Nonpeptide Tachykinin Receptor Antagonists. II. Pharmacological and Pharmacokinetic Profile of SB-222200, a Central Nervous System Penetrant, Potent and Selective NK-3 Receptor Antagonist

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ABSTRACT

The pharmacological and pharmacokinetic profile of SB-222200 ([S](-)-N-(α-ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide), a human NK-3 receptor (hNK-3R) antagonist, was determined. SB-222200 inhibited [125I]-[MePhe7]neurokinin B (NKB) binding to Chinese hamster ovary (CHO) cell membranes stably expressing the hNK-3 receptor (CHO-hNK-3R) with a Kᵢ = 4.4 nM and antagonized NKB-induced Ca²⁺ mobilization in HEK 293 cells stably expressing the hNK-3 receptor (HEK 293-hNK-3R) with an IC₅₀ = 18.4 nM. SB-222200 was selective for hNK-3 receptors compared with hNK-1 (Kᵢ > 100,000 nM) and hNK-2 receptors (Kᵢ = 250 nM). In HEK 293 cells transiently expressing murine NK-3 receptors (HEK 293-mNK-3R), SB-222200 inhibited binding of [125I]-[MePhe7]NKB (Kᵢ = 174 nM) and antagonized NKB (1 nM)-induced calcium mobilization (IC₅₀ = 265 nM). In mice oral administration of SB-222200 produced dose-dependent inhibition of behavioral responses induced by i.p. or intracerebral ventricular administration of the NK-3 receptor-selective agonist, senktide, with ED₅₀ values of approximately 5 mg/kg. SB-222200 effectively crossed the blood-brain barrier in the mouse and rat. The inhibitory effect of SB-222200 against senktide-induced behavioral responses in the mouse correlated significantly with brain, but not plasma, concentrations of the compound. Pharmacokinetic evaluation of SB-222200 in rat after oral administration (8 mg/kg) indicated sustained plasma concentrations (Cₘₐₓ = about 400 ng/ml) and bioavailability of 46%. The preclinical profile of SB-222200, demonstrating high affinity, selectivity, reversibility, oral activity, and central nervous system penetration, suggests that it will be a useful tool compound to define the physiological and pathophysiological roles of NK-3 receptors, in particular in the central nervous system.

The mammalian tachykinins, or neurokinins, are a family of small peptides, notably Substance P, neurokinin A (NKA), and neurokinin B (NKB), which share the common carboxy-terminal region Phe-Xaa-Gly-Leu-Met-NH₂ (Maggio, 1988; Maggi et al., 1993). The tachykinins are localized in both the central and peripheral nervous systems and have been proposed to play a pathophysiological role in several diseases (Otsuka and Yoshioka, 1993; Maggi, 1995, 1996). The biological effects of the tachykinins are mediated via three tachykinin receptor subtypes, neurokinin-1 (NK-1R), NK-2R, and NK-3R, which are members of the superfamily of G-protein-coupled, seven transmembrane-spanning receptors (Maggio, 1988; Nakaniishi, 1991; Maggi et al., 1993). The human variants of the three tachykinin receptors have been cloned and expressed (Gerard et al., 1990; Buell et al., 1992; Huang et al., 1992). The tachykinins and their receptors have been extensively studied for many years, with the focus on the NK-1R and Substance P, and to a lesser extent the NK-2R and NKA. A

ABBREVIATIONS: NKA, neurokinin A; NKB, neurokinin B; NK-1, neurokinin 1; NK-1R, neurokinin 1 receptor; NK-2, neurokinin 2; NK-2R, neurokinin 2 receptor; NK-3, neurokinin 3; NK-3R, neurokinin 3 receptor; CHO, Chinese hamster ovary; CHO-hNK-3R, CHO cells stably expressing the human NK-3 receptor; CHO-hNK-2R, CHO cells stably expressing the human NK-2 receptor; CHO-hNK-1R, CHO cells expressing the human NK-1 receptor; HEK, human embryonic kidney; HEK 293-hNK-3R, HEK 293 cells stably expressing the human NK-3 receptor; HEK 293-mNK-3R, HEK 293 cells transiently expressing the murine NK-3 receptor; Kᵢ, dissociation constant; ED₅₀, dose of antagonist producing 50% inhibition of the agonist response; pA₂, −log antagonist dissociation constant; LC/MS/MS, liquid chromatography with triple quadrupole mass spectrometric detection; CNS, central nervous system; PEG-400, polyethylene glycol-400.
milestone in the area of tachykinin biology was the identification of potent and selective, nonpeptide antagonists for the NK-1 and NK-2 receptors (Snider et al., 1991; Desai et al., 1992; Emonds-Alt et al., 1992; McLean et al., 1993), which have assisted in the study of the pathophysiological roles of these receptors and the potential therapeutic uses of their antagonists (Lowe and Snider, 1993; Mantyh et al., 1994; Ishizuka et al., 1995; Walsh et al., 1995). In 1995 the first potent and selective, nonpeptide NK-3R antagonist, SR-142801, was described (Emonds-Alt et al., 1995; Oury-Donat et al., 1995). Subsequently, a new chemical class of potent, competitive, and selective nonpeptide NK-3R antagonists, which are based on the 4-quinolinecarboxamide backbone, was reported (Giardina et al., 1996). A member of this class, SB-223412, has been characterized pharmacologically and pharmacokinetically, with the results indicating that it is a potent and selective, orally active NK-3R antagonist (Sarau et al., 1997). However, SB-223412 is only moderately central nervous system (CNS) penetrant in the rat. To investigate the potential pathophysiological role of the NK-3R in the CNS, and therapeutic utility of NK-3R antagonists in CNS disorders, it is important to identify compounds that are more CNS penetrant than SB-223412. In this report we describe the pharmacological and pharmacokinetic profile of an analog of SB-223412, SB-222200 [(S)-(-)-N-(α-ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide; Fig. 1], which is a potent and selective, orally active NK-3R antagonist that effectively crosses the blood-brain barrier in the rat and mouse.

**Experimental Procedures**

All procedures were performed in accordance with protocols approved by the SmithKline Beecham 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.

**Materials.** 125I-[MePhe7]NKB (specific activity, 2200 Ci/mmol), 125I-NKA (specific activity, 2200 Ci/mmol), and [3H]Substance P (specific activity, 34 Ci/mmol) were obtained from NEN Life Science Products (Boston, MA). NKA, NKB, Substance P, and [MePhe7]NKB were purchased from Peninsula Laboratories (Belmont, CA) and senktide (succinyl-[Asp5MePhe8]SP[6-13]) from California Peptide Research, Inc. (Napa, CA). Polyethylene glycol-400 (PEG-400) was purchased from Aldrich Chemical Co. (Milwaukee, WI). SB-222200 isomers and racemate, its metabolite, SB-227734 [N-(α-acetylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide], SR-142801 [(S)-(+)-N-[3-(1-benzoyl-3,4-dichlorophenyl)piperidine-3-yl]prop-1-yl-4-phenylpipеридин-4-yl-N-methylacetamide], and CP-99994 [(+)-(2S,3S)-(cis-2-methoxybenzylamino)-2-phenylpiperidine dihydrochloride] were synthesized in the Department of Medicinal Chemistry, SmithKline Beecham SpA, Milan, Italy. Atropine was obtained from BDH Chemicals (Poole, UK) and carbachol from Sigma (Poole, UK).

**Receptor Cloning and Expression of Human and Mouse Tachykinin Receptors.** The human (h) NK-1R, NK-2R, and NK-3R, and mouse (m) NK-3R were isolated, cloned, and expressed in Chinese hamster ovary (CHO) or human embryonic kidney (HEK) 293 cell lines, as outlined previously (Sarau et al., 1997; H. M. Sarau, J. A. Field, R. A. Ames, M. E. Brawner, D. Bergsma, N. A. Elshourbagy, P. Rao, D. B. Schmidt, J. J. Foley, M. A. Luttmann, G. A. M. Giardina, and D. W. P. Hay, submitted for publication). The clonal cell line producing the highest number of receptors per cell for each receptor was identified and utilized in the ligand binding and cellular calcium assays.

**Radioligand Binding Assays.** Receptor binding assays were performed with crude membranes from CHO cells stably expressing the human NK-1R (CHO-hNK-1R), NK-2R (CHO-hNK-2R), and NK-3Rs (CHO-hNK-3R) and membranes from HEK 293 cells transiently expressing the mNK-3R (HEK 293-mNK-3R) as detailed previously (Sarau et al., 1997; H. M. Sarau, J. A. Field, R. A. Ames, M. E. Brawner, D. Bergsma, N. A. Elshourbagy, P. Rao, D. B. Schmidt, J. J. Foley, M. A. Luttmann, G. A. M. Giardina, and D. W. P. Hay, submitted for publication).

For all binding studies percent inhibition of specific binding was determined for each concentration of compound and the IC50, defined as the concentration required to inhibit 50% of the specific binding, obtained from concentration-response curves. Values presented are the apparent inhibition constant (Kb), which was calculated from the IC50 as described by Cheng and Prusoff (1973).

**Calcium Mobilization Assay.** Tachykinin-induced Ca2+ mobilization in HEK 293 cells stably expressing the hNK-1R, hNK-2R, and hNK-3R receptor (HEK 293-hNK-3R), and in HEK 293 cells transiently expressing the mNK-3R, were used to investigate the functional antagonist activity of the compounds (Sarau et al., 1997; H. M. Sarau, J. A. Field, R. A. Ames, M. E. Brawner, D. Bergsma, N. A. Elshourbagy, P. Rao, D. B. Schmidt, J. J. Foley, M. A. Luttmann, G. A. M. Giardina, and D. W. P. Hay, submitted for publication).

**Senktide-Induced Contraction in Rabbit Isolated Iris Sphincter Muscle.** The effect of SB-222200 on senktide-induced contraction of rabbit iris sphincter muscle strips was determined as described previously (Medhurst et al., 1997). Tissues were exposed to SB-222200 (300 nM) or vehicle (dimethyl sulfoxide) for 120 min before cumulative concentration-effect curves to senktide were obtained. Responses to senktide were expressed as a percentage of the carbachol-induced contraction. The dissociation constant, Kb, for the antagonist-NK-3 receptor complex was calculated from the equation: Kb = [B]/CR – 1, where CR is the concentration ratio of agonist used in the presence and absence of antagonist, B.

**Senktide-Induced Behavioral Activity.** Studies were conducted using male Balb/c inbred mice (six mice per group; weight = 20–25 g), obtained from Charles River Breeding Laboratories (Raleigh, NC), which were maintained in a barrier-sustained facility. Animals were orally administered various concentrations of SB-222200 or vehicle before challenge with the NK-3R-selective agonist, senktide, which was administered via s.c. or i.c.v. routes. For the s.c. studies, 30 min after administration of SB-222200 or vehicle (50%)

**Fig. 1. Structure of SB-222200.**
PEG-400/1% methylcellulose), the mice were challenged with senktide (1.0 mg/kg, s.c.) and 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 10 min (Stoessel et al., 1987, 1990; Sarau et al., 1997). For the i.c.v. experiments, mice were anesthetized with an isoflurane mixture (95% oxygen/5% isoflurane); heads were shaved and a midline incision made into the scalp. Brain 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) using a 27-gauge needle and micromanipulator. Senktide or vehicle (sterile isotonic saline; 5-μl volume) were administered i.c.v. 30 min after administration of oral SB-222200 (5 mg/kg) or vehicle. Several doses of senktide (i.e., 0.01, 0.025, and 0.05 nmol) were administered to different mice to produce an agonist dose-response relationship; head shakes and tail whips were recorded as described above.

For the mouse behavioral experiments, the mean and S.E.M. for each group were determined, and Student’s t test was used to investigate statistical significance; a P value of .05 or lower was considered significant. The ED₅₀ for oral SB-222200 was calculated from analysis of the dose-response curve by regression analysis software using BioStatistics P57 software.

**Pharmacokinetic Studies in Rat.** Bioavailability evaluations were carried out in rat using crossover experimental designs. Indwelling femoral vein (for drug infusion) and artery catheters (for blood sampling) were placed in male Sprague-Dawley rats (300–400 g; n = 3) under ketamine/xylazine anesthesia a week before the studies. 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. An HPLC/UV analytical method using reversed phase chromatography on an octadecylsilica column with detection at 333 nM, was used to analyze rat plasma samples.

Systemic plasma clearance in rats was determined after i.v. infusion of 2.5 mg/kg SB-222200. Studies to assess the oral bioavailability of SB-222200 were conducted 1 week later after administration of SB-222200 in solution (10 mg/kg at 1.0 mg/ml in 50% PEG-400, 49.5% water, 0.5% carboxymethylcellulose) to the same rats (fasted).

CNS penetration studies were performed by i.v. infusion of SB-222200 to rats (n = 3) for 6 h at 1 mg/kg/h to approach steady-state conditions. Blood samples were collected at 30-min intervals during the final 2 h of infusion. Immediately upon completion of the infusion, the animals were euthanized and the entire brain was removed and then homogenized in saline. Plasma and brain tissue homogenate samples were stored at –30°C, until analysis for concentrations of SB-222200 with quantitative liquid chromatography with triple quadrupole mass spectrometric detection (LC/MS/MS). Plasma concentrations of SB-222200 are expressed as nanograms/ml, whereas brain concentrations are given as nanograms of SB-222200 per gram of total brain weight.

**Pharmacodynamic Study in Mouse.** A pharmacodynamic study was performed in mice to explore the correlation between the plasma and brain concentrations of SB-222200 after oral administration and inhibition of s.c. senktide-induced behavioral responses. Male BALB/c mice (19–21 g; n = 3–6) were pretreated with oral SB-222200 (5 mg/kg) at various times (30 min, 1 h, 2 h, 4 h, and 8 h) before challenge with senktide (1.0 mg/kg, s.c.); the head twitches and tail whips induced by senktide were counted over 10 min as outlined above. Immediately upon completion of the experiment, the animals were euthanized, blood samples (about 0.5 ml) were drawn, and the entire brain removed. Blood samples were collected from each mouse into heparinized microcentrifuge tubes, placed on crushed ice, and then centrifuged to isolate plasma. Two 50-μl plasma aliquots from each sample were dispensed into polypropylene microcentrifuge tubes and frozen on dry ice. The brain from each mouse was weighed and placed in a volume of chilled isotonic saline equal to four times the weight of tissue. Each sample was homogenized individually with a polytron (Brinkmann Instrument Co., Switzerland) and frozen on dry ice. Plasma and brain tissue homogenate samples were stored at –30°C, until analysis for concentrations of SB-222200 using quantitative LC/MS/MS analysis. The limits of detection for the assays used were 25 and 10 ng/ml for the HPLC/UV and LC/MS/MS methods, respectively, using 50 μl of plasma.

**Results**

**Pharmacokinetic Characterization**

The pharmacokinetic profile of SB-222200 (in 50% PEG-400, 49.5% water, 0.5% carboxymethylcellulose) was assessed in rats after oral and i.v. (infusion) administration (Table 1). When administered by oral gavage (10 mg/kg) sustained plasma concentrations of SB-222200 were measured, with peak values attained over the first 4 h, of 427 ± 232 ng/ml (n = 3). Bioavailability was determined to be 46 ± 19%, with a terminal half-life (t₁/₂) of about 2 h (Fig. 2A and Table 1). The systemic plasma clearance of SB-222200 in the rat is high (56 ml/min/kg) with a moderately large volume of distribution (3–5 l/kg).

CNS penetration of SB-222200 was assessed directly in the rat. After 6 h of continuous i.v. infusion of SB-222200 at 1 mg/kg/h, brain tissue concentrations were 504 ± 43 ng/g (n = 3). Plasma concentrations of SB-222200 were relatively constant over the last 2 h of the infusion and at the 6-h time point had a mean value of 378 ± 30 ng/ml, yielding a brain tissue:plasma concentration ratio of approximately 1.3 (data not shown).

Analytical assessment revealed a circulating metabolite of SB-222200 after oral administration (10 mg/kg) to rats; the metabolite, SB-227734 was formed by σ = 1 oxidation of SB-222200. For example, 6 h after administration of SB-222200, the apparent brain tissue concentration of the metabolite, SB-227734, was lower, 70 ± 20 ng/g, than the mean plasma concentration, 247 ± 126 ng/ml (n = 3), producing a brain tissue:plasma concentration ratio of 0.28 (data not shown).

In the mouse pharmacodynamic study, both brain and plasma concentrations of SB-222200 after oral administration (5 mg/kg) had peaked by 30 min, which was the first time point examined (Fig. 2B); the brain concentration 30 min after administration was 122.4 ± 17.8 ng/g. The brain concentrations of SB-222200 were maintained at levels >80 ng/g after 60 and 120 min (Fig. 2B).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Pharmacokinetic assessment of SB-222200 after i.v. or p.o. administration to the rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values are given as the mean ± S.E.M. for the results of p.o. studies (n = 3) and as the individual values and average (given in parentheses) of the results of i.v. studies (n = 2).</td>
<td></td>
</tr>
<tr>
<td><strong>Dose (mg/kg)</strong></td>
<td>i.v. Values (Average)</td>
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<tr>
<td>---</td>
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</tr>
<tr>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>36, 58 (47)</td>
<td>93.5 ± 42.6</td>
</tr>
<tr>
<td>43, 69 (56)</td>
<td>122 ± 52</td>
</tr>
<tr>
<td>2.4, 1.5 (1.9) min</td>
<td>2.1 ± 0.4 h</td>
</tr>
<tr>
<td><strong>t₁/₂ (min or h)</strong></td>
<td>N.A.</td>
</tr>
<tr>
<td><strong>Cmax (ng/ml)</strong></td>
<td><strong>CLp (ml/min/kg)</strong></td>
</tr>
<tr>
<td>NA</td>
<td>1/2 (min or h)</td>
</tr>
<tr>
<td><strong>tmax (min)</strong></td>
<td><strong>Tmax (min)</strong></td>
</tr>
<tr>
<td><strong>CLp (ml/min/kg)</strong></td>
<td><strong>t½ (min or h)</strong></td>
</tr>
<tr>
<td><strong>F (%)</strong></td>
<td><strong>NA</strong></td>
</tr>
</tbody>
</table>

**CLp,** plasma clearance; AUC, area under the plasma concentration vs. time curve; **Cmax,** maximum plasma concentration; **Tmax,** time at which maximum concentration was observed; **t½,** apparent terminal half-life; **F,** bioavailability; N.A., not applicable.
Pharmacological Characterization

In Vitro Studies. Binding experiments. SB-222200 produced an enantioselective inhibition of the binding of \(^{125}\text{I}-[\text{MePhe}^7]\text{NKB}\) to CHO-hNK-3R cell membranes. Thus, the active S-enantiomer, SB-222200, inhibited the binding of \(^{125}\text{I}-[\text{MePhe}^7]\text{NKB}\) to CHO-hNK-3R cell membranes with a \(K_i\) of 4.4 ± 0.7 nM (n = 4), whereas the racemate, SB-221275, and the less potent R-isomer, SB-222201, had \(K_i\) values of 9.8 ± 1.2 nM (n = 3) and 138 ± 7 nM (n = 3), respectively (Fig. 3). The metabolite of SB-222200 that is formed after oral administration to the rat, SB-227734 (R/S), is also a potent hNK-3R antagonist, competing with the binding of \(^{125}\text{I}-[\text{MePhe}^7]\text{NKB}\) to CHO-hNK-3R cell membranes, with a \(K_i = 5.8 \pm 0.5\) nM (n = 5).

The affinity of SB-222200 for mNK-3R was also examined. SB-222200 inhibited the binding of \(^{125}\text{I}-[\text{MePhe}^7]\text{NKB}\) to HEK 293-mNK-3R cell membranes with a \(K_i\) of 174 ± 55 nM (n = 3). SB-227734, the metabolite of SB-222200, inhibited \(^{125}\text{I}-[\text{MePhe}^7]\text{NKB}\) binding to HEK 293-mNK-3R cell membranes (IC\(_{50} = 360\) nM; n = 1).

The affinity of SB-222200 for the rat and guinea pig NK-3Rs was assessed with \(^{125}\text{I}-[\text{MePhe}^7]\text{NKB}\) binding to brain cortical membrane preparations. SB-222200 had a similar high affinity for the guinea pig NK-3R (\(K_i = 3.0 \pm 0.5\) nM; n = 4) as the hNK-3R, but lower affinity for the rat NK-3R (\(K_i = 88 \pm 20\) nM; n = 4). Similar results were obtained with the metabolite, SB-227734: \(K_i = 8.2\) nM (n = 2) in the guinea pig NK-3R binding assay and \(K_i = 182\) nM (n = 2) in the rat NK-3R binding assay.

Selectivity profile. Selectivity of SB-222200 for the hNK-3R relative to other tachykinin receptors was determined by competitive binding experiments using membranes prepared from CHO cells stably expressing the human NK-2R (CHO-hNK-2R) and human NK-1R (CHO-hNK-1R) cells and \(^{125}\text{I}-\text{NKA}\) and \(^3\text{H}\text{Substance P}\), respectively. SB-222200 had moderate potency for inhibition of \(^{125}\text{I}-\text{NKA}\) binding to CHO-hNK-2R with a \(K_i = 250 \pm 49\) nM (n = 4), but was without effect, in concentrations up to 100 \(\mu\)M, on the binding of \(^3\text{H}\text{Substance P}\) to CHO-hNK-1R (n = 2).

SB-222200, at concentrations up to 1 or 10 \(\mu\)M, was without effect in 33 receptor binding and enzyme assays, including endothelin (ET\(_A\), ET\(_B\)), interleukin-8 (CXCR1, CXCR2), C5a, LTD\(_4\), LTB\(_4\), adenosine (A\(_1\), A\(_2\)), serotonin (5HT\(_{1A}\), 5-HT\(_{1D}\)), 5-HT\(_{2A}\), 5-HT\(_{2C}\), 5-HT\(_{4}\), opiate...
mountable inhibition of NKB-induced Ca\(^{2+}\) mobilization in HEK 293-hNK-3R cells; Schild plot analysis of the data revealed a pA\(_2\) of 8.4 (n = 2) and a slope not significantly different from 1 (0.92), indicative of competitive antagonism (Fig. 4B). SB-222200 inhibited 1 nM NKB-induced Ca\(^{2+}\) mobilization in HEK 293-mNK-3R cells with an IC\(_{50}\) of 265 ± 28 nM (n = 3).

**Senktide-induced contraction in rabbit isolated iris sphincter muscle.** An additional functional study, utilizing the rabbit isolated iris sphincter muscle, also demonstrated the competitive nature of the profile of inhibition of SB-222200. Thus, SB-222200 (300 nM) surmountably antagonized the contractile response induced by the NK-3R-selective agonist, senktide, with a K\(_i\) of 3.3 ± 0.7 nM (n = 4) (data not shown).

**Reversibility and time dependence of antagonist activity.** The cellular functional NK-3R antagonist activity of SB-222200 was not time-dependent, i.e., the inhibition of NKB (1 nM)-induced calcium mobilization was identical with 5-s (IC\(_{50}\) = 18.4 ± 3.0 nM; n = 4) or 5-min (IC\(_{50}\) = 17.6 ± 2.2 nM; n = 3) pretreatment with antagonist (Fig. 5A). Furthermore, inhibition of the Ca\(^{2+}\) response induced by NKB (1 nM) in HEK 293-NK-3R cells was rapidly reversible, because treatment with varying concentrations of SB-222200 for 5 min followed by two washes and resuspension in fresh buffer without antagonist over 30 min results in significant loss of the inhibitory activity (IC\(_{50}\) = 7800 nM; n = 2) (Fig. 5C). In contrast, the inhibitory effects of SR-142801, an NK-3R antagonist from a chemical series different from SB-222200, for inhibition of NKB-induced Ca\(^{2+}\) mobilization were time-dependent and not reversed by washing. Thus, the IC\(_{50}\) values for SR-142801 after 5 s and 5 min of pretreatment were 155 ± 28 nM (n = 3) and 15.1 ± 3.6 nM (n = 3), respectively (Fig. 5B). Furthermore, in another series of experiments the inhibitory effects of SR-142801 were not rapidly reversed, i.e., the inhibition was similar after 5-min pretreatment (16.3 nM; n = 2) compared with pretreatment for 5 min followed by two washes and resuspension in fresh buffer without antagonist over 30 or 180 min (IC\(_{50}\) = 22.6 nM after 30 min; n = 2) (Fig. 5D).

**In Vivo Studies.** NK-3R-induced behavioral responses in mouse. Subcutaneous (s.c.) or i.c.v. administration of the

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**Fig. 3.** Competition binding of \(^{125}\)I-[MePhe\(^7\)]NKB to CHO-hNK-3Rs by SB-222200, the less active enantiomer SB-222201, and the racemate, SB-221275. CHO-hNK-3R membranes were incubated with 0.1 nM \(^{125}\)I-[MePhe\(^7\)]NKB in the absence and presence of various concentrations of NKB (●), SB-222200 (○), the less active enantiomer SB-222201 (□), or the racemate SB-221275 (◇) as described under Experimental Procedures. Values presented are the mean ± S.E.M. of three to seven experiments.

**Fig. 4.** A, inhibition of calcium mobilization in HEK 293-hNK-3R cells by SB-222200, the less active enantiomer SB-222201, and the racemate SB-221275. Fura-2-loaded HEK 293-hNK-3R cells were stimulated with 1 nM NKB after the addition of increasing concentrations of SB-222200 (●), SB-222201 (○), or SB-221275 (◇). The results are expressed as a percentage of the maximum Ca\(^{2+}\) concentrations elicited by 1 nM NKB in the absence of any antagonist. Values presented are the mean ± S.E.M. of three to six experiments. B, kinetics of antagonism by SB-222200 of NKB-induced Ca\(^{2+}\) mobilization in HEK 293-hNK-3R cells. Concentration-response curves to NKB were generated using Fura-2-loaded HEK 293-hNK-3R cells in the absence (●) and presence of 10 nM (◇), 33 nM (□), 100 nM (△), 330 nM (◇), and 1000 nM (◇) SB-222200. Results presented are the mean of duplicate samples of a single representative of two experiments.
NK-3R-selective ligand, senktide, produces a set of behaviors in rodents that appears to be mediated by serotonin release in brain and spinal cord (Stoessl et al., 1987, 1990). In the current study the effects of oral SB-222200 against behavioral responses induced by s.c. or i.c.v. senktide in mouse was explored. Oral SB-222200 (5–20 mg/kg in 50% PEG-400/1% methylcellulose, 30 min pretreatment; n = 6) produced a dose-related inhibition of s.c. senktide (1 mg/kg)-induced behavioral effects (rapid head shakes and tail whips) with an ED50 of 5.6 mg/kg (data not shown). Oral administration of the active metabolite, SB-227734 (5 mg/kg; 30-min pretreatment), produced 57% inhibition of senktide-induced behavioral responses in mice (n = 6).

Intracerebral ventricular administration of senktide (0.01, 0.025, and 0.05 nmol) produced a dose-related inhibition of s.c. senktide (1 mg/kg)-induced behavioral effects (rapid head shakes and tail whips) with an ED50 of 5.6 mg/kg (data not shown). Oral administration of the active metabolite, SB-227734 (5 mg/kg; 30 min pretreatment), produced 57% inhibition of senktide-induced behavioral responses in mice (n = 6).

Intracerebral ventricular administration of senktide (0.01, 0.025, and 0.05 nmol) produced a dose-related increase in head shakes and tail whips in the mouse (Fig. 6); no behavioral effects of i.c.v. saline administration were observed. Oral SB-222200 (5 mg/kg; 30-min pretreatment) blocked the effects of i.c.v. administration of senktide; both head shakes and tail whips induced by the various concentrations of senktide were significantly antagonized (28–84%) by SB-222200 (Fig. 6).

Pharmacodynamic assessment demonstrated that the inhibitory effect of oral SB-222200 (5 mg/kg) against senktide-induced behavioral responses correlated with the brain, but not the plasma, concentrations of the compound (Fig. 7). Significant inhibition (57%; P < .01) was evident for up to 1 h after administration of SB-222200, with a trend toward inhibition at 2 h (data not shown).

Discussion

There is a paucity of information on the biology of the NK-3R, especially its potential pathophysiological role(s). This deficit in this particular area of tachykinin research can be attributed, in large part, to the lack of availability, until relatively recently, of potent and selective antagonists of the NK-3R. In 1995 information was provided on the first potent and selective, nonpeptide NK-3R antagonist SR-142801 (Emonds-Alt et al., 1995; Oury-Donat et al., 1995). More recently, another series of potent and selective compounds were identified as NK-3R antagonists (Giardina et al., 1996; Sarau et al., 1997). NK-3Rs are present in both the CNS and peripheral nervous systems, where they may modulate the release of various neurotransmitters (Stoessl et al., 1990; Arenas et al., 1991; Schemann and Kayser, 1991; Ramirez et al., 1994). To ascertain the physiological and pathophysiological roles of activation of this receptor, it is necessary to identify compounds that have different abilities to enter the CNS. The results of the present study indicate that SB-222200 is a potent and selective NK-3R antagonist that effectively enters the CNS and is efficacious in a CNS model of NK-3R activation. Accordingly, SB-222200 would appear to be an appropriate tool compound with which to assess the role of NK-3Rs in animal models of CNS diseases.

SB-222200 belongs to the recently described class of nonpeptide NK-3R antagonists, which are based on the 4-quino-linecarboxamide backbone (Giardina et al., 1996). Functional and binding studies indicate that SB-222200 is a high affinity antagonist for the hNK-3R: IC50 = 18.4 nM for inhibition of NKB-induced calcium mobilization in HEK 293-hNK-3 cells. A and B, Fura-2-loaded HEK 293-hNK-3 cells were stimulated with 1 nM NKB after 5-s (●, □) or 5-min (○, △) pretreatment with SB-222200 (A) or SR-142801 (B). C and D, Fura-2-loaded cells were treated with the indicated concentrations of SB-222200 (C) or SR-142801 (D) and washed twice and after 30 min (●) or 180 min (△) stimulated with 1 nM NKB. The control cells (○) were treated as the washed groups except that the antagonists were added 5 min before 1 nM NKB challenge. The values presented are the means of duplicate samples for an individual experiment that was representative of two or three experiments.
Results are given as the mean mg/kg; 30-min pretreatment) (data are from same mice utilized in A). Whips induced by i.c.v. senktide were antagonized by oral SB-222200 (5 mg/kg; 30-min pretreatment). B, the increases in tail whips induced by i.c.v. senktide were antagonized by oral administration of SB-222200. A, dose-related inhibition by oral SB-222200 of senktide-induced behavioral effects (head shakes and tail whips) in mouse. Although, to our knowledge, the inhibition of senktide-induced behavioral responses in mouse by oral SB-222200.

The results of binding and calcium mobilization studies indicate that the inhibitory effects of SB-222200 are not time-dependent and are reversed rapidly by washout. Furthermore, the antagonism of senktide-induced contraction in rabbit isolated iris sphincter smooth muscle produced by the compound, as well as the inhibition of NKB-induced calcium mobilization, is surmountable. Similar effects were obtained with SB-223412 (Sarau et al., 1997). In contrast, the effects of SR-142801, a compound from a different structural class than SB-222200 or SB-223412, against NK-3R-induced calcium mobilization in HEK 293-hNK-3R cells are time-dependent and not reversed by washout. Furthermore, the functional actions of SR-142801 against NK-3R-induced contractions in guinea pig isolated ileum longitudinal muscle preparations have been reported previously to be essentially irreversible and surmountable (i.e., not reversed by washing out for up to 2 h) and time-dependent (Patachini et al., 1995). Collectively, these data suggest that, based upon their functional effects, differences may exist in the competitive nature of the NK-3R antagonism produced by SB-222200 and SB-223412 (classically competitive) compared with SR 124801 (noncompetitive).

The pharmacokinetic characteristics of SB-222200 after i.v. and/or oral administration were assessed in rats and mice. SB-222200 was subject to high plasma clearance in the rat with oral bioavailability of about 45%. Note, preliminary studies in the dog demonstrated high and sustained plasma levels after intraduodenal administration of SB-222200 (5 mg/kg), with bioavailability of 43% and a half-life of 9.2 h; the systemic plasma clearance of SB-222200 in the dog is moderate (unpublished observations). SB-222200 was CNS penetrant in the rat and mouse. In the latter species, which was used for in vivo studies, peak brain levels of 290 ng/g SB-222200 were obtained 0.5 h after oral administration (5 mg/kg) and were maintained at >80 ng/g for at least 2 h. A major metabolite of SB-222200, formed by \( \sigma - 1 \) oxidation of the parent compound, was demonstrated after oral administration in rats. Binding studies revealed that the metabolite, SB-227734, had a similar affinity to SB-222200 in the hNK-3R, hNK-2R, hNK-1R, and mNK-3R assays. Furthermore, SB-227734 had a similar potency to SB-222200 for antagonism of hNK-3R-induced calcium mobilization in transfected HEK cells. Collectively, these data suggest that the metabolite SB-227734 may contribute to the pharmacological effects of SB-222200 in vivo.

In agreement with the drug metabolism and pharmacokinetic studies, indicating appreciable plasma concentrations after oral administration and high CNS penetration, SB-222200 inhibited NK-3R-induced behavioral effects (head shakes and tail whips) in mouse. Although, to our knowledge, the ability of senktide to enter the CNS has not been assessed directly, this characteristic effect of NK-3R agonists, including senktide, has been attributed, at least in part, to the release of 5-HT from the CNS (Stoessel et al., 1990). Of note was the ability of oral SB-222200 to inhibit behavioral responses induced by either s.c. or i.c.v. administration of senktide. Thus, the results provide direct pharmacokinetic and functional evidence that SB-222200 effectively enters the mouse brain in sufficient concentrations to inhibit responses due to NK-3R activation. In support of a CNS site of action of

![Fig. 6. Behavioral effects of central NK-3R stimulation by i.c.v. senktide and inhibition by oral administration of SB-222200. A, dose-related increases in head shakes induced by i.c.v. senktide were antagonized by oral SB-222200 (5 mg/kg; 30-min pretreatment). B, the increases in tail whips induced by i.c.v. senktide were antagonized by oral SB-222200 (5 mg/kg; 30-min pretreatment) (data are from same mice utilized in A). Results are given as the mean ± S.E.M.; n = 5–12 mice per group; **P < .01; *P < .05.](image-url)
semitide and SB-222200 is the demonstration that the inhibitory influence of oral SB-222200 against semitide-induced behavioral responses in mouse are correlated with the brain, but not the plasma, concentrations of the compound.

NK-3Rs have been demonstrated using pharmacological, electrophysiological, biochemical, and/or molecular biological techniques, in the CNS of several species, including rats (Dam et al., 1990; Stoessl et al., 1990; Keegan et al., 1992; Mason and Elliott, 1992; Ding et al., 1996; Shughrue et al., 1996) and humans (Buell et al., 1992). In the latter, polymerase chain reaction analysis revealed the presence of NK-3R mRNA in all regions of human brain analyzed (frontal cortex, temporal cortex, parietal cortex, locus niger, hippocampus, and striatum). However, to date there has been controversy and debate regarding the presence of NK-3Rs in human brain. Two recent studies, utilized autoradiographic and/or immunohistochemical techniques to demonstrate the presence of the NK-3R (Mileusnic et al., 1999a,b) and NKB-containing neurons (Mileusnic et al., 1999b) in human brain.

There were differences in the cellular and anatomical distribution of the NK-3R between rat and human brain; in the latter, NK-3Rs were localized to superficial cortical layers, pyramidal neurons, and astrocytes in the neocortex and white matter. The physiological and pathophysiological roles of NK-3 receptors in mammalian CNS are unknown, although there is evidence from several studies in animal that activation of this tachykinin receptor modulates the release of a variety of neurotransmitters, including 5-HT (Stoessl et al., 1990), acetylcholine (Arenas et al., 1991), dopamine (Stoessl et al., 1991; Bannon et al., 1995), and vasopressin (Saigo et al., 1993). Based on such information, in addition to results describing changes in the expression of the NK-3R and/or NKB and the effects of tachykinin ligands in vivo, it has been speculated that NK-3Rs may play a pathophysiological role in various diseases, including epilepsy (Roder et al., 1994), anxiety (Ribeiro et al., 1999), and Parkinson’s disease (Bannon et al., 1995).

In summary, the data indicate that SB-222200 is a high affinity, selective, reversible, and competitive antagonist of hNK-3Rs. It penetrates the CNS effectively and is orally active in an NK-3-induced CNS behavioral model in mouse. The preclinical pharmacodynamic profile of SB-222200 suggests that it will be a useful tool compound to assist in the elucidation of the physiological and pathophysiological roles of NK-3R activation, in particular in the CNS.

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Fig. 7. Pharmacodynamic study with oral SB-222200 in mouse: correlation between plasma concentrations (A) or brain concentrations (B) of SB-222200 and inhibition of senktide (s.c.)-induced behavioral effects. Mice were pretreated orally with SB-222200 (5 mg/kg) at various times (30 min, 1 h, 2 h, 4 h, and 8 h) before challenge with senktide (1.0 mg/kg, s.c.); the head twitches and tail writhes induced by senktide were counted over 10 min. Plasma and brain tissue homogenate samples were stored at -30°C, until analysis for concentrations of SB-222200 as described under Experimental Procedures. Results are expressed as senktide-induced response (number of events) against SB-222200 concentration (ng/ml for plasma and ng/g for brain) and are given as the mean ± S.E.M.; n = 5–6 mice per group; **P < 0.1; *P < 0.5.

References


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