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SCH 206272: a potent, orally active tachykinin NK_1, NK_2, and NK_3 receptor antagonist

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Abstract

Experiments were performed to characterize the pharmacology of SCH 206272 [(*R*,*R*)-1′[5-[(3,5-dichlorobenzoyl)methylamino]-3-(3,4-dichlorophenyl)-4(*Z*)-(methoxyimino)pentyl]-*N*-methyl-2-oxo-[1,4′ bipiperidine]-3-acetamide] as a potent and selective antagonist of tachykinin (NK) NK_1, NK_2, and NK_3 receptors. SCH 206272 inhibited binding at human tachykinin NK_1, NK_2, and NK_3 receptors ($K_i = 1.3$, 0.4, and 0.3 nM, respectively) and antagonized $[Ca^{2+}]_i$ mobilization in Chinese hamster ovary (CHO) cells expressing the cloned human tachykinin NK_1, NK_2, or NK_3 receptors. SCH 206272 inhibited relaxation of the human pulmonary artery ($pK_b = 7.7 \pm 0.3$) induced by the tachykinin NK_1 receptor agonist, [Met-O-Me] substance P and contraction of the human bronchus ($pK_b = 8.2 \pm 0.3$) induced by the tachykinin NK_2 receptor agonist, neurokinin A. In isolated guinea pig tissues, SCH 206272 inhibited substance P-induced enhancement of electrical field stimulated contractions of the vas deferens, ($pK_b = 7.6 \pm 0.2$), NKA-induced contraction of the bronchus ($pK_b = 7.7 \pm 0.2$), and senktide-induced contraction of the ileum. In vivo, oral SCH 206272 (0.1–10 mg/kg, p.o.) inhibited substance P-induced airway microvascular leakage and neurokinin A-induced bronchospasm in the guinea pig. In a canine in vivo model, SCH 206272 (0.1–3 mg/kg, p.o.) inhibited NK_1 and NK_2 activities induced by exogenous substance P and neurokinin A. Furthermore, in guinea pig models involving endogenously released tachykinins, SCH 206272 inhibited hyperventilation-induced bronchospasm, capsaicin-induced cough, and airway microvascular leakage induced by nebulized hypertonic saline. These data demonstrate that SCH 206272 is a potent, orally active tachykinin NK_1, NK_2, and NK_3 receptor antagonist. This compound may have beneficial effects in diseases thought to be mediated by tachykinins, such as cough, asthma, and chronic obstructive pulmonary disease.

Keywords: Tachykinin; Pulmonary; Tachykinin NK_1 receptor; Tachykinin NK_2 receptor; Tachykinin NK_3 receptor

1. Introduction

The three main mammalian tachykinins are substance P, neurokinin, A and neurokinin B. These tachykinins are located in the central and peripheral nervous systems, with a primary location in capsaicin-sensitive primary afferent neurons. These neurons innervate multiple organs including the airways, gastrointestinal, and urinary tract (Otsuka and Yoshioka, 1993). The biological effects of the tachykinins are mediated through distinct G protein-coupled receptors (NK_1, NK_2, and NK_3). Although all the endogenous tachykinins are known to interact with all three receptor subtypes, there is a rank order of potency with substance P having the highest affinity for the tachykinin NK_1 receptor, neurokinin A for the tachykinin NK_2 receptor, and neurokinin B for the tachykinin NK_3 receptor.

Tachykinins have a variety of effects in the lungs. They are potent constrictors of airway smooth muscle (Ellis et al., 1993; Foulon et al., 1993; Sheldrick et al., 1995), produce

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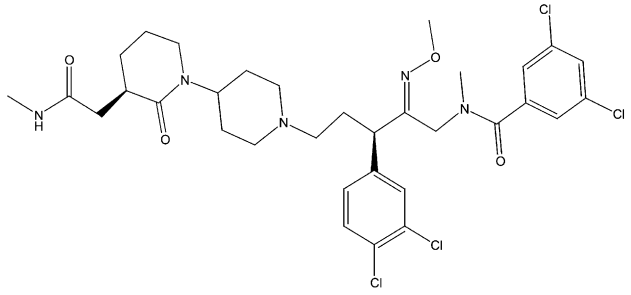

Fig. 1. The structure of SCH 206272.

vasodilation and microvascular leakage of airway blood vessels (Rogers et al., 1988; Piedimonte et al., 1993), are potent secretagogues of airway mucus glands and cells (Rogers, 1995), and are chemotactic and activate several inflammatory and immune cells in respiratory tissues (Chapman et al., 1998). Additionally, tachykinins modulate a variety of pulmonary reflexes including the cough reflex (Ujie et al., 1993; Bolser et al., 1997; Daoui et al., 1998) and the parasympathetic, cholinergic, bronchoconstrictor reflex (Haxhiu et al., 1989; Myers and Undem, 1993; Hey et al., 1996). On the basis of these findings, tachykinins have been implicated in the pathophysiology of asthma, chronic bronchitis, and rhinitis (Barnes et al., 1991). The pulmonary effects of tachykinins are mediated predominantly by tachykinin NK_1 and NK_2 receptor activation (Chapman et al., 1998) and tachykinin NK_3 receptors have been implicated in the physiology of cough (Daoui et al., 1998). A compound that possessed combined tachykinin NK_1, NK_2, and NK_3 receptor antagonist activity would be extremely useful to evaluate the role of tachykinins in pulmonary diseases. Various peptide and nonpeptide tachykinin receptor antagonists that selectively inhibit NK_1, NK_2, or NK_3 receptors have been described (Emonds-Alt et al., 1992; Mussap et al., 1993; McLean, 1996; Rumsey et al., 2001). In addition, dual nonpeptide antagonists with equal affinity for NK_1 and NK_2 receptors have been synthesized (Morimoto et al., 1992; Patacchini et al., 1995; Robineau et al., 1995; Kudlacz et al., 1996).

This study describes the pharmacology of SCH 206272 (Fig. 1), a potent human tachykinin NK_1, NK_2, and NK_3 receptor antagonist that is orally active in a variety of animal models that involve tachykinin pathophysiology.

2. Materials and methods

2.1. Materials and animals

SCH 206272 [(*R*,*R*)-1′[5-[(3,5-dichlorobenzoyl)methylamino]-3-(3,4-dichlorophenyl)-4(*Z*)-(methoxyimino)pentyl]-*N*-methyl-2-oxo-[1,4′bipiperidine]-3-acetamide], CP 99994 [(2*S*,3*S*)-3-(2-methoxybenzylamino-2-phenyl-piperidine] (tachykinin NK_1 receptor antagonist), SR 48968 [(*S*)-*N*-methyl-*N*[(4-acetylamino-4-phenylpiperidino-2-(3,4)dichlorophenyl)butyl]benzamide]) (tachykinin NK_2 receptor antagonist), and SR 142801 ([1-{2-[3-(3,4-dichlorophenyl-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]etyl}-4-phenyl-1-azoniabicyclo[2.2.2]octane,chloride]) (tachykinin NK_3 receptor antagonist) were synthesized by the chemistry department of Schering-Plough Research Institute (Kenilworth, NJ, USA). [^{3}H][Sar^9,Met(O$_2$)^{11}]substance P (specific activity 41 Ci/mmol) and [^{125}I][MePhe^7] neurokinin B (specific activity 2200 Ci/mmol) were purchased from NEN Life Sciences (Boston, MA, USA). [^{3}H]-neurokinin A (specific activity 114 Ci/mmol) was purchased from Zeneca Pharmaceuticals (Cheshire, UK). Phosphoramidon and chymostatin were obtained from Boehringer-Mannheim (Indianapolis, IN, USA). Indomethacin, carbachol, chlorpheniramine, and MK-571 were obtained from Sigma (St. Louis, MO, USA). Substance P, [Sar^9,Met(O$_2$)^{11}] substance P, neurokinin A, [βAla^8]-neurokinin A (4–10), [Met-O-Me^{11}] substance P, and [MePhe^7] neurokinin B were obtained from Peninsula Labs (Belmont, CA, USA).

In vivo studies were conducted in male Hartley guinea pigs (Charles River Labs, Wilmington, MA, USA; weight range 350–650 g) and male beagle dogs (Schering-Plough, Lafayette, NJ, USA; weight range 9–15 kg). The animals were fasted overnight before study but were allowed water ad libitum. The studies were conducted in accordance with the NIH Guide to the Care and Use of Laboratory Animals and the Animal Welfare Act. All protocols were reviewed and approved by the Animal Care and Use Committee of SPRI.

For in vitro experiments, stock solutions of compounds were prepared in dimethyl sulfoxide or saline and then diluted with the appropriate physiological buffer to the final desired concentration. All control samples contained an equal amount of dimethylsulfoxide that did not exceed a final concentration of 0.5%. For in vivo experiments in guinea pigs, compounds were solubilized in water or prepared in 0.4% methylcellulose in isotonic saline before oral (p.o.) administration. For studies in dogs, compounds were given orally as a dry powder in a gelatin capsule.

2.2. In vitro assays

2.2.1. Receptor binding assays

Chinese hamster ovary (CHO) cells transfected with the coding regions for the human and rat tachykinin (NK_1, NK_2, and NK_3) receptors were obtained from Dr. Jim Krause (Neurogen, Branford, CT, USA). Cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum, 0.1 mM nonessential amino acids, 2 mM glutamine, 100 units/ml of penicillin and streptomycin, and 0.8 mg/ml of G418 at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were detached from T-175 flasks with a sterile solution containing 5 mM EDTA in phosphate buffered saline, har-

vested by centrifugation and washed in RPMI media at 4 °C for 5 min. The pellet was resuspended in Tris–HCl (pH 7.4) containing 1 μM phoshoramidon and 4 μg/ml of chymostatin at a cell density of 30×10^6 cells/ml. The suspension was then homogenized in a Brinkman Polytron (setting 5) for 30–45 s. The homogenate was centrifuged at $800 \times g$ for 5 min at 4 °C to collect unbroken cells and nuclei. The supernatant was centrifuged in a Sorvall RC5C at 19,000 rpm ($44{,}000 \times g$) for 30 min at 4 °C. The pellet was resuspended, an aliquot was removed for a protein determination and washed again. The resulting pellet (10 mg/ml) was stored at −80 °C.

To assay tachykinin NK_1 and NK_2 receptor binding, 50 μl of either [^{3}H][Sar^9,Met(O$_2$)^{11}]substance P (0.8 nM) or [^{3}H] neurokinin A (1.0 nM) were added to wells of a deep-well microplate containing buffer (50 mM Tris–HCl [pH 7.4] with 1 mM $MnCl_2$, 1 mM $MgCl_2$, and 0.2% bovine serum albumin) and either dimethyl sulfoxide or various concentrations of test compounds. Binding was initiated by the addition of 100 μl of membrane (10–20 μg) expressing the human tachykinin NK_1 or NK_2 receptor in a final volume of 200 μl. After 40 min at room temperature, the reaction was stopped by rapid filtration onto Whatman GF/B filters which had been presoaked in 0.3% polyethylenimine. Filtration was accomplished using a TOMTEC automated harvester (Orange, CT, USA). Filters were washed three times with cold 50 mM Tris–HCl (pH 7. 4). Filter mats were dried in a microwave oven for 2×2.5 min. Meltilex solid scintillant was applied to filters and scintillation counting carried out in a Betaplate counter (Wallac, Gaithersburg, MD, USA). Nonspecific binding was determined by the addition of either CP 99994, SR 48968, or SR 142801 at a concentration of 1 μM. To assay tachykinin NK_3 receptor binding, a scintillation proximity assay was developed. Briefly, for each assay point, 10 μg of membrane was preincubated with 200 μg of wheat germ agglutinin scintillation proximity assay beads in buffer (50 mM Tris–HCl [pH 7.4] with 1 mM $MnCl_2$ and $MgCl_2$ and 0.2% bovine serum albumin) at 4 °C for 1 h. The membrane-bead suspension was centrifuged at $200 \times g$ for 5 min. The resulting membrane bound scintillation proximity assay beads (100 μl) were added to a Packard 96-well microtitre well with 50 μl of [^{125}I]MePhe^7 neurokinin B (0.1 nM) and either 50 μl of either dimethylsulfoxide, SCH 206272, or 1 μM SR 142801 to define nonspecific binding. The plates were counted overnight on a Packard Top Count. IC_{50} values were determined from competition binding curves and K_i values were determined according to Cheng and Prusoff (1973). K_d and B_{max} values were determined by nonlinear regression using the program PRISM (GraphPad Software, San Diego, CA, USA).

2.2.2. Measurement of $[Ca^{2+}]_i$

Cells were seeded in clear, flat-bottomed, black-wall 96-well plates 1–3 days prior to assay for a final density of 50,000 cells/well. On the day of the assay, the medium was removed and cells were incubated in Hank's buffered saline solution, 20 mM HEPES, 0.4% bovine serum albumin, 2.5 mM probenecid, 10% pluronic acid, and 4 μM Fluo-3 AM for 1 h at 37°. Cells were washed four times with buffer utilizing a Labsystems Cellwash plate washer leaving 100 μl of buffer in each well. Solutions containing the appropriate concentration of test compound or buffer were added to the cells and incubated at 37° for 15 min before addition of [Sar^9,Met(O$_2$)^{11}]substance P, neurokinin A, or [MePhe^7]neurokinin B which were given in 50 μl volume at a final concentration 1, 2, and 1 nM, respectively. Changes in intracellular calcium were measured with a Fluorometric Imaging Plate Reader (Sunnyvale, CA, USA) by excitation of the calcium-sensitive fluorescent dye with an argon laser with excitation at 488 nm and emission in the 500–560 nm range. Data is presented as a percent of maximum response of the peak heights obtained from the given concentration of agonist.

2.2.3. NK_1 and NK_2 activity on human smooth muscle

The procedure for studying tachykinin NK_1 receptor mediated vasorelaxation of the pulmonary artery has been described (Corboz et al., 1998). Briefly, human lungs were procured by the International Institute for the Advancement of Medicine (Exton, PA, USA) and cryopreservation and thawing procedures were performed using previously described techniques (Rizzo et al., 1999). Small pulmonary arterial rings were dissected free from surrounding tissue and were placed in chambers filled with Krebs-Ringer buffer maintained at 37 °C and aerated with 95%O_2–5%CO_2. A resting force of 1 g was applied and tissues were allowed to equilibrate for 60 min. At the end of this equilibration period, test compounds (three concentrations) or vehicle were added to the bath followed 10 min later by the addition of the α_1-adrenergic receptor agonist phenylephrine (30 μM) to induce a sustained increase in vascular tone. This was followed by the addition of increasing log increments of [Met-O-Me] substance P at concentrations of 0.1–100 nM. Relaxant responses were measured at the end of each period. Relaxation to acetylcholine (100 nM) was measured at the end of each experiment to normalize the relaxant response.

The procedure for studying NK_2 receptor-mediated contraction of the isolated epithelium-denuded bronchus has been described (Rizzo et al., 1999). The bronchial tissues were placed under 1 g (3–5-mm diameter) or 2 g (>5 mm in diameter) passive tension and allowed to equilibrate for 90 min. Thirty minutes before the end of the equilibration, chlorpheniramine (1 μM), MK-571 (3 μM), indomethacin (3 μM), and phosphoramidon (3 μM) were added to eliminate endogenous histamine- and LTD_4-mediated tone, and block the release of cyclo-oxygenase products and inhibit the degradation of endoge-

nous tachykinins, respectively. Test compounds (three concentrations) were then given to the organ bath and 2 h later, contractile response to cumulative concentrations of neurokinin A (0.3 nM–300 μM) were measured. Contraction to carbachol (1 mM) was measured at the end of each experiment to normalize the contractile response.

2.2.4. NK_1–NK_2 and NK_3 activity on guinea pig smooth muscle

Tachykinin NK_1 receptor antagonist mediated activity was measured as the inhibition of substance P-induced potentiation of electrically field stimulated contraction of the guinea pig vas deferens (Hall and Morton, 1991). Submaximal contractile responses to electrical field stimulation (1-ms pulse duration, 50 Hz, 0.03 trains/s) were obtained by varying the train duration (0–100 ms) and stimulus intensity (0–25 V). The electrical field stimulation response before substance P was first measured and then followed by the cumulative additions of substance P (0.1 nM–10.0 μM) after each electrical field stimulation-induced contraction. Addition of substance P was stopped when the magnitude of the electrical field stimulation response was approximately a 200% increase over the baseline response. Test compounds (three concentrations) or vehicle were then added to the tissues 30 min before the first dose of substance P.

Tachykinin NK_2 receptor antagonist activity was measured as the inhibition of neurokinin A-induced contraction of the isolated guinea pig bronchus. Main bronchus segments were cleaned of connective tissue and the epithelium was removed. Ring preparations were anchored in jacketed organ baths and attached to Grass FT-03 force displacement transducers. Transducers were connected to a physiograph for recording isometric tension. Experiments were performed in 37 °C Krebs buffer with 2 μM indomethacin and 1 μM phosphoramidon following a 90-min equilibration at 1.0-g initial tension. Carbachol (10 μM) was used to test for tissue responsiveness during the equilibration period. Vehicle or test compounds (three concentrations) were added to the bath 2 h before the bronchus rings were contracted by cumulative additions of neurokinin A (0.3 nM–10.0 μM or until a maximum response was achieved). Upon completion, 80 mM KCl was added to obtain a maximum tension for normalizing neurokinin A-induced contractions.

Tachykinin NK_3 receptor antagonist activity was evaluated as the inhibition of senktide-induced contractions of isolated guinea pig whole ileum. Segments of the ileum, approximately 2 cm in length, were isolated 10–20 cm from the ileocecal junction. The ileal segments were mounted longitudinally in organ baths using 6-0 silk and connected to Grass FT-03 force displacement transducers. The transducers were attached to a physiograph for continuous recording of isometric tension. Experiments were performed at 37 °C in Krebs buffer supplemented with CP 99994 (0.3 μM). The tissues were equilibrated at 0.3-g passive tension and test compounds were added to the bath 2 h before addition of senktide (0.1 nM–1 μM). At the end of the study, carbachol (10 nM) was added to maximally contract the tissue. Contractions were assessed as peak grams tension increase over baseline and normalized as percent of carbachol maximum.

2.3. In vivo assays

2.3.1. NK_1 and NK_2 activity in guinea pigs

The procedure for inducing tachykinin NK_1 receptor-mediated increase in airway microvascular leakage in guinea pigs has been described (Danko et al., 1992). Guinea pigs were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and prepared with tracheal and jugular venous catheters. The guinea pigs were mechanically ventilated during these studies. Airway microvascular leakage was produced by injecting substance P (1 μg/kg) intravenously and measuring the leakage of Evans blue dye in the trachea and bronchi. Guinea pigs were orally dosed with compound or vehicle 2 h before challenge with substance P. The increase in airway microvascular leakage due to substance P was measured in both vehicle- and drug-treated animals and the percent inhibition due to drug measured.

Tachykinin NK_2 receptor antagonist activity was measured as the inhibition of bronchoconstriction induced by intravenous [βAla^8]-neurokinin A (4–10) (Hirayama et al., 1993). Guinea pigs were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and prepared with tracheal, esophageal, and jugular venous catheters. The guinea pigs were placed inside a whole body plethysmograph for the measurement of pulmonary resistance as previously described (Mauser et al., 1997). Mechanical ventilation was maintained throughout these experiments. Bronchoconstrictor responses to rising i.v. doses (0.1–10 μg/kg) of [βAla^8]-neurokinin A (4–10) were measured in both vehicle- and drug-treated animals. The percent increase in pulmonary resistance due to 0.3 μg/kg of [βAla^8]-neurokinin A (4–10), which is the dose producing the maximum bronchoconstriction in normal animals, was measured in both the vehicle- and drug-treated groups. Guinea pigs were orally dosed with compound or vehicle 2 h before challenge with [βAla^8]-neurokinin A (4–10). The percent inhibition of the bronchoconstriction to [βAla^8]-neurokinin A (4–10) was measured in drug-treated animals relative to that seen in the vehicle-treated group.

2.3.2. Hyperventilation-induced bronchoconstriction in guinea pigs

Hyperventilation-induced bronchoconstriction was induced in guinea pigs using experimental procedures similar to that previously described by Chapman and

Danko (1985). The guinea pigs were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and prepared with tracheal and esophageal catheters. The guinea pigs were placed inside a whole body plethysmograph for the measurement of pulmonary resistance as previously described (Mauser et al., 1997). Mechanical ventilation was maintained throughout these experiments. Hyperventilation-induced bronchoconstriction was induced by increasing the respiratory rate from 50 to 185 breaths/min for 10 min. Tidal volume (4 ml) was not changed throughout the study. After a 10-min period of hyperventilation, the respiratory rate was returned to 50 breaths/min. Measurements were obtained before the start of the hyperventilation period and for up to 10 min after the end of the hyperventilation. Peak increases in pulmonary resistance due to hyperventilation normally occur within this 10-min period.

Table 1
Receptor binding affinity of tachykinin antagonists for the cloned human tachykinin receptors

Compound	K_i (nM)		
	NK_1	NK_2	NK_3
SCH 206272	1.3 ± 0.15	0.4 ± 0.03	0.3 ± 0.01
CP 99994	0.27 ± 0.07	>10,000	>10,000
SR 48968	273 ± 16.1	0.4 ± 0.1	1282 ± 566
SR 142801	291 ± 18.1	20.4 ± 5.7	1.7 ± 0.7

CHO cell membranes expressing human tachykinin receptors were assayed with the appropriate ligand as described in Materials and methods. K_i values (mean ± S.E.M.) were obtained from three to six experiments performed in duplicate.

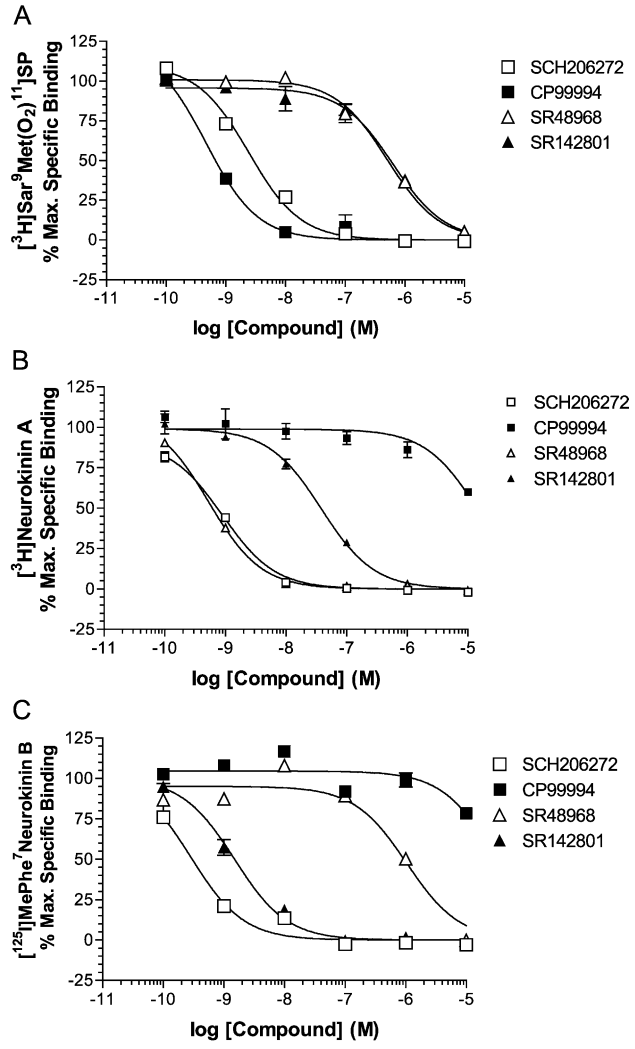

Fig. 2. Inhibition of [^{3}H][Sar^9,Met(O$_2$)^{11}]substance P, [^{3}H]neurokinin A, or [^{125}I][MePhe^7]neurokinin B binding to cloned human tachykinin receptors by SCH 206272, CP 99994, SR 48968, and SR 142801. CHO cell membranes expressing human tachykinin (A) NK_1, (B) NK_2, or (C) NK_3 receptors were incubated with [^{3}H][Sar^9,Met(O$_2$)^{11}]substance P (0.8 nM), [^{3}H] neurokinin A (1.0 nM), or [^{125}I][MePhe^7]neurokinin B (0.1 nM) in the presence of increasing concentrations of test compound. Nonspecific binding was determined with 1 μM of CP 99994, SR 48968, or SR 142801 for the NK_1, NK_2, or NK_3 receptors, respectively.

2.3.3. Capsaicin-induced cough in guinea pigs

Capsaicin-induced cough in guinea pigs was produced using procedures similar to that previously described (Bolser et al., 1997). Guinea pigs were placed in a 12 in. by 14 in. transparent chamber and exposed to aerosolized capsaicin (300 μM for 4 min) using an Ultra-Neb 99 DeVilbiss nebulizer (Somerset, PA, USA) to elicit cough. Each guinea pig was exposed only once to capsaicin. The number of coughs was detected using a microphone placed in the chamber and verified by a trained observer. The signal from the microphone was relayed to a polygraph which provided a record of the number of coughs. The ED_{30} (dose that inhibited capsaicin-induced cough by 30%) of SCH 206272, SR 48968, and CP 99994 were determined by regression analysis.

2.3.4. Hypertonic saline-induced airway microvascular leakage in guinea pigs

Airway microvascular leakage was measured using the Evans blue dye technique as previously described (see Section 2.3.1). Guinea pigs were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and catheters were placed into the trachea and jugular vein. Mechanical ventilation was performed throughout the studies. Aerosols of normal saline (0.9% NaCl) or hypertonic saline (7% NaCl) were generated by an ultrasonic nebulizer (model Ultra-Neb 99, DeVilbiss, MA, USA) and delivered for 2 min through the inspiratory circuit of the ventilator. Evans blue dye (30 mg/kg, i.v.) was injected 1 min before each aerosol challenge. Ten minutes after the aerosol challenge, the chest was opened, and the intravascular dye removed and airway microvascular leakage was measured in the trachea and bronchi using techniques previously described (Danko et al., 1992).

2.3.5. NK_1 and NK_2 activity in dogs

The dogs used in these experiments were selected on the basis of a positive bronchoconstrictor response to inhaled neurokinin A and a fall in blood pressure and increase in minute volume in response to challenge with i.v. substance P (Sherwood et al., 1998). The methodology for inducing these cardiopulmonary responses to neurokinin A and substance P in dogs and the utility of this model for the evaluation of tachykinin NK_1 and NK_2 receptor antagonists have been previously described (Sherwood et al., 1998). Specifically, tachykinin NK_1 receptor antagonists inhibit the fall in mean arterial blood pressure and increase in minute volume due to i.v. substance P (100 ng/kg) and tachykinin NK_2 receptor antagonists inhibit the bronchoconstriction induced by challenge with aerosolized neurokinin A (1%). SCH 206272 was given orally at various times before challenge with neurokinin A and substance P. A 2-h pretreatment time was used for the dose–response studies and duration studies were performed at 2, 6, 12, and 24 h after oral dosing. Each dog received either SCH 206272 or an empty capsule minus the drug as part of a cross-over experimental design. A 2-week interval between studies was allowed for individual dogs.

Table 2
Inhibition of $[Ca^{2+}]_i$ by SCH 206272 and selective tachykinin receptor antagonists at the human tachykinin NK_1, NK_2, and NK_3 receptors

Compound	IC_{50} (nM)		
	NK_1	NK_2	NK_3
SCH 206272	110 ± 36	8 ± 4.4	12 ± 4.3
CP 99994	5.4 ± 1.7	>1000	>1000
SR 48968	>1000	2.3 ± 0.9	>1000
SR 142801	>1000	>1000	29 ± 2.7

CHO cells expressing human tachykinin NK_1, NK_2, or NK_3 receptors were assayed for $[Ca^{2+}]_i$ with the appropriate ligand as described in Materials and methods. IC_{50} values (mean ± S.E.M.) were obtained from three to six experiments performed in triplicate.

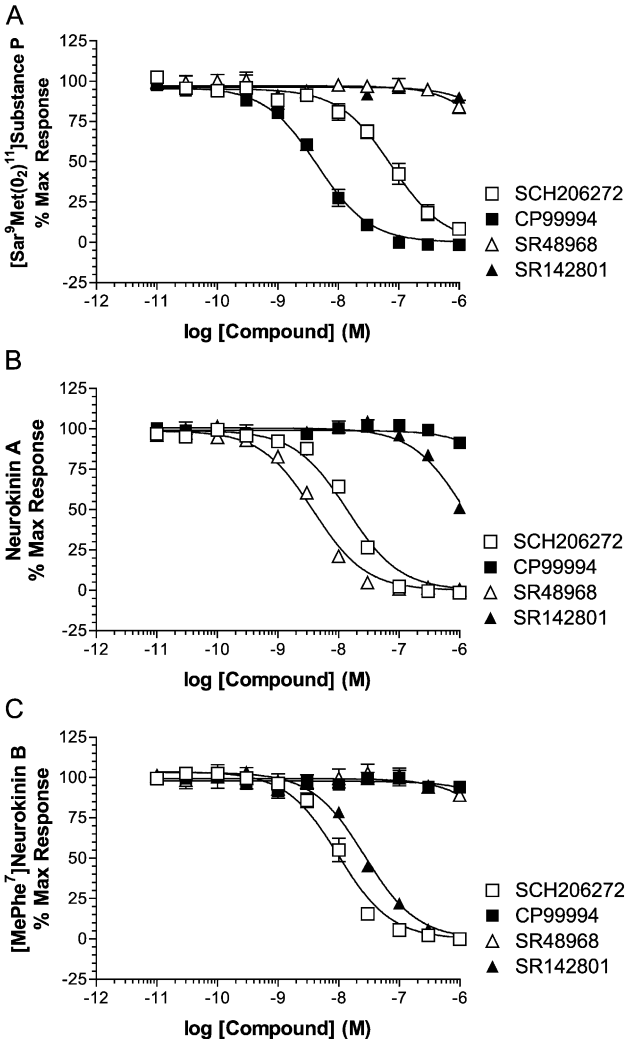

Fig. 3. Inhibition of [Sar^9,Met(O$_2$)^{11}]substance P, neurokinin A, or [MePhe^7]neurokinin B induced increases in $[Ca^{2+}]_i$ in CHO cells expressing the human tachykinin receptors by SCH 206272, CP 99994, SR 48968, and SR 142801. CHO cells expressing human tachykinin (A) NK_1, (B) NK_2, or (C) NK_3 receptors were loaded with Fura-2 dye as described in Materials and methods. Compounds were incubated for 15 min and stimulated with 1 nM [Sar^9,Met(O$_2$)^{11}]substance P, 2 nM neurokinin A, or 1.0 nM [MePhe^7]neurokinin B. Fluorescent intensity was measured relative to the signal obtained with the respective ligands. Data are the mean ± S.E.M. of a representative experiment performed in triplicate.

2.4. Data analysis and statistics

For in vitro studies, agonist potency was expressed as EC_{50} (concentration causing 50% of the maximal agonist response) estimated using linear regression of the agonist concentration–response curves. For competitive style antagonist activity, antagonist potency was expressed as a pK_b ($-\log$ of K_b) where $K_b=[B]/(\text{dose ratio}-1)$ ($B=$ concentration of the antagonist) and dose ratio$=[A']/[A]$, where A' and A are agonist EC_{50}'s in the respective presence and absence of the antagonist. For noncompetitive antagonist activity, responses were compared and IC_{50} (concentration producing half-maximal inhibition of the agonist response) was estimated assuming 100% maximum inhibition. A Mann–Whitney nonparametric analysis or Kruskal–Wallis nonparametric multiple group analysis was used to determine statistically significant effect of drugs.

For in vivo studies in guinea pigs, an analysis of variance was performed on the different treatment groups to determine significant effects of the treatments. Post hoc analysis between the different treatment groups was performed using Fischers protected least square difference using Statview for Macintosh (version 4.5, Abacus Concepts, CA, USA). In dogs, statistical significance of drugs was assessed using *t*-tests based on model-estimated standard errors. Comparisons with $p<0.05$ were considered evidence of significant treatment effects.

3. Results

3.1. In vitro assays

3.1.1. Receptor binding

The respective K_d values for [^{3}H]Sar^9,Met(O$_2$)^{11}] substance P, [^{3}H]neurokinin A, and [^{125}I][MePhe^7] neurokinin B to bind to human tachykinin NK_1, NK_2, and NK_3 receptors expressed on CHO cells were 0.8 ± 0.1, 2.4 ± 0.6, and 0.2 ± 0.1 nM ($n = 3$). Competition for agonist binding with SCH 206272, CP 99994, SR 48968, and SR 142801 to the human tachykinin receptors is presented in Fig. 2 and Table 1. SCH 206272 inhibited [^{3}H][Sar^9,Met(O$_2$)^{11}]substance P, [^{3}H]neurokinin A, and [^{125}I][MePhe^7]-neurokinin B binding to the tachykinin NK_1, NK_2, and NK_3 receptors with K_i values of 1.3 ± 0.15, 0.4 ± 0.03, and 0.3 ± 0.01 nM, respectively.

3.1.2. $[Ca^{2+}]_i$ flux in CHO cells

[Sar^9,Met(O$_2$)^{11}]substance P, neurokinin A, and [MePhe^7]neurokinin B induced an increase in $[Ca^{2+}]_i$ in intact CHO cells expressing the human tachykinin NK_1, NK_2, and NK_3 receptors. SCH 206272 antagonized [Sar^9,Met(O$_2$)^{11}]substance P, neurokinin A, and [MePhe^7]neurokinin B induced increases in $[Ca^{2+}]_i$ with IC_{50} values of 110 ± 36, 8 ± 4.4, and 12 ± 4.3 nM at the tachykinin NK_1, NK_2, and NK_3 receptors, respectively (Fig. 3 and Table 2). SCH 206272 was at least 10-fold less potent at the tachykinin NK_1 receptor than at the NK_2 and NK_3 receptor in this assay. These data demonstrate that SCH 206272 is a functional antagonist of the human tachykinin NK_1, NK_2, and NK_3 receptor.

3.1.3. NK_1 and NK_2 activity in human pulmonary artery and bronchus

[Met-O-Me^{11}] substance P produced a concentration-dependent relaxation in phenylephrine-preconstricted human pulmonary arteries ($EC_{50} = 7.9 \pm 3.0$ nM). Dose-dependent inhibition of [Met-O-Me^{11}] substance P-induced relaxation occurred with SCH 206272 ($pK_b = 7.7 \pm 0.3$) (Table 3). The relaxation induced by [Met-O-Me^{11}] substance P was also inhibited by the tachykinin NK_1 receptor antagonist, CP 99994 ($pK_b = 9.3 \pm 0.2$) but not by SR 48968 at a concentration of 1 μM. Neurokinin A induced a concentration-dependent contraction of the isolated human bronchus ($EC_{50} = 88.0 \pm 30.8$ nM). Both SCH 206272 and SR 48968 inhibited neurokinin A-induced contractions with pK_bs of 8.2 ± 0.3 and 9.5 ± 0.3, respectively (Table 3). On the other hand, CP 99994 (1 μM) had no effect on neurokinin A-induced contractions.

Table 3
Effect of SCH 206272 and selective tachykinin NK_1 and NK_2 receptor antagonists on smooth muscle contractile and relaxant responses in human and guinea pig tissue

Compound	NK_1 (pK_b)		NK_2 (pK_b)	
	Human	Guinea pig	Human	Guinea pig
SCH 206272	7.7 ± 0.3	7.6 ± 0.2	8.2 ± 0.3	7.7 ± 0.2
CP 99994	9.3 ± 0.2	8.6 ± 0.2	NE	NE
SR 48968	NE	NE	9.5 ± 0.3	9.1 ± 0.2

Values represent pK_b estimates (mean ± S.E.M.) which were determined in 4–14 individual preparations as described in Materials and methods.
NE—no effect at 1 μM.

3.1.4. NK_1, NK_2, and NK_3 receptor activity in guinea pig vas deferens, bronchus, and ileum

Substance P enhanced electrical field stimulation-induced contraction of the isolated guinea pig vas deferens. Dose-dependent inhibition of substance P enhanced electrical field stimulation contractions occurred with SCH 206272 ($pK_b = 7.6 \pm 0.2$) and CP 99994 ($pK_b = 8.6 \pm 0.2$), whereas SR 48968 (1 μM) was inactive (Table 3). Neurokinin A induced a concentration dependent contraction of guinea pig bronchus ($EC_{50} = 2.2 \pm 0.3$ nM). Dose-dependent inhibition of the neurokinin A bronchial contractions occurred with SCH 206272 ($pK_b = 7.7 \pm 0.2$) and SR 48968 ($pK_b = 9.1 \pm 0.2$), whereas CP 99994 (1 μM) was inactive (Table 3). Senktide induced a concentration-dependent contraction of the isolated guinea pig ileum ($EC_{50} = 1.1 \pm 0.2$ nM) with maximal contraction occurring at a concentration of 100 nM. SCH 206272 noncompetitively inhibited the contractile response of 10 nM senktide by 63% and 92%, at concentrations of 10 and 100 nM, respectively, whereas the inhibition produced by SR 142801 at 10 and 100 nM was 29% and 88%, respectively. SR 48968 (100 nM) inhibited the senktide contractile response by only 20%.

3.2. In vivo studies

3.2.1. NK_1 and NK_2 activity in guinea pigs

Intravenous administration of substance P (1 μg/kg) to guinea pigs increased airway microvascular leakage as measured by the concentration of Evans blue dye in the trachea and bronchi. Treatment of guinea pigs with SCH 206272 (0.1–10 mg/kg, p.o.) dose-dependently inhibited the airway concentration of Evans blue dye after substance P (Table 4). Almost complete inhibition (85 ± 4% inhibition) was produced by 10 mg/kg p.o. of SCH 206272. In this assay, CP 99994 (10 mg/kg, p.o.) produced 85 ± 2% inhibition of the airway microvascular leakage induced by SP ($n = 4$).

Intravenous administration of [βAla^8]-neurokinin A (4–10) produced a dose-dependent bronchoconstriction in guinea pigs. Peak increases in pulmonary resistance in control animals occurred at 0.3 μg/kg of [βAla^8]-neurokinin A (4–10) and averaged 500–700% increase over baseline values. Treatment with SCH 206272 (1–10 mg/kg, p.o.) dose-dependently inhibited the increase in pulmonary resistance due to [βAla^8]-neurokinin A (4–10) and almost complete inhibition (96 ± 2% inhibition) was produced by 10 mg/kg p.o. of SCH 206272. In this assay, SR 48968

Table 4
Inhibition by SCH 206272 of substance P-induced airway microvascular leakage and β-Ala-neurokinin A induced bronchoconstriction in guinea pigs

SCH 206272 (mg/kg, p.o.)	Percent inhibition	
	Substance P-induced airway microvascular leakage	β-Ala-NKA-induced bronchospasm
0.1	30 ± 4[a]	NT
1	36 ± 4[a]	0
3	NT	48 ± 10[a]
10	85 ± 4[a]	96 ± 2[a]

Compounds were given 2 h before challenge with substance P (1 μg/kg, i.v.) or β-Ala-neurokinin A (0.3 μg/kg, i.v.). Values represent the mean ± S.E.M. ($n = 6–12$ per group).
NT = not tested.
[a] $p < 0.05$ compared to vehicle.

(3 mg/kg, p.o.) completely blocked (100% inhibition) the bronchospasm due to [βAla^8]-neurokinin A (4–10), but CP 99994 (30 mg/kg, p.o.) had no effect.

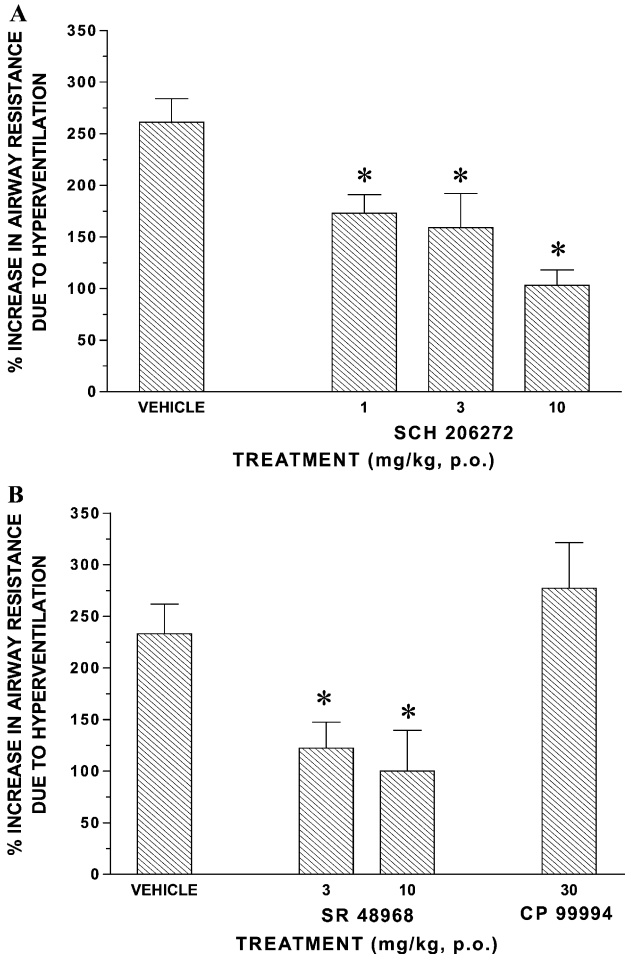

Fig. 4. Effect of compounds on hyperventilation-induced bronchospasm. Values represent mean ± S.E.M. ($n = 5–12$ per treatment) percent increase in airway resistance due to hyperventilation. * $p < 0.05$ compared to vehicle.

3.2.2. Hyperventilation-induced bronchospasm in guinea pigs

Oral pretreatment of guinea pigs with SCH 206272 (1–10 mg/kg) dose-dependently inhibited the increase in pulmonary resistance due to hyperventilation (Fig. 4A). Treatment with SR 48968 (3 and 10 mg/kg, p.o.) also inhibited guinea pig hyperventilation-induced bronchoconstriction, but CP 99994 at 30 mg/kg p.o. was inactive (Fig. 4B).

3.2.3. Capsaicin-induced cough in guinea pigs

In vehicle-treated animals, aerosolized capsaicin (300 μM for 4 min) produced 9.3 ± 1 coughs. Pretreatment of guinea pigs with SCH 206272 (1–30 mg/kg, p.o.) dose-dependently inhibited capsaicin-induced cough (Table 5). The potency of SCH 206272 to inhibit cough ($ED_{30} = 5.9$ mg/kg, p.o.) was similar to that of SR 48968 ($ED_{30} = 5.7$ mg/kg, p.o.) and approximately 2-fold less than that of CP 99994 ($ED_{30} = 2.9$ mg/kg, p.o.). SR 142801 (3 and 10 mg/kg, i.p.) was inactive.

3.2.4. Airway microvascular leakage

Inhalation of hypertonic saline aerosol (7% NaCl) increased the concentration of Evans blue dye in the trachea and bronchi by approximately 400% over that seen after challenge with 0.9% NaCl or in animals exposed to no aerosol challenge. Pretreatment of guinea pigs with SCH 206272 (1–10 mg/kg, p.o.) inhibited the airway microvascular leakage induced by 7% NaCl aerosol (Fig. 5) and the inhibition produced by 10 mg/kg of SCH 206272 was 75 ± 10%. Treatment with CP 99994 (10 mg/kg, p.o.) also inhibited (75 ± 8% inhibition, $n = 4$) the airway microvascular leakage induced by hypertonic saline. There was also a partial inhibition (41 ± 4% inhibition) of the airway microvascular leakage by SR 48968 (10 mg/kg, p.o., $n = 4$) ($p < 0.05$).

Table 5
Effect of SCH 206272, SR 48968, CP 99994, and SR 142801 on capsaicin-induced cough

Compound[a]	Dose (mg/kg)	Route	Percent inhibition cough
SCH 206272	1	p.o.	0 ± 0
	3		19 ± 14
	10		27 ± 5
	30		68 ± 10[b]
SR 48968	1	p.o.	9 ± 14
	3		26 ± 14
	10		31 ± 9[b]
	30		52 ± 7[b]
CP 99994	1	p.o.	15 ± 26
	3		27 ± 10
	10		51 ± 9[b]
SR 142801	3	i.p.	0 ± 9
	10		3 ± 8

Values represent mean ± S.E.M. ($n = 6–12$ per group).
[a] SCH 206272, SR 48968, and CP 99994 were given p.o. 2 h and SR 142801 was given i.p. 1 h before capsaicin challenge.
[b] $p < 0.05$ compared to placebo.

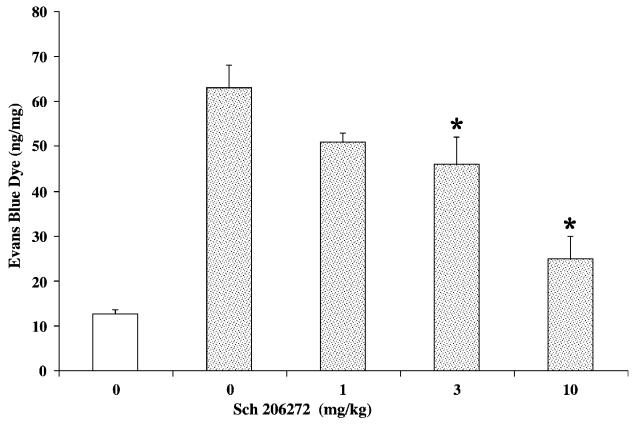

Fig. 5. Effect on SCH 206272 on the airway microvascular leakage induced by hypertonic saline. Values represent mean ± S.E.M. ($n = 8$ per treatment) concentration of Evans Blue Dye after exposure to aerosolized hypertonic (7%) saline (▨). Negative control group received normal (0.9%) saline (▭) as an aerosol. * $p < 0.05$ compared to zero drug.

3.2.5. NK_1 and NK_2 activity in dogs

In the dogs challenged with 1% aerosolized neurokinin A, pulmonary resistance increased by 100–200% above baseline values, and dynamic lung compliance fell by 55–65% below baseline values. These changes in pulmonary resistance and dynamic lung compliance due to neurokinin A were dose-dependently (0.1–3 mg/kg, p.o.) inhibited by SCH 206272 (Fig. 6). Complete inhibition of the neurokinin A-induced bronchospasm by SCH 206272 occurred at and above 1 mg/kg p.o. of the drug. SCH 206272 (0.1–3 mg/kg, p.o.) also inhibited the decrease in mean arterial pressure and increase in minute volume due to i.v. substance P (100 ng/kg) and statistically significant inhibition was seen at and above 1 mg/kg p.o. (Table 6).

To determine the duration of tachykinin NK_1 and NK_2 receptor antagonist activities of SCH 206272 in the dog, cardiopulmonary responses to substance P and neurokinin A

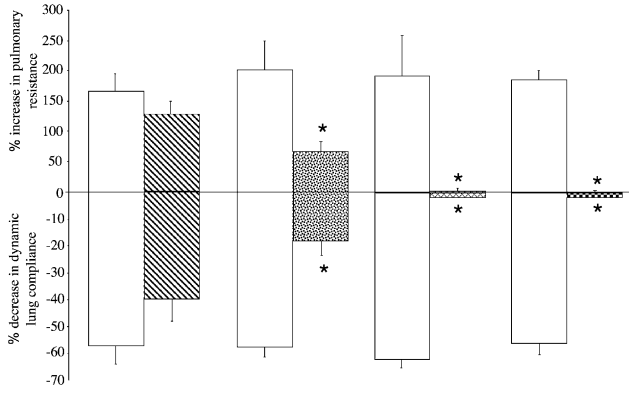

Fig. 6. Effect of SCH 206272 on neurokinin A-induced bronchospasm in dogs. Data show the percentage change in pulmonary resistance and dynamic lung compliance after challenge with aerosolized neurokinin A (1%). SCH 206272 (▨ 0.1 mg/kg; ▩ 0.3 mg/kg; ▩ 1 mg/kg; ▩ 3 mg/kg) or vehicle (▭) were given orally 2 h before neurokinin A challenge. Values represent mean ± S.E.M. ($n = 6$ per group). * $p < 0.05$ compared to vehicle.

Table 6
Effect of SCH 206272 on the blood pressure and minute volume response to substance P

SCH 206272[a] (mg/kg, p.o.)	Percent change due to substance P[b]	
	Mean arterial pressure	Minute volume
0	−51 ± 5	+166 ± 51
0.1	−42 ± 2	+140 ± 26
0	−46 ± 3	+123 ± 25
0.3	−42 ± 5	+113 ± 36
0	−50 ± 3	+157 ± 54
1	−35 ± 3[c]	+35 ± 7[c]
0	−57 ± 4	+198 ± 28
3	−22 ± 4[c]	+42 ± 31[c]

[a] SCH 206272 given orally 2 h before challenge with i.v. substance P (100 ng/kg).
[b] Values represent mean ± SEM ($n = 6$ per group).
[c] $p < 0.05$ compared to zero drug.

challenge were measured at various times after the p.o. administration of 3 mg/kg of SCH 206272. The tachykinin NK_1 receptor antagonist activity of SCH 206272 declined from 79% inhibition at 2 h to 53% inhibition at 6 h, 47% inhibition at 12 h, and 0% inhibition at 24 h. The tachykinin NK_2 receptor antagonist activity of SCH 206272 was 100% at 2 h, 88–94% at 6 and 12 h, and 48% at 24 h (data not shown).

4. Discussion

In this report, we identify SCH 206272 as a combined tachykinin NK_1, NK_2, and NK_3 receptor antagonist. Selective tachykinin NK_1, NK_2, and NK_3 receptor antagonists, dual tachykinin NK_1, NK_2 receptor antagonists and, most recently, a tachykinin NK_1, NK_2, and NK_3 receptor antagonist have been described (Rumsey et al., 2001). SCH 206272 is a potent inhibitor in recombinant human tachykinin NK_1, NK_2, and NK_3 receptor binding and functional assays. Furthermore, using in vitro studies on guinea pig and human tissues that endogenously express tachykinin NK_1, NK_2, and NK_3 receptors, SCH 206272 is an antagonist of all three tachykinin receptor subtypes. In vivo, SCH 206272 is a potent and orally active antagonist of tachykinin NK_1 and NK_2 receptors when these responses are triggered by exogenously administered tachykinins in guinea pigs and dogs. SCH 206272 also inhibits bronchospasm, cough, and airway microvascular leakage in guinea pigs when these responses are triggered by endogenously released tachykinins.

In receptor binding assays with cloned human tachykinin NK_1, NK_2, or NK_3 receptors, SCH 206272 has an affinity similar to that seen with CP 99994, SR 48968, and SR 142801 which are selective antagonists for the tachykinin NK_1, NK_2, and NK_3 receptors, respectively (Emonds-Alt et al., 1992; McLean et al., 1993; Oury-Donat et al., 1995). Furthermore, SCH 206272 is an antagonist at recombinant human tachykinin NK_1, NK_2,

or NK_3 receptors and demonstrates functional antagonism of these receptors in human and guinea pig smooth muscle contraction and relaxation assays.

In vivo, SCH 206272 inhibited the airway microvascular leakage induced by substance P and the bronchoconstriction induced by β-Ala-neurokinin A in guinea pigs. These responses are mediated by activation of tachykinin NK_1 and NK_2 receptors, respectively, and can be blocked by selective antagonists of the tachykinin NK_1 and NK_2 receptors (Danko et al., 1992; Hirayama et al., 1993). SCH 206272 was equipotent with the tachykinin NK_1 receptor antagonist, CP 99994, for blocking the airway microvascular leakage induced by substance P, but approximately 5-fold less potent than the tachykinin NK_2 receptor antagonist, SR 48968 for inhibition of the bronchospasm induced by β-Ala-neurokinin A. This latter finding may reflect the exquisite sensitivity of the guinea pig tachykinin NK_2 receptor to SR 48968 because a similar rank order potency between SCH 206272 and SR 48968 is seen with in vitro assays such as the isolated guinea pig bronchus contracted with neurokinin A.

SCH 206272 also inhibited the pharmacological responses to exogenous tachykinins in dogs measured by the fall in mean arterial pressure and the increase in minute volume induced by substance P and the bronchoconstriction induced by neurokinin A. These responses are mediated by activation of the tachykinin NK_1 and NK_2 receptor subtypes, respectively (Sherwood et al., 1997, 1998). It is important to note that, unlike the guinea pig (Foulon et al., 1993), combined tachykinin NK_1 and NK_2 receptor antagonists do not act synergistically on neurokinin A-induced bronchospasm in dogs (Chapman et al., 1999). Therefore, the potent effect of SCH 206272 on neurokinin A-induced bronchospasm in dogs would not be the result of synergistic interactions between tachykinin NK_1 and NK_2 receptors. In the dog, SCH 206272 had a duration of activity of 12 h after oral dosing with 3 mg/kg.

SCH 206272 was also active in a variety of models involving endogenously released tachykinins. For example, the bronchoconstriction induced by hyperventilation with dry isocapnic gas (Ray et al., 1989) or room air (Chapman and Danko, 1985) in guinea pigs is mediated, in part, by the release of tachykinins from bronchopulmonary C-fibers (Ray et al., 1989; Mauser et al., 1995; Solway et al., 1993; Yang et al., 1997). In this study, hyperventilation-induced bronchoconstriction was attenuated by SCH 206272 and SR 48968 but not by CP 99994. The only known effect of NK_3 receptors in the lung is inhibition of cholinergic neurotransmission (Myers and Undem, 1993), but this mechanism is unlikely to play a major role in the actions of SCH 206272 because activation of cholinergic airway nerves does not contribute significantly to guinea pig hyperventilation-induced bronchoconstriction (Chapman and Danko, 1985). Therefore, it is likely that SCH 206272 inhibits guinea pig hyperventilation-induced bronchospasm predominantly via tachykinin NK_2 receptor antagonism.

Inhalation of aerosolized capsaicin induces cough in guinea pigs (Kohrogi et al., 1988; Bolser et al., 1997) and this effect is blocked with tachykinin receptor antagonists (Advenier et al., 1993; Bolser et al., 1997). Both NK_1 and NK_2 receptor antagonists block cough and both CP 99994 and SR 48968 were active in this study. NK_3 receptor antagonists have been shown to display antitussive effects in guinea pigs (Daoui et al., 1998), but, surprisingly, we found no activity of SR 142801 to inhibit capsaicin-induced cough. It is important to note that in the study by Daoui et al. (1998), cough challenge was performed with inhaled citric acid which mechanistically may differ from the capsaicin challenge used in our study. Bolser et al. (1997) have presented data indicating that the primary site of action of tachykinin receptor antagonists is in the central nervous system. Therefore, SCH 206272 may produce its antitussive effects by acting at the level of the central nervous system. Nevertheless, capsaicin is a potent stimulant of pulmonary C-fibers and releases tachykinins from the C-fiber nerve terminals into the lungs (Otsuka and Yoshioka, 1993). Therefore, a peripheral site of action of SCH 206272 to block cough cannot be ruled out.

Airway microvascular leakage induced by nebulized hypertonic saline in guinea pigs involves the release of tachykinins from airway sensory nerves (Piedimonte et al., 1993), and, in this study, the airway microvascular leakage induced by hypertonic saline was blocked by CP 99994 and partially by SR 48968. Previous studies identify an important role for NK_1 receptors in the airway microvascular leakage induced by hypertonic saline (Piedimonte et al., 1993), but this is the first report of inhibition by an NK_2 receptor antagonist. In previous studies in guinea pigs, Daoui et al. (1998, 2000, 2001) reported that tachykinin NK_3 receptors augment airway microvascular leakage, indicating an important role for the tachykinin NK_3 receptor subtype. Therefore, inhibition of the airway microvascular leakage induced by nebulized hypertonic saline by SCH 206272 may result from combined antagonism of tachykinin NK_1, NK_2, and NK_3 receptor subtypes.

In conclusion, these studies identify SCH 206272 as a potent tachykinin receptor antagonist that is active across several species, including humans. Furthermore, SCH 206272 is orally active in a variety of animal models that involve tachykinin pathophysiology.

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