_2, 5% CO_2 gas mixture. Then S 16474 was added and incubated for 30 min. The incubation media were centrifuged and supernatants were collected for histamine content determination (radioimmunoassay; Immunotech Int. Marseille, France). Histamine release was expressed as a percentage of total lung fragments histamine content after perchloric acid extraction.

2.4. Substance P-, neurokinin A- and capsaicin-induced bronchoconstriction

Male Hartley guinea-pigs (360–460 g, Charles River) were anaesthetized with urethane (1.5 g/kg i.p.) and the trachea, left jugular vein and right carotid artery were cannulated. Body temperature was maintained at 37 ± 1°C using a blanket control unit (Harvard). The animal was attached to a respiratory pump (Apelex), artificially ventilated (60 breaths/min, tidal volume 10 ml/kg), and curarized (gallamine triethiodide 2 mg/kg i.v.) to prevent interference from spontaneous respiration.

Pulmonary inflation pressure was recorded on a breath-by-breath basis using a Statham pressure transducer connected to a side arm of the tracheal cannula. Carotid blood pressure was measured with a similar transducer. Both transducers were connected to amplifiers (Gould) and to a recorder (Gould).

All animals were pretreated with mepyramine (1 mg/kg i.v.) to block the effects of histamine release that may be induced by tachykinins. In substance P-induced bronchoconstriction studies, propranolol (1 mg/kg i.v.) was also given. Substance P (2 nmol/kg i.v.) or neurokinin A (1.5 nmol/kg i.v.) was injected 15 min before intravenous treatment with either saline or S 16474 (1–10 μmol/kg). Responses to respective agonists were then examined 3 min, 20 min and 40 min following drug treatment. The effects of a specific tachykinin NK_2 receptor antagonist (SR48968, 0.1–1.0 μmol/kg i.v.) and of a specific tachykinin NK_1 receptor antagonist (CP99,994, 0.05–1.00 μmol/kg i.v.) were also studied under the same conditions. Capsaicin (10 μg/kg i.v.) was administered 30 min before treatment with either saline (control group), or SR48968 (1 μmol/kg i.v.), or CP99,994 (1 μmol/kg i.v.), or both antagonists (SR48968, 1 μmol/kg i.v. and CP99,994, 1 μmol/kg i.v.) or S 16474 (10–20 μmol/kg i.v.). Capsaicin was reinjected 3 min later.

Responses to agonists were expressed as increases in pulmonary inflation pressure. For each group, the results were presented as percentages of the mean response in the control group at the same time (mean ± S.E.M., $n = 5$). An analysis of variance and, if a significant difference was found among groups, a Newman-Keuls' test, were performed. Significance level was set at $P < 0.05$.

2.5. Vagus nerve stimulation-induced neurogenic plasma extravasation

Male Hartley guinea-pigs (360–460 g, Charles River) were anaesthetized and mechanically ventilated as described above. Left jugular vein and right carotid artery were cannulated for administration of drugs and monitoring of systemic arterial pressure, respectively. Both vagus nerves were carefully dissected free and sectioned. All animals were pretreated with atropine (1 mg/kg i.v.) to block muscarinic receptors. After a 15 min rest period, the animals were injected i.v. with S 16474 (10 μmol/kg) or saline (time 0), and then, 3 min later, with Evans blue dye (30 mg/kg). The peripheral ends of vagus nerves were positioned over small electrodes and, at $t = 4$ min, the nerves were stimulated (10V, 5 Hz, 5 ms duration) for 3 min (S88 stimulator, Grass). At $t = 9$ min, the animals were disconnected from the ventilator. The chest was opened and a cannula was inserted into the aorta through the left ventricle. Intravascular dye was eliminated using a perfusion through this cannula with 100 ml saline at a pressure of 80 mm Hg. Main bronchi were then excised, cleaned, blotted, weighed, placed in 2 ml formamide and incubated at 37°C for 16 h. Absorption at 620 nm was measured in a spectrophotometer (PU 8740 UNICAM).

The results were expressed as ng Evans blue/mg wet tissue (mean ± S.E.M., $n = 6$) and were analysed using a two-ways analysis of variance, with significance levels set at $P < 0.05$.

2.6. Substance P-induced salivary secretion in rats

Male Sprague-Dawley rats (200–225 g, Charles River) were anaesthetized with sodium pentobarbital (40 mg/kg i.p.), placed on a thermal pad to maintain body temperature, artificially ventilated (80 breaths/min, tidal volume 10 ml/kg, Apelex Respiratory pump) and pretreated with mepyramine (1 mg/kg i.v.). Animals were injected i.v. with S 16474 (10 μmol/kg) or saline, and 5 min later a single injection of substance P (2 nmol/kg in a volume of 1 ml/kg) was given intravenously in the tail vein. Salivation was measured by placing cotton swabs in the rat's mouth and quantitating the amount of saliva secreted by the difference in the weight of the cotton swab before and after the 5 min collection period. Results were expressed as means ± S.E.M. ($n = 6$).

3. Results

3.1. Binding assays

Binding assays on human tachykinin NK_1 and NK_2 receptors revealed that S 16474 binds specifically to both receptors with IC_{50} values of 85 ± 15 nM and

129 ± 13 nM, respectively. However, S 16474 exhibited 170-fold higher affinity for the human tachykinin NK_1 receptor than for the rat NK_1 receptor (IC_{50} value for the rat of 14450 ± 1450 nM). Such a species selectivity was not observed for tachykinin NK_2 receptors since S 16474 showed only a 3-fold higher affinity for the human NK_2 receptor than for the rat NK_2 receptor (IC_{50} value of 388 ± 30 nM for rat NK_2 receptor).

S 16474 bound to human tachykinin NK_3 receptor with an IC_{50} value of about 3000 nM, much higher than IC_{50} values for human NK_1 and NK_2 receptors.

S 16474 showed no appreciable affinity ($IC_{50} > 10$ μM) for dopamine (D_1, D_2), noradrenaline (α_1, α_2, β), serotonin (5-HT_1, 5-HT_2, 5-HT_3), histamine (H_1, H_2), acetylcholine (muscarinic, nicotinic), opiate (μ), glutamate receptors and verapamil recognition sites (data not shown).

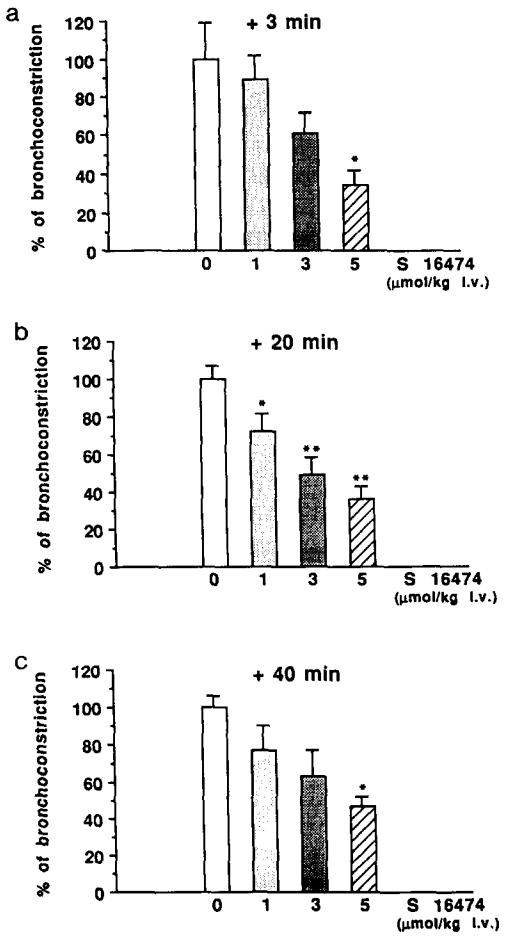

Fig. 2. Effects of S 16474 on substance P-induced bronchoconstriction in guinea-pigs. S 16474 was given i.v. (a) 3 min, (b) 20 min and (c) 40 min prior to substance P (2 nmol/kg i.v.). Data (means ± S.E.M., $n = 5$) are expressed in percent of the control values (mean value of five animals treated with vehicle). $^*P < 0.05$, $^{**}P < 0.01$ compared with the Newman-Keuls' test.

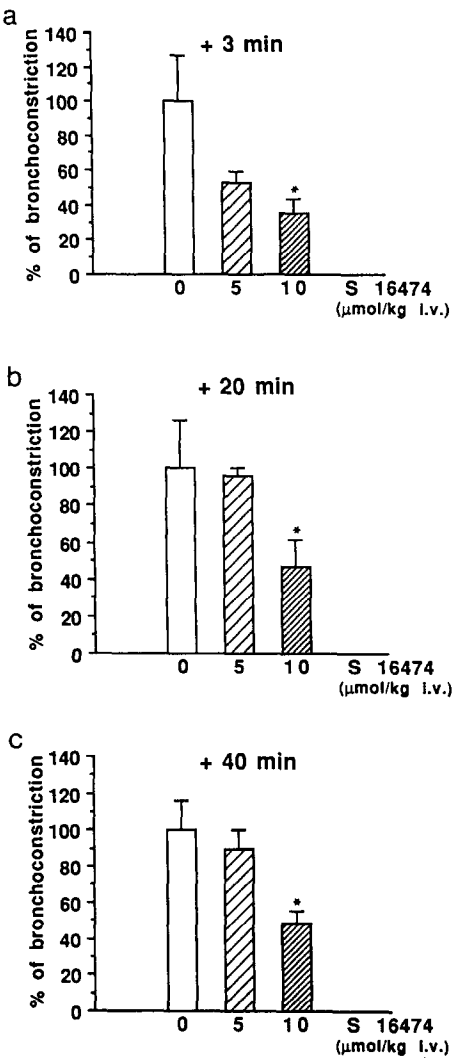

Fig. 3. Effects of S 16474 on neurokinin A-induced bronchoconstriction in guinea-pigs. S 16474 was given i.v. (a) 3 min, (b) 20 min and (c) 40 min prior to neurokinin A (1.5 nmol/kg i.v.). Data (means ± S.E.M., $n = 5$) are expressed in percent of the control values (mean value of five animals treated with vehicle). $^*P < 0.05$ compared with the Newman-Keuls' test.

3.2. Bioassays

In bioassays experiments, S 16474 dose dependently inhibited selective agonist-induced contractions of rabbit vena cava (tachykinin NK_1 receptor preparation) and rabbit pulmonary artery (tachykinin NK_2 receptor preparation). The pA_2 values were 7.03 ± 0.7 on the tachykinin NK_1 selective agonist-induced contraction of rabbit vena cava and 5.6 ± 0.4 on the myotropic effect of [β-Ala^8]neurokinin A-(4–10) on the rabbit pulmonary artery.

3.3. Mast cell degranulation

S 16474 was tested for its ability to promote degranulation of rat peritoneal mast cells and human lung

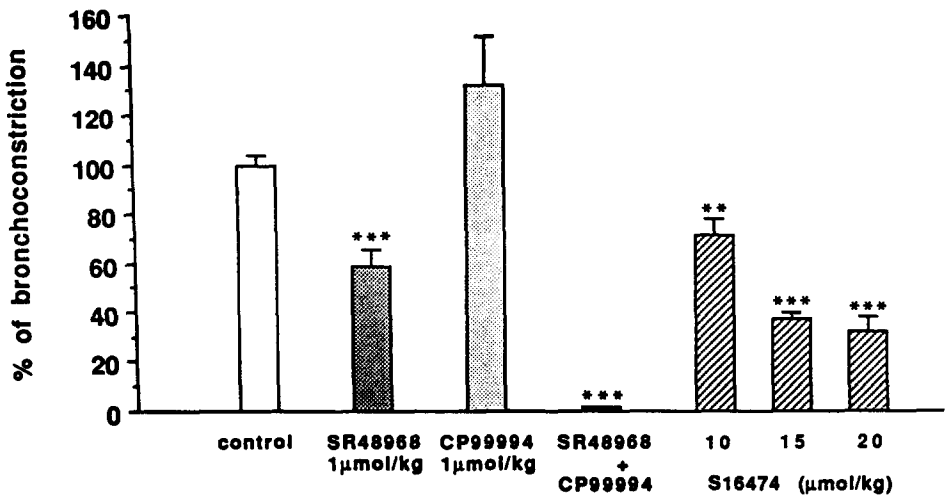

Fig. 4. Effects of SR48968 (1 μmol/kg i.v.), CP99,994 (1 μmol/kg i.v.), the combination of SR48968 and of CP99,994 (1 μmol/kg i.v. of each antagonist), and S 16474 (10, 15 or 20 μmol/kg i.v.) on capsaicin-induced bronchoconstriction in guinea-pigs. All drugs were given 3 min prior to capsaicin (10 μg/kg i.v.). Data (means ± S.E.M., $n = 5$) are expressed in percent of the control values (mean value of five animals treated with vehicle). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the Newman-Keuls' test.

mast cells. No degranulation in either mast cell preparations could be detected with S 16474 when applied in concentrations up to 100 μM.

3.4. Substance P- and neurokinin A-induced bronchoconstrictions in guinea-pigs

The effects of S 16474 (0, 1, 3, 5 μmol/kg i.v.) on substance P-induced bronchoconstriction were investigated in four groups of animals ($n = 5$ in each group). Results obtained at 3 min, 20 min and 40 min after treatment are reported in Fig. 2. S 16474 was found to significantly inhibit responses to substance P with an ID_{50} of approximately 4 μmol/kg. An inhibition was still present 40 min after treatment with S 16474 (10 μmol/kg). In addition, as expected from in vitro results, S 16474 also dose dependently inhibited neurokinin A-induced bronchoconstriction with an ID_{50} of approximately 7 μmol/kg (Fig. 3). CP99,994 inhibited responses to substance P with an ID_{50} of approximately 0.05 μmol/kg (i.v.), but at 1 μmol/kg, failed to reduce response to neurokinin A (% of bronchoconstriction at 3 min: 89 ± 4). Similarly, SR48968 inhibited neurokinin A-induced bronchoconstriction with an ID_{50} of approximately 0.1 μmol/kg (i.v.) and, at 1 μmol/kg, did not significantly change responses to substance P (% of bronchoconstriction at 3 min: 74 ± 7).

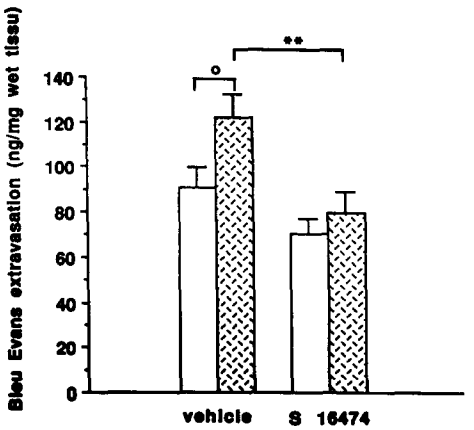

Fig. 5. Effects of S 16474 (10 μmol/kg i.v.) on plasma extravasation induced by electrical stimulation of the vagus nerve, in main bronchi of guinea-pigs. Results are expressed as means ± S.E.M. ($n = 6$). Open columns: sham-stimulated groups; dotted columns: electrical stimulated groups. Different ($P < 0.05$) from sham-stimulated group, **different ($P < 0.01$) from vehicle-treated group.

3.5. Capsaicin-induced bronchoconstriction and vagus nerve stimulation-induced neurogenic plasma extravasation in guinea-pigs

In order to determine the efficiency of S 16474 on the effects of endogenously released tachykinins, we studied its activity in capsaicin-induced bronchoconstriction, and in neurogenic plasma extravasation induced in the bronchi by vagus nerve stimulation.

As shown in Fig. 4, S 16474 (10–20 μmol/kg i.v.) strongly and dose dependently reduced capsaicin-induced bronchoconstriction. In our experimental conditions, only 40% of the capsaicin-induced response was inhibited by SR48968 (1 μmol/kg i.v.) while CP99,994 (1 μmol/kg i.v.) was inactive; the association of both antagonists at the same doses suppressed bronchoconstriction completely.

The effect of S 16474 on the plasma extravasation induced by vagus nerve stimulation was tested using

four groups of guinea-pigs (n = 6 per group), treated as follows: control solution and sham stimulation, S 16474 (10 μmol/kg i.v.) and sham stimulation, control solution and vagal stimulation, S 16474 (10 μmol/kg i.v.) and vagal stimulation. Vagal stimulation evoked in main bronchi a significant plasma extravasation, as shown by an increase in Evans blue dye in the tissue in the stimulated control group, compared to sham-stimulated controls (Fig. 5). S 16474 (10 μmol/kg i.v.) totally abolished the extravasation due to vagal stimulation (Fig. 5).

3.6. Substance P-induced salivation in rats

To confirm, in vivo, the species specificity for the human with respect to the rat tachykinin NK_1 receptor previously shown in binding assays, S 16474 was tested for antagonism of the sialogogic response to substance P in the rat. As expected from in vitro data, S 16474 (10 μmol/kg i.v.) was devoid of any significant inhibiting activity (amount of saliva secreted: 442.5 ± 34.1 mg vs. 531.9 ± 41.7 mg in control group).

4. Discussion

Our present data demonstrate that S 16474 is a novel water-soluble cyclopeptide tachykinin antagonist which interacts with both NK_1 and NK_2 receptors. This compound does not behave as a partial agonist, nor does induce histamine release from rat peritoneal or human lung mast cells in contrast to some previously described peptidic substance P analogs (Lowman et al., 1988). Furthermore, S 16474 is a selective antagonist for tachykinin NK_1 and NK_2 receptors, having a poor affinity for human tachykinin NK_3 receptors and showing no appreciable affinity ($IC_{50} > 10$ μM) for the other assayed receptors.

According to binding assays, the affinities of S 16474 for human NK_1 and NK_2 receptors are very close (IC_{50} values: 85 ± 15 and 129 ± 13 nM, respectively). In guinea pigs in vivo, substance P- and neurokinin A-induced bronchoconstriction were inhibited by S 16474 with ID_{50} of 4 and 7 μmol/kg i.v., respectively. S 16474 thus appears to be a real bipotent antagonist. However, results of bioassays – conducted in rabbit vessels – showed a difference in pA_2 values (7.0 ± 0.7 and 5.6 ± 0.4 on NK_1 and NK_2 preparations, respectively). We ignore the reasons of this discrepancy, but a pharmacological difference between tachykinin NK_2 receptors present in rabbit vessels and in human tissues has already been observed by Maggi et al. (1992).

With regard to species differences for tachykinin NK_1 receptors, S 16474 shows approximately 170-fold higher affinity for the human than for the rat NK_1 receptor. Furthermore, as expected from in vitro results, S 16474 also exhibits an in vivo selectivity, as shown by its lack of effectiveness in inhibiting substance P-induced salivary secretion in rats, while being active on substance P-induced bronchoconstriction in the guinea-pig, a species in which tachykinin NK_1 receptor antagonists are as active as in man. Heterogeneity of the tachykinin NK_1 receptor between species has been previously suggested on the basis of different affinities observed with non-peptide antagonists of the tachykinin receptors as opposed to the similar affinities for the natural peptide ligands, i.e. CP96,345 shows selectivity for the human NK_1 receptor, whereas RP67580 is selective for the rat NK_1 receptor (Snider et al., 1991; Fardin et al., 1993; Fong, 1992). Since species selectivity has been shown for CP96,345 and FK888 to depend critically on the same amino acid residues – namely residues 116 and 290 in transmembrane segments TMIII and TMVII respectively (Jensen et al., 1994) –, it would be of interest to know if this holds true for S 16474 which possesses an even more remarkable species selectivity than CP96,345 and FK888 (170-fold, 22-fold and 76-fold higher affinity for the human tachykinin NK_1 receptor respectively as compared to the rat NK_1 receptor).

Although S 16474 is less potent than some specific tachykinin NK_1 or NK_2 receptor antagonists, we found that, in vivo, S 16474 inhibits airway constriction induced by either substance P or neurokinin A. In contrast, CP99,994, a very potent and specific tachykinin NK_1 receptor antagonist (McLean et al., 1993) with a K_i value on NK_1 receptors (IM-9 cells) of 0.25 nM and a pA_2 value on rabbit vena cava of 9.3 (Cogé and Regoli, 1994) is only active on substance P-induced bronchoconstriction, and SR48968, a very potent and specific tachykinin NK_2 receptor antagonist (Emonds-Alt et al., 1992) with a K_i value on human NK_2 receptors of 0.7 nM and a pA_2 value on rabbit pulmonary artery of 9.6 (Cogé and Regoli, 1994) is only active on neurokinin A-induced bronchoconstriction. In guinea-pigs, i.v.-administered capsaicin, as well as vagus nerve stimulation, induced a conjoined release of substance P and neurokinin A from lung sensory nerves, leading to both a tachykinin NK_1 receptor-mediated increase in vascular extravasation in the airways and a predominantly NK_2 receptor-mediated bronchoconstriction. As shown in Fig. 4, capsaicin-induced bronchoconstriction is reduced by SR48968 but not by CP99,994, and is fully inhibited by the association of both antagonists, a finding consistent with the data reported by Bertrand et al. (1993). On the other hand, inhibition of neurogenic airway plasma extravasation is achieved with CP99,994, but not with SR48968 (data not shown). These results confirm that in guinea-pigs tachykinins-induced airway edema is mediated mainly via tachykinin NK_1 receptors, whereas bronchocon-

striction is mediated via tachykinin NK_2 and possibly NK_1 receptors.

The inhibition by S 16474 of both airway bronchoconstriction and mucosal plasma extravasation, produced either by exogenous substance P and neurokinin A or by endogenous tachykinins released by neurogenic stimulation, provides further evidence in vivo that this compound is a dual tachykinin NK_1 and NK_2 receptors antagonist. Assuming that NK_1 and NK_2 receptors play similar roles in human, these results emphasize the therapeutic potential interest of bipotent tachykinin receptors antagonist such as S 16474.

The discovery of an antagonist active both on tachykinin NK_1 and NK_2 receptors has already been reported (Morimoto et al., 1992; Murai et al., 1992). In vitro, FK224 was described to bind to the human tachykinin NK_1 receptor with an IC_{50} value of 85 ± 4 nM (Cogé et Regoli, 1994), quite similar to the value that we obtained with S 16474. The affinity of FK224 for the human NK_2 receptor is not known. Bioassays on guinea-pig trachea showed that FK224 inhibited substance P- and neurokinin A-induced contractions, with IC_{50} values of 2.6×10^{-6} and 1.3×10^{-6} M, respectively (Murai et al., 1992). In guinea-pigs in vivo, the first dose of FK224 reported to inhibit substance P- and neurokinin A-induced bronchoconstriction is 1 mg/kg – or 0.95 μmol/kg – i.v. (Murai et al., 1992). Thus FK224 and S 16474, although they have quite different chemical structures, are both dual antagonists of approximately similar potencies. As water-soluble, S 16474 may be more suitable than FK224 for local applications such as the inhalation route.

In fact substance P and neurokinin A are present in primary afferent sensory nerves innervating the airways, and may be released together by pathophysiologic agents or stimuli known to activate sensory C-fibers (Solway and Leff, 1991). Both tachykinin NK_1 and NK_2 receptors are present in human and guinea-pig airways (Advenier et al., 1987; Floch and al., 1994; Walsh and al., 1994). Since NK_1 receptors regulate airway vascular leakage and mucus secretion and NK_2 receptors regulate airway smooth muscle contraction and antigen-induced airway hyperresponsiveness (Boichot et al., 1995), dual antagonists for both tachykinin NK_1 and NK_2 receptors could be clinically more useful for treatment of chronic inflammatory airway diseases than highly specific tachykinin NK_1 or NK_2 receptor antagonists.

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