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Nonpeptide Tachykinin Receptor Antagonists. III. SB 235375, a Low Central Nervous System-Penetrant, Potent and Selective Neurokinin-3 Receptor Antagonist, Inhibits Citric Acid-Induced Cough and Airways Hyper-reactivity in Guinea Pigs

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ABSTRACT

In this report the in vitro and in vivo pharmacological and pharmacokinetic profile of (−)-(*S*)-*N*-(α-ethylbenzyl)-3-(carboxymethoxy)-2-phenylquinoline-4-carboxamide (SB 235375), a low central nervous system (CNS)-penetrant, human neurokinin-3 (NK-3) receptor (hNK-3R) antagonist, is described. SB 235375 inhibited ^{125}I-[MePhe^7]-neurokinin B (NKB) binding to membranes of Chinese hamster ovary (CHO) cells expressing the hNK-3R (CHO-hNK-3R) with a K_i = 2.2 nM and antagonized competitively NKB-induced Ca^{2+} mobilization in human embryonic kidney (HEK) 293 cells expressing the hNK-3R (HEK 293-hNK-3R) with a K_b = 12 nM. SB 235375 antagonized senktide (NK-3R)-induced contractions in rabbit isolated iris sphincter (pA_2 = 8.1) and guinea pig ileal circular smooth muscles (pA_2 = 8.3). SB 235375 was selective for the hNK-3R compared with hNK-1 (K_i > 100,000 nM) and hNK-2 receptors (K_i = 209 nM), and was without effect, at 1 μM, in 68 other receptor, enzyme, and ion channel assays. Intravenous SB 235375 produced a dose-related inhibition of miosis induced by i.v. senktide in the rabbit (ED_{50} of 0.56 mg/kg). Intraperitoneal SB 235375 (10–30 mg/kg) inhibited citric acid-induced cough and airways hyper-reactivity in guinea pigs. In mice oral SB 235375 (3–30 mg/kg) was without significant effect on the behavioral responses induced by intracerebral ventricular administration of senktide. Pharmacokinetic evaluation in the mouse and rat revealed that oral SB 235375 was well absorbed systemically but did not effectively cross the blood-brain barrier. The preclinical profile of SB 235375, encompassing high affinity, selectivity, oral activity, and low CNS penetration, suggests that it is an appropriate tool compound to define the pathophysiological roles of the NK-3Rs in the peripheral nervous system.

The tachykinins, or neurokinins, are a family of small peptides: the main mammalian members are substance P (11 amino acids), neurokinin A (NKA; 10 amino acids), and neurokinin B (NKB; 10 amino acids), which are distributed in both the central (CNS) and peripheral nervous systems. A major location of the tachykinins is in capsaicin-sensitive, primary afferent neurons (unmyelinated sensory fibers) that are predominant in various sites, including the lung, skin, and gastrointestinal tract (Otsuka and Yoshioka, 1993; Maggi, 1996). It has been speculated that the tachykinins play a pathophysiological role in several diseases (Maggio, 1988; Nakanishi, 1991; Maggi et al., 1993; Maggi, 1995). The diverse biological effects of the tachykinins are mediated via three tachykinin receptor subtypes, neurokinin-1 (NK-1R), NK-2R, and NK-3R, which belong to the superfamily of G protein-coupled, seven transmembrane-spanning receptors (Nakanishi, 1991; Gerard et al., 1993; Maggi, 1995).

Of the tachykinins receptors the NK-3R has been the least

ABBREVIATIONS: NKA, neurokinin A; NKB, neurokinin B; CNS, central nervous system; NK-1R, neurokinin-1 receptor; NK-2R, neurokinin-2 receptor; NK-3, neurokinin-3; NK-3R, neurokinin-3 receptor; PEG, polyethylene glycol; CHO, Chinese hamster ovary; CHO-hNK-3R, CHO cells stably expressing the human NK-3R; CHO-hNK-2R, CHO cells stably expressing the human NK-2R; CHO-hNK-1R, CHO cells expressing the human NK-1R; HEK, human embryonic kidney; HEK 293-hNK-3R, HEK 293 cells stably expressing the human NK-3R; HEK 293-mNK-3R, HEK 293 cells transiently expressing the murine NK-3R; HEK 293-mNK-2R, HEK 293 cells transiently expressing the murine NK-2R; IC_{50}, concentration of antagonist causing 50% inhibition of agonist response; K_i, apparent inhibition constant; K_b, dissociation constant; ANOVA, analysis of variance; 5-HT, serotonin (5-hydroxytryptamine).

widely studied, and minimal information exists on the potential relevance of this receptor to disease etiology. A key event in the NK-3R research area occurred in 1995 with the identification of the first potent and selective, nonpeptide NK-3R antagonist, SR 142,801 (Emonds-Alt et al., 1995; Oury-Donat et al., 1995). This was followed soon thereafter with the report of a novel chemical class of potent, competitive, and selective nonpeptide NK-3R antagonists, based on the 4-quinolinecarboxamide backbone (Giardina et al., 1996). Members of this class include SB 223412 (Sarau et al., 1997) and SB 222200 (Sarau et al., 2000). SB 223412 is a moderate CNS-penetrant compound, whereas SB 222200 more effectively crosses the blood-brain barrier.

The NK-3R is distributed in the rodent CNS (Ding et al., 1996; Shughrue et al., 1996; Mileusnic et al., 1999a,b) and in the human brain (Buell et al., 1992; Mileusnic et al., 1999a,b). There is also evidence, from electrophysiological, biochemical, and pharmacological analyses, for the presence of this receptor in the peripheral nervous system, including in guinea pig bronchial parasympathetic ganglia (Myers and Undem, 1993; Myers et al., 1996), guinea pig ileum (Maggi et al., 1990; Yau and Mandel, 1992), rabbit iris sphincter muscle (Medhurst et al., 1997), guinea pig gall bladder (Mawe, 1995), rat kidney (Chen and Hoover, 1993), and rat portal vein (Dion et al., 1987). However, to date there have been no reports of the presence of functional NK-3Rs in human lung. The evaluation of the potential pathophysiological roles of the NK-3R in the peripheral nervous system, and the possible therapeutic utility of NK-3R antagonists in peripheral diseases, will be assisted significantly by the identification of tool compounds that have very limited ability to enter the CNS, certainly lower than SB 223412, SB 222200, and SR 142,801. Herein, we describe the pharmacological and pharmacokinetic profile of an analog of SB 223412 and SB 222200, (−)-(*S*)-*N*-(α-ethylbenzyl)-3-(carboxymethoxy)-2-phenylquinoline-4-carboxamide (SB 235375; Fig. 1), which is a potent and selective, orally active NK-3R antagonist and is a low CNS-penetrant compound in the rat and mouse. SB 235375 is effective against citric acid-induced cough and airways hyper-reactivity in guinea pigs, providing further support for a significant influence of lung NK-3Rs in modulating pulmonary function in this species (Daoui et al., 1997, 1998, 2000).

Experimental Procedures

All in vivo procedures involving guinea pigs, rabbits, and mice were performed in accordance with protocols approved by the Glaxo-SmithKline Institutional Animal Care and Use Committee, and met or exceeded the standards of the American Association for the Accreditation of Laboratory Animal Care, the United States Department of Health and Human Services, and all local and federal animal welfare laws.

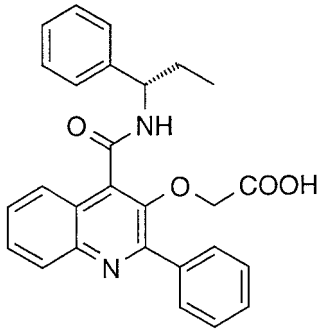

Fig. 1. Structure of SB 235375.

Materials. ^{125}I-[MePhe^7]-NKB, ^{125}I-NKA, and ^{125}I-substance P (all with specific activities of 2200 Ci/mmol) were obtained from PerkinElmer Life Sciences (Boston, MA). NKA, NKB, substance P, and [MePhe^7]-NKB were purchased from Peninsula Laboratories (Belmont, CA) and senktide {succinyl-[Asp^9 MePhe^8]-SP(6-13)}was purchased from California Peptide Research, Inc. (Napa, CA). PEG-400 was purchased from Aldrich Chemical (Milwaukee, WI). Carbachol and atropine were obtained from Sigma Chemical (St. Louis, MO). SB 235375 isomers and racemate, (*S*)-(+)-*N*-{[3-[1-benzoyl-3-(3,4-dichlorophenyl)piperidine-3-yl]prop-1-yl}-4-phenylpiperidin-4-yl}-*N*-methylacetamide (SR 142,801), (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide (SR 48,968), and (+)-(2*S*,3*S*)-*cis*-(2-methoxybenzylamino)-2-phenylpiperidine dihydrochloride (CP 99,994) were synthesized in the Department of Medicinal Chemistry, SmithKline Beecham S.p.A, Milan, Italy.

Cloning and Expression of Human and Mouse Tachykinin Receptors. The human (h) NK-1R, hNK-2R, and hNK-3R, and mouse (m) NK-3R and mNK-2R were isolated, cloned, and expressed in Chinese hamster ovary (CHO) or human embryonic kidney (HEK) 293 cell lines (Sarau et al., 1997, 2001). The human receptors were stably expressed in CHO cells for binding experiments and HEK 293 cells for calcium mobilization studies. The clonal cell line expressing the highest number of receptors per cell for each receptor was used in the ligand binding and functional assays. The murine receptors were expressed transiently in HEK 293 cells for both binding and calcium mobilization experiments.

Radioligand Binding Assays. Receptor binding assays were performed with crude membranes from CHO cells stably expressing the hNK-1R (CHO-hNK-1R), hNK-2R (CHO-hNK-2R), or hNK-3Rs (CHO-hNK-3R) and membranes from HEK 293 cells transiently expressing the mNK-3R (HEK 293-mNK-3R) or mNK-2R (HEK 293-mNK-2R), as detailed previously (Sarau et al., 1997, 2001). Competition binding studies for mNK-2R were performed using ^{125}I-NKA (0.15 nM) binding to HEK 293-mNK-2R membranes incubated in 150 μl of 25 mM Tris, pH 7.4, containing 4 mM $MnCl_2$, 1 μM phosphoramidon, and 0.1% ovalbumin, with or without antagonist, for 90 min at 25°C. Incubations were stopped by rapid filtration with a Packard Filtermate 96-well harvester (Packard Instrument Co., Meriden, CT) through Packard GF/C filters that were presoaked for 30 min in 0.1% polyethylenimine. Membranes were washed with 10 ml of ice-cold 20 mM Tris, pH 7.4, containing 0.1% bovine serum albumin, and then 50 μl of Microscint-20 was added to each well, and the radioactivity was counted in a Packard Topcount scintillation counter. Concentration-response curves for each compound were run using duplicate samples in at least three independent experiments. Specific binding was determined by subtracting nonspecific binding from total binding, which was assessed as the binding in the presence of 1 μM cold NKA.

For all binding studies percent inhibition of specific binding was determined for each concentration of compound, and the concentration required to inhibit 50% of the specific binding (IC_{50}) was obtained from concentration-response curves. The apparent inhibition constant, K_i, was calculated from the IC_{50} (Cheng and Prusoff, 1984).

NK-3R binding assays were also performed using ^{125}I-[MePhe^7]-NKB binding to a crude membrane preparation of brain tissue from male Hartley guinea pigs (450–650 g; Hazelton Research Animals, Denver, PA) and Sprague-Dawley rats (250–350 g; Charles River Breeding Laboratories, Kingston, NY) as described previously (Sarau et al., 1997).

Calcium Mobilization Assay. The functional antagonist activity of SB 235375 was determined by assessing its effects against tachykinin-induced Ca^{2+} mobilization in HEK 293 cells stably expressing the hNK-1R (HEK 293-hNK-1R), hNK-2R (HEK 293-hNK-2R), or hNK-3R receptor (HEK 293-hNK-3R), and in HEK 293 cells tran-

siently expressing the mNK-3R (HEK 293-mNK-3R) or mNK-2R (HEK 293-mNK-2R) as outlined previously (Sarau et al., 1997, 2001).

Senktide-Induced Contraction in Rabbit Isolated Iris Sphincter Muscle. The effect of SB 235375 on senktide-induced contraction of rabbit iris sphincter muscle strips from male rabbits (2–3 kg of body weight; H.A.R.E. Rabbitry, Hewitt, NJ) was determined as outlined previously (Medhurst et al., 1997). Tissues were exposed to SB 235375 (0.03–3 μM) or vehicle (distilled water) for 30 min before cumulative concentration-effect curves to senktide were obtained; atropine (1 μM) was present during construction of the senktide concentration-effect curve. Responses to senktide were expressed as a percentage of the contraction to carbachol (10 μM) added at the start of the experiment, which served as the reference response. The antagonist potency was expressed as pA_2 ($-$log of the dissociation constant), determined from Schild plot analysis of the results (Arunlakshana and Schild, 1958). The dissociation constant, K_b, for the antagonist-NK-3R complex was calculated from the equation $K_b = [B]/X - 1$, where X is the ratio of the concentration of agonist used in the presence and absence of antagonist.

Senktide-Induced Contraction in Guinea Pig Ileal Circular Smooth Muscle. Ileum was removed from male Hartley guinea pigs (weight range 450–650 g; Charles River, Portage, MI) and placed in modified Krebs-Henseleit solution (113 mM NaCl, 4.8 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $KH_2PO_4 \cdot 2H_2O$, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4 \cdot 7H_2O$, and 5.5 mM glucose) overnight at 4°C to diminish spontaneous contractile activity. On the day of the experiment rings of circular smooth muscle (4–6 mm in length) were prepared and placed in 10-ml organ baths containing Krebs-Henseleit solution, which was gassed with 95% O_2, 5% CO_2 and maintained at 37°C and pH 7.4. Preparations were connected via stainless steel hooks and silk suture to Grass FT03C force-displacement transducers. Mechanical responses were recorded isometrically by MP100WS/Acknowledge data acquisition system (BIOPAC Systems, Santa Barbara, CA) run on Macintosh computers. Tissues were equilibrated under 1.5-g resting load (Maggi et al., 1990) for at least 1 h before the start of each experiment and washed every 15 min. After the equilibration period, tissues were contracted with 10 μM carbachol and then rinsed three times over 15 min. The addition of carbachol and rinse was repeated twice to confirm the maximal response of the tissue. The mean of the three carbachol contractions served as a reference contraction for data analysis. Tissues were then rinsed three times over 30 to 40 min before the start of the experiment. Phosphoramidon (10 μM), a neutral endopeptidase inhibitor, and 1 μM CP 99,994, a NK-1R antagonist, were added to each bath before starting the experiment to block enzymatic breakdown of peptides and substance P-mediated contractile effects, respectively.

Senktide concentration-response curves were obtained by a cumulative addition of the agonist in half-log increments. Each concentration was left in contact with the preparation for 5 min before the addition of the subsequent agonist concentration; the response to each senktide concentration spiked and returned toward baseline. At the end of the experiment, tissues were exposed to 10 μM carbachol to rule out a nonspecific effect of SB 235375 on smooth muscle contractility. Paired tissues were exposed to SB 235375 (0.1–10 μM) or vehicle for 30 min before senktide concentration-response curves were generated. Agonist-induced responses for each tissue were expressed as a percentage of the mean of the three reference carbachol (10 μM)-induced contractions obtained at the beginning of the experiment (precarbachol). Geometric mean EC_{50} values (pD_2 values) were calculated from linear regression analyses of data and the pA_2 for SB 235375 determined as outlined above.

Citric Acid-Induced Cough in Guinea Pigs. Assessment of cough in male Hartley guinea pigs (500–800 g; Charles River) followed methodology previously described (Kotzer et al., 2000). Briefly, cough was induced by inhalation of an aerosol of 0.4 M citric acid, which has been shown previously to induce the cough reflex in guinea pigs (Forsberg et al., 1992). The aerosol was administered to the animals via a small-volume ultrasonic nebulizer (AeroSonic model 5000D; DeVilbiss, Somerset, PA) connected to the bias flow port immediately before the exposure chamber inlet. A volume of 2 ml of citric acid solution was placed into the ultrasonic nebulizer, and during the 1-min aerosolization period approximately 0.5 ml of the solution was nebulized. The incidence of cough over 13 min (aerosolization time + observation time) was recorded. Animals were used for only one citric acid challenge due to tachyphylaxis of the cough response (Kotzer et al., 2000). SB 235375 was administered i.p. at doses of 3, 5, 10, and 30 mg/kg, 30 min before cough challenge with citric acid.

Citric Acid-Induced Airways Hyper-reactivity in Guinea Pigs. Male Hartley guinea pigs (500–800 g) were treated i.p. with SB 235375 or vehicle, 5 min after administration of thiorphan (1 mg/kg i.p.), the neutral endopeptidase inhibitor, and 30 min before exposure to a 20-min aerosol of 0.4 M citric acid or saline. Twenty-four hours later, the animals were anesthetized with urethane (1.2 g/kg i.p.) approximately 10 min before surgery, and the jugular vein and trachea were cannulated with 50 and 260 polyethylene tubing, respectively. The animals were placed into a whole-body plethysmograph, paralyzed with i.v. succinylcholine chloride (2 mg/kg), and ventilated at 60 breaths/min and 3.75 ml/breath by a Harvard rodent respirator (model 683; Harvard Instruments, South Natick, MA). Transpulmonary pressure was measured with a differential pressure transducer ($\pm$80 cm of H_2O, model MP 45; Validyne Engineering, Northridge, CA) that was connected on the positive pressure side to a side arm pressure tap from the trachea and on the negative pressure side to a 16-gauge needle inserted parallel to the heart into the thoracic cavity. Flow through the pneumotachograph was measured with a differential pressure transducer ($\pm$2 cm of H_2O, model MP 45; Validyne Engineering). Flow and pressure signals were used to calculate R_L and C_{dyn} throughout the experiment with Modular Instruments Hardware and BioWindows software (Modular Instruments, West Chester, PA). An ascending noncumulative dose-response curve to i.v. acetylcholine (10, 20, 50, and 100 μg/kg) was determined for each animal.

[Nle^{10}]-NKA(4-10)-Induced Bronchoconstriction in Guinea Pigs. Male Hartley guinea pigs (600–700 g) were treated with SB 235375 (10 mg/kg i.p), the NK-2R antagonist SR 48968 (10 mg/kg i.p), or vehicle, 10 min before anesthesia with ketamine/rompum (60 mg/kg/10 mg/kg i.m.) followed by vascular and tracheal catheterization as described above. Animals were paralyzed with pancuronium bromide (0.1 mg/kg i.v.), ventilated, and treated with the neutral endopeptidase inhibitor phosphoramidon (1 mg/kg i.v.). Five minutes later (approximately 35 min after i.p. vehicle, SB 235375, or SR 48968), the NK-2R-selective agonist [Nle^{10}]-NK A(4-10) (0.3 and 1.0 nmol/kg) was administered i.v., and bronchospasm was recorded as described above.

Senktide-Induced Miosis in Rabbits. Senktide (25 μg in 0.2 ml of 5% dimethyl sulfoxide/95% saline) was injected via i.v. bolus in the marginal ear vein. Before injection, baseline pupil diameter measurements were taken with a Finescale Comparator focusing magnifier; the data were recorded in millimeters. Measurements were made and recorded at 2.5-, 5-, 7-, 10-, 15-, and 20-min post-senktide administration (Medhurst et al., 1997). SB 235375 (0.25–1 mg/kg i.v.) or vehicle (0.2 ml of phosphate-buffered saline) was given 2.5 min before administration of senktide.

Senktide-Induced Behavioral Activity in Mice. Male BALB/c inbred mice (20–25 g), obtained from Charles River Breeding Laboratories (Raleigh, NC), were maintained in a barrier-sustained facility. For these experiments, mice were anesthetized using an isoflurane mixture (95% oxygen, 5% isoflurane), the head was shaved, and a midline incision was made in the scalp. Injections into the right lateral ventricle were made at set coordinates from the skull landmark bregma (2 mm posterior, 2 mm lateral, and 2 mm below the skull surface) by using a 27-gauge needle and micromanipulator. Senktide (0.05 nmol) or vehicle (sterile isotonic saline; 5-μl volume) was administered i.c.v. 30 min after administration of oral SB 235375 (3–30 mg/kg) or vehicle (water for SB 235375 or 50% PEG-

400/1% methylcellulose for SB 222200). Immediately after the mice were challenged with i.c.v. senktide, the head twitches (i.e., a vigorous shake response) and/or tail whips (i.e., typically counted individually as a rattle that consists of several twitches in tandem) were counted over 15 min (Stoessl et al., 1987, 1990; Sarau et al., 1997). For comparison, the effect of oral administration of SB 222200 (3 mg/kg), the high CNS-penetrant NK-3R antagonist (Sarau et al., 2000), against responses to i.c.v. senktide was explored. For these experiments, the mean and S.E.M. for each group were determined, and data were analyzed using one-way ANOVA with Dunnett's post hoc test; a p value of 0.05 or lower was considered significant.

Pharmacokinetic Studies in Rat. Oral bioavailability evaluations in the rat were carried out using i.v./p.o. crossover experimental design. Indwelling femoral vein (for drug infusion) and artery catheters (for blood sampling) were placed in male Sprague-Dawley rats (300–400 g; $n = 6$) under ketamine/xylazine anesthesia a week before the studies. On study day 1, each rat received a single i.v. infusion of SB 235375 (1.2 mg/kg/h; 30 min; 80% PEG/20% ethanol). On study day 2, each rat was crossed over to receive a single oral solution dose of SB 235375 (3.4 mg/kg). Blood samples were collected at various times over 24 h after dosing, and plasma was prepared by centrifugation and stored at −30°C until analysis for plasma concentrations of SB 235375 by using high-performance liquid chromatography with triple quadrupole mass spectrometric detection. The lower limit of quantitation was 10 ng/ml for 50 μl of rat plasma.

CNS Penetration Experiments in Rat and Mouse. The CNS penetration study in rats involved i.v. infusion of SB 235375 (1 mg/kg/h) for 6 h to approach steady-state conditions. Blood samples (two 50-μl plasma aliquots) were collected, into heparinized microcentrifuge tubes, from each mouse at 30-min intervals during the final 2 h of infusion, placed on crushed ice, and then centrifuged to isolate plasma. Immediately upon completion of the infusion, the animals were euthanized, and the entire brain was removed and then homogenized in saline. The brain from each mouse was weighed and placed in a volume of chilled isotonic saline equal to 4 times the weight of tissue. Each sample was homogenized individually with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) and frozen on dry ice. Plasma and brain tissue homogenate samples were stored at −30°C until analysis for concentrations of SB 235375 by using quantitative liquid chromatography with triple quadrupole mass spectrometric detection analysis. Plasma concentrations of SB 235375 are expressed as nanograms per milliliter, whereas brain concentrations are given as nanograms of SB 235375 per gram of total brain weight.

A study was conducted in mouse comparing plasma and brain concentrations of SB 235375 at various times after oral administration. Male BALB/c mice (19–21 g; $n = 4$) were pretreated with oral SB 235375 (10 mg/kg) at various times (30 min and 1, 2, and 4 h); then the animals were euthanized, blood samples (about 0.5 ml) were drawn, and the entire brain was removed. Plasma and brain tissue homogenate samples were stored at −30°C, until analysis for concentrations of SB 235375 as outlined above.

Results

Pharmacological Characterization

In Vitro Studies. *Binding experiments: NK-3Rs.* SB 235375 produced enantioselective inhibition of the binding of ^{125}I-[MePhe^7]-NKB to CHO-hNK-3R cell membranes. Thus, the active S-enantiomer, SB 235375, inhibited the binding of ^{125}I-[MePhe^7]-NKB to CHO-hNK-3 cell membranes with a K_i of 2.2 ± 0.3 nM ($n = 6$), whereas the racemate, SB 280765, and the less potent R-isomer, SB 283352, had K_i values of 4.1 ± 1.1 nM ($n = 3$) and 251 ± 37 nM ($n = 3$), respectively (Fig. 2A).

The affinities of SB 235375 for the murine (m), rat, and guinea pig NK-3Rs were also determined. SB 235375 inhibited the binding of ^{125}I-[MePhe^7]-NKB to HEK 293-mNK-3R cell membranes with a K_i of 82.4 ± 19.5 nM ($n = 3$). The potency of SB 235375 for inhibition of ^{125}I-[MePhe^7]-NKB binding to brain cortical membranes was higher for the guinea pig NK-3R (K_i = 1.6 nM; $n = 1$) than for the rat NK-3R (K_i = 17.9 nM; $n = 2$).

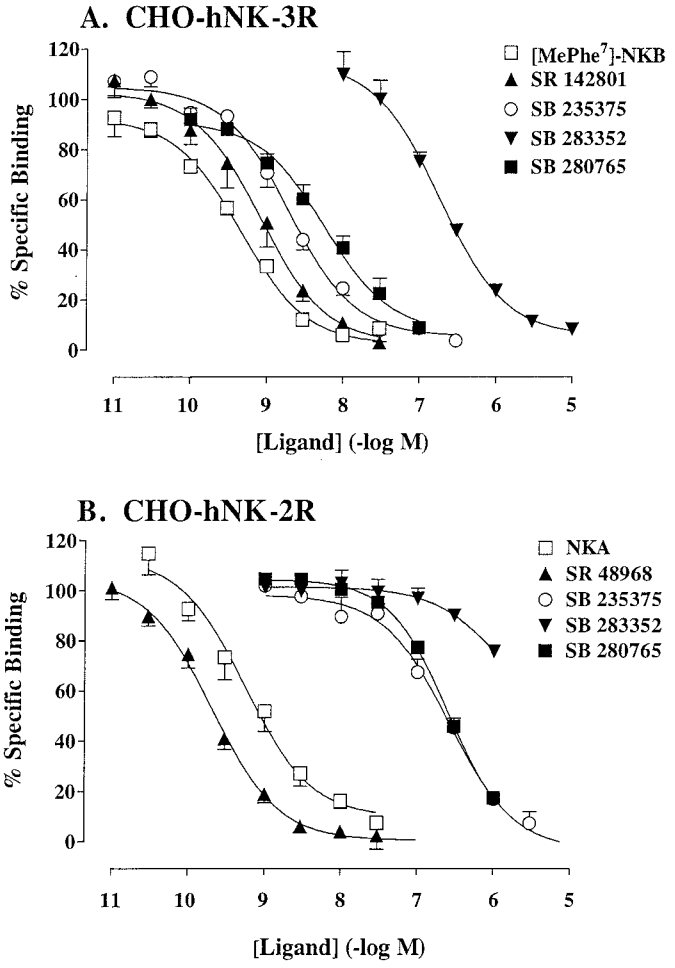

Fig. 2. A, competition binding of ^{125}I-[MePhe^7]-NKB to CHO-hNK-3 membranes by [MePhe^7]-NKB, SR 142801, the enantiomers, and racemate of SB 235375. B, competition binding of ^{125}I-NKA to CHO-hNK-2 membranes by NKA, SR 48968, the enantiomers, and racemate of SB 235375. In A, competition binding to CHO hNK-3 membranes for [MePhe^7]-NKB, racemic, R-, and S-isomers of SB 235375 was performed as described under *Experimental Procedures*. □, [MePhe^7]-NKB; ○, (S)-SB 235375; ▼, (R)-SB 283352; ■, (R,S)-SB 280765-A; ▲, SR 142,801. Values presented are the mean ± S.E.M. of three to seven experiments. In B, competition binding to CHO hNK-2 membranes for NKA, SR 48,968, R- (SB 283352) and S-isomers (SB 235375) and racemate (SB 280765-A) of SB 235375 was performed as described under *Experimental Procedures*. □, NKA; ▲, SR 48,968; ○, (S)-SB 235375; ▼, (R)-SB 283352; ■, (R,S)-SB 280765. Values presented are the mean ± S.E.M. of three to six experiments.

Ca^{2+} mobilization studies: NK-3Rs. Cellular functional NK-3R antagonist activity of SB 235375 was determined in Ca^{2+} mobilization studies by using HEK 293-hNK-3R cells. SB 235375 inhibited Ca^{2+} mobilization induced by 1 nM NKB (EC_{75} for NKB) with an IC_{50} of 81.9 ± 4.0 nM ($n = 3$). SB 235375 (33 nM-1 μM) produced a concentration-dependent, surmountable inhibition of NKB-induced Ca^{2+} mobilization in HEK 293-hNK-3 cells. Schild plot analysis of the data revealed a pA_2 of 7.9 (K_b = 12 nM; $n = 2$) and a slope of

1.1, i.e., not significantly different from 1, indicative of competitive antagonism (Fig. 3). Similar to the results of binding experiments, SB 235375 was a weak inhibitor of NKB (1 nM)-induced Ca^{2+} mobilization in HEK 293-mNK-3R with an IC_{50} = 3600 nM (n = 2).

Selectivity profile. Selectivity of SB 235375 for the hNK-3R relative to other tachykinin receptors was determined from assessment of its effects in competitive binding experiments by using binding of ^{125}I-NKA and [^{3}H]substance P to membranes prepared from CHO-hNK-2R and CHO-hNK-1R cell membranes, respectively. SB 235375 had moderate, enantioselective potency for inhibition of ^{125}I-NKA binding to CHO-hNK-2R with a K_i = 209 ± 14 nM (n = 6) (Fig. 2B) but was without effect, in concentrations up to 100 μM, on the binding of [^{3}H]substance P to CHO-hNK-1R (n = 2).

SB 235375 was a weak inhibitor of ^{125}I-NKA binding to HEK 293-mNK-2R cell membranes, with a K_i of 5253 ± 397 nM (n = 3). Furthermore, in NK-2R binding assays with guinea pig lung and rat bladder membranes, SB 235375 had low potency for inhibiting ^{125}I-NKA binding, with an IC_{50} = 95,815 ± 55,486 nM (n = 5) and a K_i = 2,332 ± 688 nM (n = 3), respectively.

Further evidence of selectivity was provided from Ca^{2+} mobilization experiments. SB 235375 weakly inhibited 10 nM NKA-induced Ca^{2+} mobilization in HEK 293-hNK-2R cells with an IC_{50} of 4200 nM (n = 2); SR 48968, the positive control NK-2R antagonist (Emonds-Alt et al., 1992), had an IC_{50} of 1.3 ± 0.4 nM (n = 5). SB 235375 was a weak antagonist at the murine NK-2R, inhibiting NKA (3.3 nM)-induced Ca^{2+} mobilization in HEK 293-mNK-2R cells with an IC_{50} of 35,000 nM (n = 2). SB 235375 had an IC_{50} > 10,000 nM (n = 2) for inhibition of substance P (1 nM)-induced Ca^{2+} mobilization in HEK 293-hNK-1R cells; CP 99,994, the positive control NK-1R antagonist (McLean et al., 1993), had an IC_{50} of 37.7 nM (n = 2).

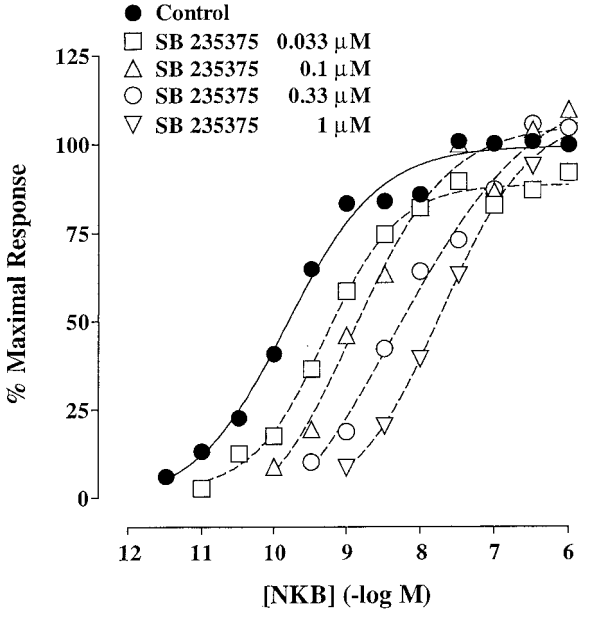

Fig. 3. Competitive nature of cellular functional activity of SB 235375. Concentration-response curves were generated for NKB using fura-2-loaded HEK 293 hNK-3 cells in the absence (●) and presence of 33 nM (□), 100 nM (△), 330 nM (○), and 1000 nM (▽) SB 235375. Results presented are a representative of two experiments.

SB 235375, at concentrations up to 1 or 10 μM, was without effect in 68 receptor-binding and enzyme assays, including endothelin ($endothelin_A$, $endothelin_B$), interleukin-8 (CXCR1, CXCR2), C5a, leukotriene D_4, leukotriene B_4, formyl-methionyl-leucyl-phenylalanine, adenosine (A_1, A_2), serotonin (5-HT_{1A}, 5-HT_{1Da}, 5-HT_{1Db}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_4), opiate (μ, δ, κ), nicotinic, muscarinic (m_1, m_2), 5-lipoxygenase, coenzyme A-independent transacylase, prostaglandin H synthetase (COX1), phopholipase A_2 [rh(LMW), rh(HMW)], protein kinase C, and phosphodiesterases (PDE1, PDE2, PDE3, PDE4, and PDE5) (data not shown).

Reversibility and time dependence of antagonist activity. The NK-3R antagonist activity of SB 235375 in the Ca^{2+} mobilization assay was rapidly reversible and not time-dependent. Thus, treatment with various concentrations of SB 235375 for 5 min, followed by two washes and resuspension in fresh buffer without antagonist over 30 min, resulted in a significant loss of the inhibitory activity for SB 235375 against NKB (1 nM)-induced calcium mobilization in HEK 293-NK-3R cells [washed, IC_{50} = 7800 nM; n = 2; control (no washing) = 82 nM; n = 3]. Furthermore, the inhibition of NKB (1 nM)-induced calcium mobilization by SB 235375 was similar with 5-s (IC_{50} = 146 nM; n = 2) or 5-min incubation (IC_{50} = 218 nM; n = 2).

Senktide-induced contraction in rabbit isolated iris sphincter muscle and guinea pig ileal circular smooth muscle. NK-3R agonists, such as senktide, potently and effectively contract rabbit isolated iris sphincter muscle (Medhurst et al., 1997) and guinea pig ileal circular smooth muscle (Maggi et al., 1990). SB 235375 (0.03–3 μM) produced a concentration-dependent and surmountable antagonism of senktide-induced contractions in rabbit isolated iris sphincter muscle. Schild plot analysis of the results revealed a pA_2 of 8.1 and a slope of 1.0, indicative of competitive antagonism (n = 5) (Fig. 4). Similar results were obtained with SB 235375 (0.1–10 μM) against senktide-induced contractions in guinea pig ileal circular smooth muscle: pA_2 = 8.3 (slope = 0.8; n = 4) (data not shown).

In Vivo Studies. *Senktide (NK-3R)-induced miosis in rabbits.* A rabbit model of senktide-induced miosis (pupil con-

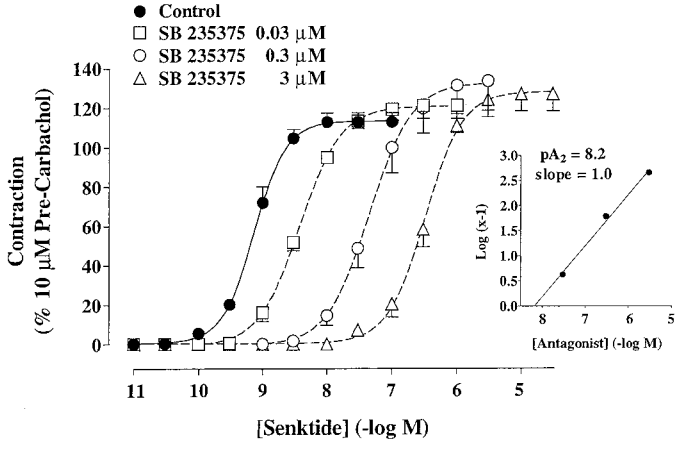

Fig. 4. Antagonism of senktide-induced contraction in rabbit isolated iris sphincter muscle. Concentration-response curves were generated for senktide in the absence (●) and presence of SB 235375 (□, 30 nM; ○, 300 nM; and △, 3 μM). Results are expressed as a percentage of the response to 10 μM carbachol and are expressed as the mean ± S.E.M. of five experiments. The inset represents the Schild plot analysis of the data.

striction) was developed, based upon the contractile effects of NK-3R agonists in the rabbit isolated iris sphincter muscle preparation (Medhurst et al., 1997). Intravenous SB 235375 (0.25–1 mg/kg) produced a potent, dose-related inhibition of i.v. senktide (25 μg)-induced miosis in the rabbit with an ED_{50} of 0.56 mg/kg (n = 3) (Fig. 5).

Citric acid-induced cough in guinea pigs. Aerosol administration of citric acid to guinea pigs produces cough; this model is used routinely to evaluate potential antitussives. In this study, exposure of saline-treated animals to 0.4 M citric acid for 1 min resulted in 16 ± 0.8 coughs in the 13-min measurement period (n = 8; data not shown). Prior treatment with SB 235375 (3–30 mg/kg i.p.) produced a dose-related inhibition of citric acid-induced cough in guinea pigs with an ID_{50} of 14.2 mg/kg (Fig. 6A).

Citric acid-induced airways hyper-reactivity in guinea pigs. Exposure to citric acid (0.4 M for 20 min) resulted in a significantly enhanced response to i.v. acetylcholine (50 or 100 μg/kg i.v.), assessed 24 h after citric acid challenge, compared with aerosol saline-exposed animals (Fig. 6B; n = 4–5, $P < 0.05$). When animals were pretreated with SB 235375 (10 mg/kg i.p., 35 min before citric acid), the citric acid-induced hyper-responsiveness to acetylcholine was attenuated; thus, the acetylcholine dose response was normalized to that observed in saline-exposed animals (Fig. 6B; n = 5, $P > 0.05$).

Nle^{10}-NKA (4-10) (NK-2R)-induced bronchospasm in guinea pigs. To ensure that the in vivo effects of SB 235375 were not due to NK-2R antagonism, the activity of the compound against NK-2R-mediated bronchoconstriction in guinea pigs was evaluated. The NK-2R-selective agonist [Nle^{10}]-NKA (4-10), produced dose-related increases in airway resistance when administered intravenously (0.3–1 nmol/kg) to anesthetized, ventilated guinea pigs that had been treated with vehicle (Fig. 7). [Nle^{10}]-NKA (4-10)-induced bronchoconstriction was abolished by pretreatment with the NK-2R antagonist SR 48968, but unaffected by SB 235375 (10 mg/kg i.p.) (Fig. 7).

Senktide (NK-3R)-induced behavioral responses in mice. Administration of NK-3R-selective ligands, such as senktide, results in a characteristic set of serotonin-mediated behaviors in rodents (Stoessl et al., 1987, 1990). In the current study the effects of oral SB 235375 against behavioral responses induced by i.c.v. senktide in mouse was explored. Oral SB 235375 (3–30 mg/kg; 30-min pretreatment) was without significant effect on the increase in head shakes and tail whips induced by i.c.v. senktide (0.05 nmol); there was a trend toward inhibition at the highest dose of SB 235375 (Fig. 8). In contrast, a low oral dose of SB 222200 (3 mg/kg), a high CNS-penetrant NK-3R antagonist, significantly inhibited, by about 50%, senktide-induced behavioral responses (Fig. 8).

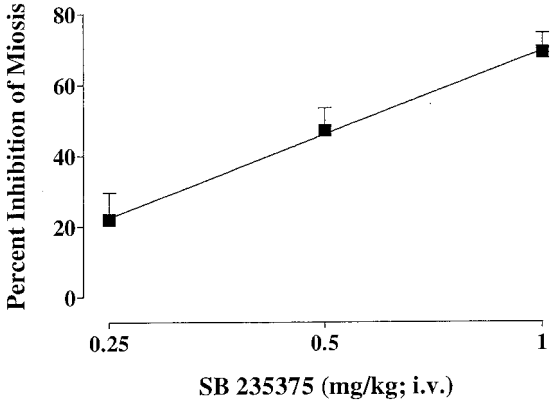

Fig. 5. Effects of SB 235375 (0.25–1.0 mg/kg i.v.) against senktide-induced miosis in rabbit. Animals were treated with vehicle (0.2 ml of Dulbecco's phosphate-buffered saline) or SB 235375 (0.25, 0.5, or 1.0 mg/kg i.v.) 2.5 min before exposure to senktide. Results are expressed as a percentage of the control response as the mean ± S.E.M. of three experiments.

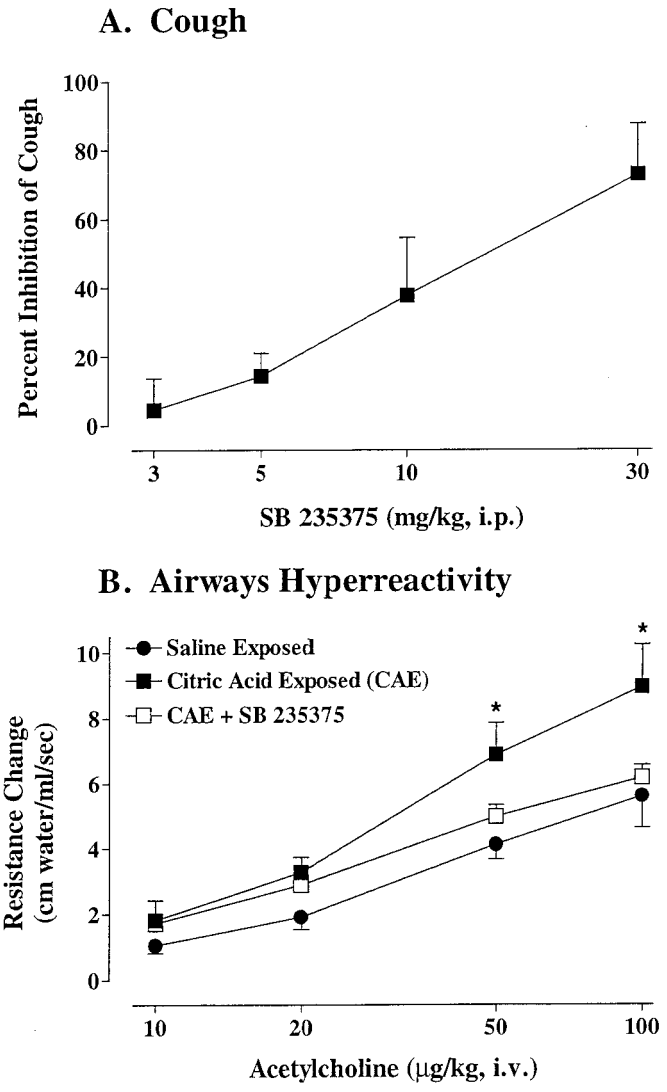

Fig. 6. Effect of SB 235375 on citric acid-induced cough (A) and airways hyper-reactivity (B) in guinea pigs. A, animals were treated with vehicle or SB 235375 (3–30 mg/kg i.p.), 30 min before exposure to aerosolized citric acid (0.4 M) for 1 min. The results are expressed as percentage of inhibition of the citric acid-induced cough response compared with vehicle-treated animals. The results are expressed as the mean ± S.E.M; n = 5. B, animals were treated with SB 235375 (□, 10 mg/kg i.p.) or vehicle (■) 5 min after administration of thiorphan (1 mg/kg i.p.) and 30 min before exposure to a 20-min aerosol of 0.4 M citric acid. An aerosol control group was exposed to 0.9% sterile saline for 20 min (●). Twenty-four hours later, the responsiveness to acetylcholine (10, 20, 50, and 100 μg/kg i.v.) was assessed. Results are expressed as mean ± S.E.M; n = 4 to 5; *$P < 0.05$, ANOVA.

Pharmacokinetic Characterization

The pharmacokinetic profile of SB 235375 in rats (n = 6) was assessed after oral administration (3.4 mg/kg; solution

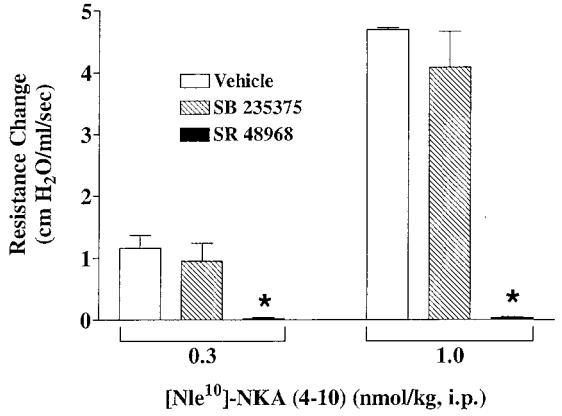

Fig. 7. Effect of SB 235375 on [Nle^{10}]-NKA (4-10)-induced bronchoconstriction in guinea pigs. Animals were treated with vehicle, SB 235375 (10 mg/kg i.p.), or SR 48968 (10 mg/kg i.p.) before anesthesia, cannulation, and ventilation, and then challenged with the NK-2R-selective agonist [Nle^{10}]-NKA (4-10) (0.3 and 1.0 nmol/kg i.v.) 35 min later. Bronchoconstriction was determined as lung resistance changes from baseline (cm of H_2O/ml/s) and expressed as mean ± S.E.M. (n = 6–7, $^*P < 0.001$, ANOVA).

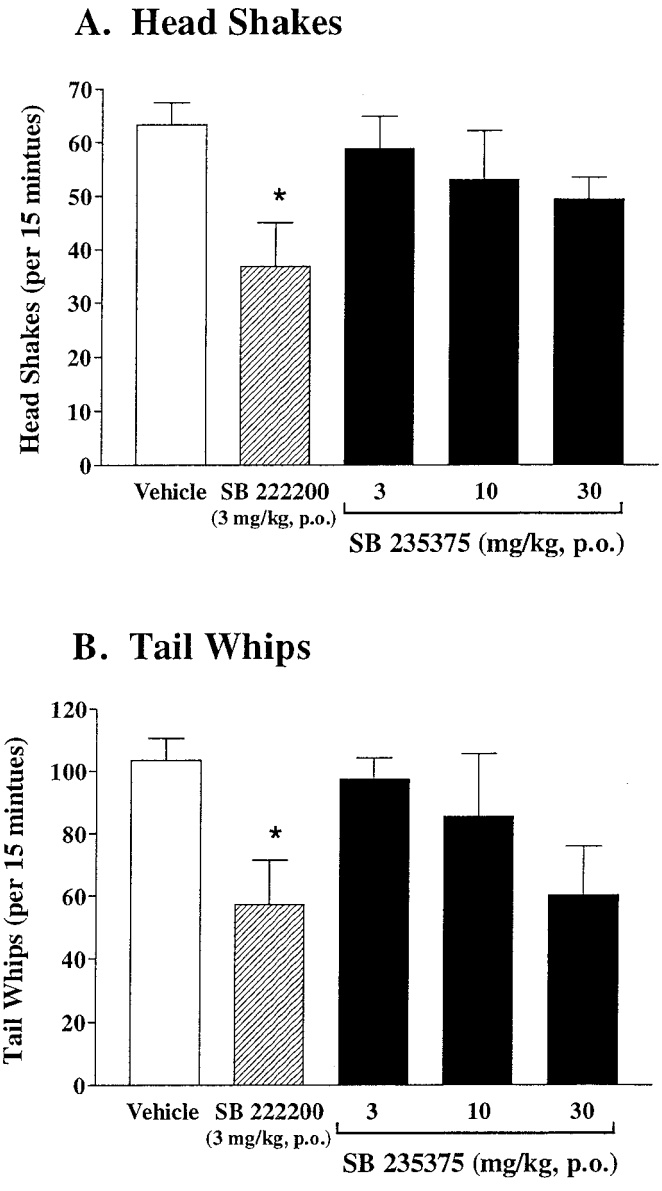

Fig. 8. Effect of oral administration of SB 235375 on behavioral effects of central NK-3R stimulation by i.c.v. senktide. Oral SB 235375 (3–30 mg/kg; 30-min pretreatment) was without effect on the increase in head shakes (A) and tail whips (B) induced by i.c.v. senktide (0.05 nmol). In contrast, oral SB 222200 (3 mg/kg), a high CNS-penetrant NK-3R antagonist, inhibited senktide-induced behavioral responses. Results are given as the mean ± S.E.M.; n = 5 to 8 mice/group; $^*P < 0.05$.

dose) and i.v. infusion (1.2 mg/kg). Systemic plasma clearance of SB 235375 in the rat was moderate (22.0 ± 3.5 ml/min/kg) with a volume of distribution approximately 3 times total body water (2.7 ± 1.0 l/kg), and the terminal half-life was approximately 3 to 5 h. After oral administration, maximum plasma concentrations were obtained at approximately 75 min after dosing, and oral bioavailability was determined to be 23 ± 4%. No significant amounts of circulating metabolites were detected by high-performance liquid chromatography/UV analysis of plasma samples. Preliminary assessment in dogs and monkeys revealed a similar pharmacokinetic profile of SB 235375 in these species: low clearance and oral bioavailability of 40% in monkeys and moderate clearance and oral bioavailability of 39% in dogs (data not shown).

CNS penetration of SB 235375 was assessed in the rat (n = 3). After a 6-h continuous i.v. infusion of SB 235375 (1 mg/kg/h), brain tissue concentrations of SB 235375 were below the limit of detection. Plasma concentrations of SB 235375 were relatively constant during the last 2 h of the infusion (range 670-1400 ng/ml), indicating that steady-state conditions had been achieved.

After oral administration of SB 235375 (10 mg/kg) to mice, plasma concentrations >100 ng/ml were obtained at all times points (0.5, 1, 2, and 4 h), whereas no SB 235375 was detected in brain homogenates at any time postdosing (Fig. 9). At the time used to assess the effects of SB 235375 (10 mg/kg) against senktide-induced behavioral effects, i.e., 30 min after administration, plasma concentrations of SB 235375 were 654 ± 77.6 ng/ml, which is equivalent to 1.4 μM.

Discussion

It is only relatively recently that potent and selective, nonpeptide NK-3R antagonists have been identified (Emonds-Alt et al., 1995; Oury-Donat et al., 1995; Giardina et al., 1996; Sarau et al., 1997). It is anticipated that these tool compounds will assist greatly in the elucidation of the potential pathophysiological roles of the NK-3Rs, for which there is currently limited information. NK-3Rs have been demonstrated in both the CNS and the peripheral nervous system, where their activation modulates the release of various neurotransmitters (Maggi et al., 1990; Stoessl et al., 1990; Arenas et al., 1991; Schemann and Kayser, 1991; Ramirez et al., 1994). Electrophysiological analysis has also provided evidence of a neuromodulatory role of the NK-3R in the guinea pig tracheal and bronchial parasympathetic ganglia (Myers and Undem, 1993; Myers et al., 1996) and gall bladder (Mawe, 1995). The elucidation of the physiological and pathophysiological roles of the NK-3R requires the utility of compounds with different abilities to enter the CNS. SR 142,801 (Emonds-Alt et al., 1995; Oury-Donat et al., 1995), SB 222200 (Sarau et al., 2000), and, to a lesser extent, SB 223412 (Sarau et al., 1997) effectively enter the CNS. The

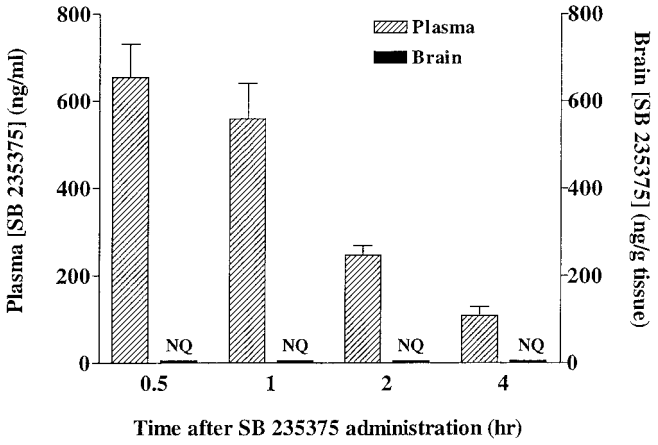

Fig. 9. Comparison of plasma and brain concentrations of SB 235375 after oral administration in the mouse. Mice were administered 10 mg/kg SB 235375 by gavage. Plasma (▨) and brain concentrations (■) of SB 235375 were measured at 0.5, 1, 2, or 4 h as described under *Experimental Procedures*. Results are expressed as mean ± S.E.M.; $n = 4$. NQ, not quantifiable, i.e., below the limit of detection.

current study identifies SB 235375 as a potent and selective NK-3R antagonist that is a low CNS-penetrant compound in the rat. Accordingly, SB 235375 would appear to be an appropriate tool compound with which to assess the role of NK-3Rs in peripheral diseases.

SB 235375 belongs to the class of nonpeptide NK-3R antagonists that are based on the 4-quinolinecarboxamide backbone (Giardina et al., 1996). Functional and binding studies indicate that SB 235375 is a high-affinity antagonist for the hNK-3R: $pA_2 = 7.9$ for inhibition of NKB-induced calcium mobilization in HEK 293-NK-3R cells, and $K_i = 2.2$ nM for inhibition of ^{125}I-MePhe^7-NKB binding to CHO-hNK-3R cell membranes. In addition, the data indicate that SB 235375 is a selective NK-3R antagonist. Thus, with respect to the tachykinin receptors, SB 235375 has about 100-fold selectivity for the hNK-3R versus the hNK-2R and over 100,000-fold selectivity versus the hNK-1R. Furthermore, SB 235375 was without effect, in concentrations of 1 or 10 μM, in 68 receptor binding, ion channel, and enzyme assays. Overall, SB 235375 has a pharmacological and selectivity profile similar to those of SB 223412 (Sarau et al., 1997) and SB 222200 (Sarau et al., 2000).

Various techniques have demonstrated the presence of NK-3Rs in mammalian CNS, including humans, and also in the peripheral nervous system (Dion et al., 1987; Maggi et al., 1990; Buell et al., 1992; Yau and Mandel, 1992; Chen and Hoover, 1993; Myers and Undem, 1993; Mawe, 1995; Ding et al., 1996; Myers et al., 1996; Shughrue et al., 1996; Medhurst et al., 1997; Mileusnic et al., 1999a,b). NK-3R activation modulates the release of several neurotransmitters, including acetylcholine in the CNS (Arenas et al., 1991) and the gastrointestinal tract (Maggi et al., 1990; Schemann and Kayser, 1991; Ramirez et al., 1994). This suggests that NK-3R antagonists may have therapeutic utility in diseases that are characterized by dysfunction in neuronal inputs. However, there is no clinical information available on the effects of NK-3R antagonists in any disorder and minimal data from the evaluation of compounds in animal models of disease. Regarding peripheral diseases, SR 142,801, the NK-3R antagonist (Emonds-Alt et al., 1995), has demonstrated efficacy in guinea pig (Mazelin et al., 1998) and rat models of gastrointestinal tract diseases (Julia et al., 1999).

There is increasing evidence that NK-3Rs play a significant role in controlling pulmonary function in guinea pigs via an influence on nerve inputs. For example, comprehensive electrophysiological analysis of guinea pig tracheal or bronchial parasympathetic ganglia, including desensitization experiments with an NK-3R-selective agonist and the use of SR 142,801, revealed that exogenous administration of tachykinins or endogenous release using capsaicin increases membrane depolarization by activation of NK-3Rs (Myers and Undem, 1993; Myers et al., 1996). Thus, the evidence suggests that tachykinins released from afferent nerve fibers modulate nerve inputs, including parasympathetic drive, via NK-3R stimulation. These results form part of the growing body of data that local ganglionic reflexes occur in the airways. In vivo studies provide additional support for the in vitro findings. For example, aerosol administration of NKB and NK-3R-selective agonists elicited airways hyper-responsiveness in guinea pigs (Daoui et al., 2000). In addition, in guinea pigs SR 142,801 inhibited substance P-induced airways hyper-reactivity (Daoui et al., 1997) and significantly attenuated citric acid-induced cough, and the airways hyper-reactivity and potentiation of histamine-induced microvascular permeability induced by citric acid (Daoui et al., 1998). The mechanism for the inhibition of airways hyper-responsiveness by the NK-3R antagonists is not clear. The citric acid-induced hyper-responsiveness may involve a serial process involving more than one tachykinin receptor as suggested previously (Daoui et al., 2000). It has been demonstrated that NK-3R-induced airways hyper-responsiveness is not related to bronchoconstriction because the former phenomenon is observed with selective NK-3R agonists at doses that are not bronchoconstrictive (Daoui et al., 2000). In the current study, SB 235375, which is chemically distinct from SR 142,801, also inhibits citric acid-induced cough and airways hyper-responsiveness in guinea pigs. Although SB 235375 has moderate potency at human and guinea pig NK-2Rs, it had had no effect on bronchoconstriction elicited by the NK-2R-selective agonist [Nle^{10}]-NKA (4-10) in guinea pigs. Overall, the findings suggest that the inhibitory effects of SR 142,801 and SB 235375 in these guinea pig models are indeed due to blockade of NK-3Rs.

A key issue relates to the site of action (peripheral versus central?) for the inhibitory actions of SR 142,801 and SB 235375 against citric acid-induced cough and airways hyper-responsiveness in guinea pigs. Based upon direct assessment of their abilities to enter the brain and/or the potent inhibition of NK-3R-mediated CNS effects in rodents, SR 142,801 (Emonds-Alt et al., 1995), as well as SB 223412 (Sarau et al., 1997) and SB 222200 (Sarau et al., 2000), effectively enter the CNS. Accordingly, they are not appropriate tool compounds to explore exclusively the peripheral roles of the NK-3R. In contrast, the current findings indicate that SB 235375 has limited ability to enter the CNS. Thus, the brain/plasma ratio for SB 235375 in the rat was very low (brain concentrations were below the limit of detection), and significantly less than for SB 222200 (Sarau et al., 2000) and SB 223412 (Sarau et al., 1997). Furthermore, despite possessing about a 2-fold higher affinity for the mNK-3R ($K_i = 82$ nM) than SB 222200 ($K_i = 174$ nM; Sarau et al., 2000), concomitant with about 2-fold higher plasma concentrations of SB

235375 over SB 222000 at 30 min after oral administration in the mouse, SB 235375, in oral doses up to 30 mg/kg, was without significant effect on i.c.v. senktide-induced behavioral effects in this species, whereas SB 222200 produced significant inhibition at 3 mg/kg p.o. Direct measurement after oral administration in the mouse demonstrated that SB 235375 concentrations in the brain were below the limit of detection, confirming that it is a low CNS-penetrant compound; in this study high plasma concentrations of SB 235375, equivalent to >1 μM at 30 min postdosing, were detected. The similar effects of SR 142,801 (Daoui et al., 1997, 1998) and SB 225375 against citric acid-induced cough and airways hyper-reactivity in guinea pigs, despite what appears to be marked differences in their abilities to enter the CNS, suggest that it is the lung, rather than the CNS, that is their site of action for producing these effects. However, this hypothesis requires additional investigation. For example, it is possible that, although SB 235375 is a poor CNS penetrant compound, it may enter the brain in sufficient concentrations to exert inhibitory effects on pulmonary function. Pharmacokinetic investigation in rats, dogs, and monkeys demonstrated that SB 235375 is a low-to-moderate clearance molecule with oral bioavailabilities ranging from 23 to 40%. Thus, SB 235375 may also have utility in evaluating the role of peripheral NK-3Rs in animal models in these three species.

The inhibitory effects of SB 235375 in calcium mobilization experiments in HEK 293-hNK-3R are not time-dependent and are reversed rapidly by washout, and the antagonism by SB 235375 of NKB-induced calcium mobilization and senktide-induced contraction in rabbit isolated iris sphincter smooth muscle and guinea pig ileal circular smooth muscle is surmountable. These data indicate the competitive nature of the antagonism by the chemical class to which SB 235375 belongs; this includes the previously described SB 223412 (Sarau et al., 1997) and SB 222200 (Sarau et al., 2000). In contrast, SR 142,801, a member of a structurally distinct chemical class (Emonds-Alt et al., 1995; Oury-Donat et al., 1995), appears to be a noncompetitive antagonist; the inhibitory effects of SR 142801 are time-dependent, insurmountable, and reversed very slowly by washout (Patacchini et al., 1995; Sarau et al., 2000).

In summary, the current study indicates that SB 235375 is a high-affinity, selective, reversible, and competitive antagonist of the hNK-3R. It is a low CNS-penetrant compound and is effective against citric acid-induced airways hyperreactivity and cough in guinea pigs. The preclinical pharmacodynamic profile of SB 235375 suggests that it will be a useful compound to assist in the elucidation of the pathophysiological roles of NK-3R activation, especially in the peripheral nervous system.

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References

Arenas E, Alberch J, Perez-Navarro E, Solsona C, and Marsal J (1991) Neurokinin receptor differentially mediate endogenous acetylcholine release evoked by tachykinins in the neostriatum. *J Neurosci* **11:**2332–2338.

Arunlakshana O and Schild HO (1958) Some quantitative uses of drug antagonists. *Br J Pharmacol* **14:**48–58.

Buell G, Schulz MF, Arkinstall SJ, Maury K, Missotten M, Adami N, Talabot F, and Kawashima E (1992) Molecular characterization, expression and localization of human neurokinin-3 receptor. *FEBS Lett* **299:**90–95.

Chen Y and Hoover DB (1993) Pharmacological characterization of the pressor response to tachykinins in isolated perfused rat kidney. *J Pharmacol Exp Ther* **267:**1176–1180.

Cheng Y-C and Prusoff WH (1984) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which cause 50 percent inhibition (IC_{50}) of an enzymatic reaction. *Biochem Pharmacol* **22:**3099–3108.

Daoui S, Cognon C, Naline E, Emonds-Alt X, and Advenier C (1998) Involvement of tachykinin NK3 receptors in citric acid-induced cough and bronchial responses in guinea pigs. *Am J Respir Crit Care Med* **158:**42–48.

Daoui S, Cui Y-Y, Lagente V, Emonds-Alt X, and Advenier C (1997) A tachykinin NK_3 receptor antagonist, SR 142801 (Osanetant), prevents substance P-induced bronchial hyperreactivity in guinea-pigs. *Pulm Pharmacol Ther* **10:**261–270.

Daoui S, Naline E, Lagente V, Emonds-Alt X, and Advenier C (2000) Neurokinin B- and specific tachykinin NK_3 receptor agonists-induced airway hyperresponsiveness in the guinea-pig. *Br J Pharmacol* **130:**49–56.

Ding Y-Q, Shigemoto R, Takada M, Ohishi H, Nakanishi S, and Mizuno N (1996) Localization of the neuromedin K receptor (NK_3) in the central nervous system of the rat. *J Comp Neurol* **364:**291–310.

Dion S, D'Orléans-Juste P, Drapeau G, Rhaleb NE, Rouissi N, Tousignant C, and Regoli D (1987) Characterization of neurokinin receptors in various isolated organs by the use of selective agonists. *Life Sci* **41:**2269–2278.

Emonds-Alt X, Bichon D, Ducoux JP, Heaulme M, Miloux B, Poncelet M, Proietto V, Van Broeck D, Vilain P, Neliat G, et al. (1995) SR 142801, the first potent non-peptide antagonist of the tachykinin NK-3 receptor. *Life Sci* **56:**PL27–PL32.

Emonds-Alt X, Vilain P, Goulaouic P, Proietto V, Van Broeck D, Advenier C, Naline E, Neliat G, Le Fur G, and Breliere JC (1992) A potent and selective non-peptide antagonist of the neurokinin A (NK-2) receptor. *Life Sci* **50:**101–106.

Forsberg K, Karlsson JA, Zackrisson C, and Persson CGA (1992) Selective inhibition of cough and bronchoconstriction in conscious guinea pigs. *Respiration* **59:**72–76.

Gerard NP, Bao L, He X-P, and Gerard C (1993) Molecular aspects of the tachykinin receptors. *Regul Pept* **43:**21–35.

Giardina GAM, Sarau HM, Farina C, Medhurst AD, Grugni M, Foley JJ, Raveglia LF, Schmidt DB, Rigolio R, Vassallo M, et al. (1996) 2-Phenyl-4-quinolinecarboxamides: a novel class of potent and selective non-peptide competitive antagonists for the human neurokinin-3 receptor. *J Med Chem* **39:**2281–2284.

Julia V, Su X, Buéno L, and Gebhart GF (1999) Role of neurokinin 3 receptors on responses to colorectal distention in the rat: electrophysiological and behavioral responses. *Gastroenterology* **116:**1124–1131.

Kotzer CJ, Hay DWP, Dondio G, Giardina G, Petrillo P, and Underwood DC (2000) The antitussive activity of delta opioid receptor stimulation in guinea pigs. *J Pharmacol Exp Ther* **292:**803–809.

Maggi CA (1995) The mammalian tachykinin receptors. *Gen Pharmacol* **26:**911–944.

Maggi CA (1996) Tachykinins in the autonomic nervous system. *Pharm Res* **33:**161–170.

Maggi CA, Patacchini R, Giachetti A, and Meli A (1990) Tachykinin receptors in the circular muscle of the guinea-pig ileum. *Br J Pharmacol* **101:**996–1000.

Maggi CA, Patacchini R, Rovero P, and Giachetti A (1993) Tachykinin receptors and tachykinin receptor antagonists. *J Auton Pharmacol* **13:**23–93.

Maggio JE (1988) Tachykinins. *Annu Rev Neurosci* **11:**13–28.

Mawe GM (1995) Tachykinins as mediators of slow EPSPs in guinea-pig gall-bladder ganglia: involvement of neurokinin-3 receptors. *J Physiol (Lond)* **485:**513–524.

Mazelin L, Theodorou V, More J, Emonds-Alt X, Fioramonti J, and Bueno L (1998) Comparative effects of nonpeptide tachykinin receptor antagonists on experimental gut inflammation in rats and guinea-pigs. *Life Sci* **63:**293–304.

McLean S, Ganong A, Seymour PA, Snider RM, Desai MC, Rosen T, Bryce DK, Longo KP, Reynolds LS, Robinson G, et al. (1993) Pharmacology of CP-99994; a nonpeptide antagonist of the tachykinin neurokinin-1 receptor. *J Pharmacol Exp Ther* **267:**472–479.

Medhurst AD, Hay DWP, Parsons AA, Martin LD, and Griswold DE (1997) *In vitro* and *in vivo* characterization of NK 3 receptor subtypes in the rabbit eye by use of selective non-peptide NK3 receptor antagonists. *Br J Pharmacol* **122:**469–476.

Mileusnic D, Lee JM, Magnuson DJ, Hejna MJ, Krause JE, Lorens JB, and Lorens SA (1999a) Neurokinin-3 receptor distribution in rat and human brain: an immunohistochemical study. *Neuroscience* **89:**1269–1290.

Mileusnic D, Magnuson DJ, Hejna MJ, Lorens JB, Lorens SA, and Lee JM (1999b) Age and species-dependent differences in the neurokinin B system in rat and human brain. *Neurobiol Aging* **20:**19–35.

Myers AC and Undem BJ (1993) Electrophysiological effects of tachykinins and capsaicin on guinea-pig bronchial parasympathetic ganglion neurones. *J Physiol (Lond)* **470:**665–679.

Myers A, Undem B, and Kummer W (1996) Anatomical and electrophysiological comparison of the sensory innervation of bronchial and tracheal parasympathetic ganglion neurons. *J Auton Nerv Syst* **61:**162–168.

Nakanishi S (1991) Mammalian tachykinin receptors. *Annu Rev Neurosci* **14:**123–136.

Otsuka M and Yoshioka K (1993) Neurotransmitter functions of mammalian tachykinins. *Physiol Rev* **73:**229–308.

Oury-Donat F, Carayon P, Thurneyssen O, Pailhon V, Emonds-Alt X, Soubrié P, and Le Fur G (1995) Functional characterization of the non-peptide neurokinin-3 (NK-3) receptor antagonist, SR 142801 on the human NK-3 receptor expressed in Chinese hamster ovary cells. *J Pharmacol Exp Ther* **274:**148–154.

Patacchini R, Barthò L, Holzer P, and Maggi CA (1995) Activity of SR 142801 at peripheral tachykinin receptors. *Eur J Pharmacol* **278:**17–25.

Ramirez MJ, Cenarruzabeitia Del Rio J, and Lasheras B (1994) Involvement of neurokinins in the non-cholinergic response to activation of 5-HT_3 and 5-HT4 receptors in guinea pig ileum. *Br J Pharmacol* **111:**419–424.

Sarau HM, Field JA, Ames RA, Brawner ME, Bergsma D, Elshourbagy NA, Rao P, Schmidt DB, Foley JJ, Luttmann MA, et al. (2001) Molecular and pharmacological characterization of the murine NK-3 receptor. *Eur J Pharmacol* **413:**143–150.

Sarau HM, Griswold DE, Potts W, Foley JJ, Schmidt DB, Webb EF, Martin LD, Brawner ME, Elshourbagy NA, Medhurst AD, et al. (1997) Nonpeptide tachykinin receptor antagonists. I. Pharmacological and pharmacokinetic characterization of SB 223412, a novel, potent and selective NK-3 receptor antagonist. *J Pharmacol Exp Ther* **281:**1303–1311.

Sarau HM, Griswold DE, Potts W, Foley JJ, Schmidt DB, Webb EF, Martin LD, Medhurst AD, Giardina GAM, and Hay DWP (2000) Nonpeptide tachykinin receptor antagonists. II. Pharmacological and pharmacokinetic profile of SB 222200, a CNS-penetrant, potent and selective NK-3 receptor antagonist. *J Pharmacol Exp Ther* **295:**373–381.

Schemann M and Kayser H (1991) Effects of tachykinins on myenteric neurons of the guinea pig gastric corpus: involvement of NK-3 receptors. *Pfluegers Arch* **419:**566–571.

Shughrue PJ, Lane MV, and Merchenthaler I (1996) In situ hybridization analysis of the distribution of neurokinin-3 mRNA in the rat central nervous system. *J Comp Neurol* **372:**395–414.

Stoessl AJ, Dourish CT, and Iversen SD (1990) Pharmacological characterization of the behavioural syndrome induced by the NK-3 tachykinin agonist senktide in rodents: evidence for mediation by endogenous 5-HT. *Brain Res* **517:**111–116.

Stoessl AJ, Dourish CT, Young SC, Williams BJ, Iversen SD, and Iversen LL (1987) Senktide, a selective neurokinin B-like agonist, elicits serotonin-mediated behavior following intracisternal administration in the mouse. *Neurosci Lett* **80:**321–326.

Yau WM and Mandel KG (1992) $Neurokinin_3$ receptor regulation of acetylcholine release from myenteric plexus. *Am J Physiol* **263:**G659–G664.

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