SSR240600 [(R)-2-{1-[2-[4-[2-[3,5-Bis(trifluoromethyl)phenyl]acetyl]-2-(3,4-dichlorophenyl)-2-morpholinyl]ethyl]-4-piperidinyl]-2-methylpropanamide], a Centrally Active Nonpeptide Antagonist of the Tachykinin Neurokinin-1 Receptor: I. Biochemical and Pharmacological Characterization

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ABSTRACT
The biochemical and pharmacological properties of a novel antagonist of the tachykinin neurokinin 1 (NK₁) receptor, SSR240600 [(R)-2-{1-[2-[4-[2-[3,5-bis(trifluoromethyl)phenyl]acetyl]-2-(3,4-dichlorophenyl)-2-morpholinyl]ethyl]-4-piperidinyl]-2-methylpropanamide], were evaluated. SSR240600 inhibited the binding of radioactive substance P to tachykinin NK₁ receptors in human lymphoblastic IM9 cells (Kᵢ = 0.0061 nM), human astrocytoma U373MG cells (Kᵢ = 0.10 nM), and human brain cortex (IC₅₀ = 0.017 nM). It also showed subnanomolar affinity for guinea pig NK₁ receptors but was less potent on rat and gerbil NK₁ receptors. SSR240600 inhibited [Sar⁹,Me⁶]substance P-induced inositol monophosphate formation in human astrocytoma U373MG cells with an IC₅₀ value of 0.66 nM (agonist concentration of 100 nM). It also antagonized substance P-induced contractions of isolated human small bronchi with a pIC₅₀ value of 8.6 (agonist concentration of 100 nM). The compound was >100- to 1000-fold more selective for tachykinin NK₁ receptors versus tachykinin NK₂ or NK₃ receptors as evaluated in binding and in vitro functional assays. In vivo antagonistic activity of SSR240600 was demonstrated on tachykinin NK₁ receptor-mediated hypotension in dogs (3 and 1 μg/kg i.v.), microvascular leakage (1 and 3 mg/kg i.p.), and bronchoconstriction (50 and 100 μg/kg i.v.) in guinea pigs. It also prevented citric acid-induced cough in guinea pigs (1–10 mg/kg i.p.), an animal model in which central endogenous tachykinins are suspected to play a major role. In conclusion, SSR240600 is a new, potent, and centrally active antagonist of the tachykinin NK₁ receptor, able to antagonize various NK₁ receptor-mediated pharmacological effects in the periphery and in the central nervous system.

Substance P belongs to a group of related neuropeptides named tachykinins, which includes neurokinin A and neurokinin B. These peptides are widely distributed in the peripheral and central nervous systems where they exert various biological actions as neuromodulators or neurotransmitters.

Biological activities of tachykinins are mediated by three different, but related, G-protein-coupled receptors with seven α-helical transmembrane segments, denoted NK₁, NK₂, and NK₃. Substance P is the natural endogenous ligand of tachykinin NK₁ receptors, whereas neurokinin A and neurokinin B are the preferential ligands of tachykinin NK₂ and NK₃ receptors, respectively (Regoli et al., 1994; Maggi, 1995; Quartara and Maggi, 1997).

ABBREVIATIONS: NK, neurokinin; SSR240600, (R)-2-{1-[2-[4-[2-[3,5-bis(trifluoromethyl)phenyl]acetyl]-2-(3,4-dichlorophenyl)-2-morpholinyl]ethyl]-4-piperidinyl]-2-methylpropanamide; CHO, Chinese hamster ovary; SR140333, (S)-1-[2-(1-[3-(3,4-dichlorophenyl)-1-[[3-(1-methyl-1-phenylmethyl)-3-(2-methoxybenzylamino)-2-phenylpiperidine; ANOVA, analysis of variance. SR144190, (R)-3-[2-(4-benzoyl-2-(3,4-difluorophenyl))-morpholin-2-yl]-ethyl]-4-phenylpiperidin-4-yl]-1-dimethylurea; SR142801, (R)-(N)-[1-[3-[1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl]propyl]-4-phenylpiperidin-4-yl]-N-methylacetamide; SSR146977, (R)-(N)-[1-[3-[1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl]propyl]-4-phenylpiperidin-4-yl]-N-dimethylurea; GR205171, (2S,3S)-2-methoxy-5-[5-(trifluoromethyl)-tetrazol]-1-yl]-benzyl-(2-phenyl-piperidin-3-yl)amine; GR73632, d-Ala-[L-Pro⁶,Me-Leu⁸]substance P(7-11).

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Over the past several years, potent nonpeptide antagonists, selective for the different tachykinin receptors, have been described and have provided tools to investigate the physiopathological role of tachykins and their receptors both in the periphery and in the central nervous system (Regoli et al., 1994; Quartara and Maggi, 1997, 1998; Lagente and Advenier, 1998; Stout et al., 2001). Based on the more recent pharmacological data, confirmed by preliminary clinical trials, it has emerged that blockade of tachykinin NK<sub>1</sub> receptors may provide a novel treatment of major depression (Kramer et al., 1998; Rupniak and Kramer, 1999) and emesis (Rupniak and Kramer, 1999; Diemunsch and Grélot, 2000). These activities of tachykinin NK<sub>1</sub> receptor antagonists are essentially dependent on their ability to penetrate the brain (Rupniak et al., 1997; Kramer et al., 1998; Diemunsch and Grélot, 2000). We now describe some general biochemical and pharmacological activities of a novel nonpeptide tachykinin NK<sub>1</sub> receptor antagonist, SSR240600 [(R)-2-{1-[2-[4-[2-[3,5-bis(trifluoromethyl)phenyl]acyetyl]-2-(3,4-dichlorophenyl)-2-morpholinyl]ethyl]-4-piperidinyl}-2-methylpropanamide] (Fig. 1). Its activity in various tests predictive of an antidepressant activity is described in the accompanying paper (Steinberg et al., 2002).

Materials and Methods

Binding Assays. The affinity of SSR240600 for tachykinin receptors was evaluated in several receptor-radioligand binding assays: 1) binding of [<sup>35</sup>I]Bolton-Hunter region-labeled substance P to tachykinin NK<sub>1</sub> receptors of rat cortex, guinea pig, and gerbil ileum, human lymphoblast cells (IM9), and human astrocytoma cells (U373MG, STTG1); 2) binding of [<sup>125</sup>I]iodohistidyl-neurokinin A (or [<sup>125</sup>I]neuropeptide γ) to tachykinin NK<sub>2</sub> receptors of rat or hamster urinary bladder or guinea pig ileum as well as to human receptors, stably expressed in CHO cells; and 3) binding of [<sup>125</sup>I]iodohistidyl-[MePhe<sup>7</sup>]neurokinin B (or [<sup>125</sup>I]eleidoisin) to rat, guinea pig, and gerbil brain cortex tachykinin NK<sub>3</sub> receptors and human NK<sub>3</sub> receptor, cloned and stably expressed in CHO cells. All these binding assays were conducted and analyzed as previously described in detail (Emonds-Alt et al., 1993, 1995, 1997).

The affinity of SSR240600 for tachykinin receptors was also investigated on the binding of [<sup>35</sup>I]substance P to tachykinin NK<sub>1</sub> receptors of human brain cortex. Brain cortex was obtained from a 49-year-old man, 48 h after death due to pulmonary edema. Tissue was homogenized at 4°C in 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM KCl and 10 mM EDTA. The homogenate was centrifuged at 28,000g for 15 min at 4°C. The pellet was homogenized and incubated for 30 min at 4°C in 50 mM Tris-HCl buffer, pH 7.4, containing 300 mM KCl and 10 mM EDTA. This homogenate was centrifuged at 40,000g for 15 min and the pellet was suspended in 50 mM Tris-HCl buffer, pH 7.4. Membranes were stored at −20°C until use. Before use in binding assays, the membranes were diluted at 4°C in 50 mM Tris-HCl buffer, pH 7.4, and centrifuged at 40,000g for 15 min. The final pellet was suspended in 50 mM Tris-HCl buffer, pH 7.4, containing 0.2 mg/ml bovine serum albumin, 40 μg/ml bacitracin, 4 μg/ml leupeptin, 4 μg/ml chymostatin, and 3 mM MnCl<sub>2</sub>. Binding assays were conducted in “low binding” tubes (NUNC A/S, Roskilde, Denmark). Human brain cortex membranes (10 mg) and [<sup>3</sup>H]substance P (0.5 nM) in 500 μl of assay buffer (50 mM Tris-HCl buffer, pH 7.4, containing 0.2 mg/ml bovine serum albumin, 40 μg/ml bacitracin, 4 μg/ml leupeptin, 4 μg/ml chymostatin, 3 mM MnCl<sub>2</sub>) were incubated at 25°C for 60 min with various concentrations of SSR240600. At the end of the incubation, separation of bound and free ligand was done after dilution [3 ml of cold (4°C)] 50 mM Tris-HCl buffer, pH 7.4, containing 0.2 mg/ml bovine serum albumin and rapid filtration on Whatman (Maidstone, Kent, UK) GF/C filters pretreated with 50 mM Tris-HCl buffer, pH 7.4, containing 0.2 mg/ml bovine serum albumin and 0.25% polyethyleneimine. The filters were washed three times at 4°C with 50 mM Tris-HCl buffer, pH 7.4, containing 0.2 mg/ml bovine serum albumin. The radioactivity was counted in a β liquid scintillation counter. Specific binding was determined by subtraction of the nonspecific binding, which was determined in the presence of 1 μM unlabeled [Sar<sup>3</sup>Met(O<sup>2</sup>)<sup>11</sup>]substance P.

In addition, the selectivity of SSR240600 was evaluated in a large variety of ion channel- and receptor-binding assays as well as enzyme assays. This was performed by MDS Panlabs Pharmacology Services (Bothell, WA) and Cerep (Celles L’Evescault, France).

In Vitro Functional Assays. Antagonistic activity of SSR240600 was first determined by measuring inhibition of inositol phosphate-1 formation elicited by tachykinin NK<sub>1</sub> receptor activation with specific agonists ([Sar<sup>3</sup>Met(O<sup>2</sup>)<sup>11</sup>]substance P and GR73632) in human astrocytoma U373MG cells. The effect of SSR240600 on inositol phosphate-1 formation was also measured using CHO cells stably expressing either human tachykinin NK<sub>2</sub> or NK<sub>3</sub> receptor in response to [Nle<sup>10</sup>]neurokinin A-(4-10) or [MePhe<sup>7</sup>]neurokinin B, respectively. All these assays were conducted as previously described in detail (Oury-Donat et al., 1994, 1995).

The in vitro pharmacological profile of SSR240600 was then investigated using several functional bioassays specific for the three tachykinin receptor subtypes (Regoli et al., 1994): [Sar<sup>3</sup>Met(O<sup>2</sup>)<sup>11</sup>]substance P-induced endothelium-dependent relaxation of rabbit pulmonary artery, precontracted by 0.1 μM norepinephrine (specific for NK<sub>1</sub> receptors), [Ala<sup>8</sup>]neurokinin A-(4-10)-induced contractions of endothelium-denuded rabbit pulmonary artery (specific for NK<sub>2</sub> receptors), and [MePhe<sup>7</sup>]neurokinin B-induced contractions of guinea pig ileum (specific for NK<sub>3</sub> receptors). All these assays were conducted and analyzed as previously described in detail (Emonds-Alt et al., 1993). As already reported and discussed for other nonpeptide antagonists of the tachykinin receptor subtypes, SSR240600 was only observed after prolonged incubation with the tissue. Therefore, SSR240600 was added 120 min before the agonist in all experiments.

Finally, SSR240600 antagonist activity for tachykinin NK<sub>1</sub> receptors was evaluated by measuring inhibition of [Sar<sup>3</sup>Met(O<sup>2</sup>)<sup>11</sup>]substance P-induced contractions of human isolated small bronchi (diameter <1 mm) as described by Naline et al. (1996). Bronchial tissues were removed from patients (25 men, previous smokers, mean age 64 ± 2 years) with lung cancer at the time of the surgical operation. Just after removal, segments of bronchi with an inner diameter of 0.5 to 1 mm were taken from an area as far as possible from the malignancy and stored overnight at 4°C in Krebs-Henseleit solution.

In Vivo Assays. All protocols have been approved by the Comité d’Expérimentation Animale (Animal Care and Use Committee) of Sanofi-Synthélabo Recherche and are in accordance with the principles of the Declaration of Helsinki. The in vivo pharmacological profile of SSR240600 was investigated in three animal models in which the role of tachykinin NK<sub>1</sub> receptor has been well characterized: hypotension, bronchoconstriction, and plasma extravasation.

Fig. 1. Structure of SSR240600 [(R)-2-{1-[2-[4-[2-[3,5-bis(trifluoromethyl)phenyl]acyetyl]-2-(3,4-dichlorophenyl)-2-morpholinyl]ethyl]-4-piperidinyl}-2-methylpropanamide].
### Table 1

Inhibition constants ($K_i$) of SSR240600 in radioligand binding assays for tachykinin receptors

<table>
<thead>
<tr>
<th>Receptors</th>
<th>NK1</th>
<th>NK2</th>
<th>NK3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human brain cortex</td>
<td>0.65 ± 0.23</td>
<td>0.0043 ± 0.0012</td>
<td>0.053 ± 0.001</td>
</tr>
<tr>
<td>IM9 (human)</td>
<td>0.044 ± 0.008</td>
<td>0.0061 ± 0.0004</td>
<td>0.007 ± 0.001</td>
</tr>
<tr>
<td>U373MG (human)</td>
<td>0.31 ± 0.06</td>
<td>0.10 ± 0.01</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>STTG1 (human)</td>
<td>0.33 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>Rat ileum</td>
<td>0.054 ± 0.006</td>
<td>1.07 ± 0.07</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Gerbil ileum</td>
<td>0.042 ± 0.003</td>
<td>1.15 ± 0.072</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Guinea pig ileum</td>
<td>0.059 ± 0.007</td>
<td>0.23 ± 0.03</td>
<td>206 ± 9</td>
</tr>
<tr>
<td>Human (CHO cells)</td>
<td>0.48 ± 0.01</td>
<td>24 ± 2</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Guinea pig ileum</td>
<td>2.30 ± 0.49</td>
<td>71 ± 5</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Rat urinary bladder</td>
<td>0.76 ± 0.03</td>
<td>34 ± 3</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Human (CHO cells)</td>
<td>0.34 ± 0.05</td>
<td>206 ± 9</td>
<td>206 ± 9</td>
</tr>
<tr>
<td>Guinea pig brain cortex</td>
<td>0.43 ± 0.05</td>
<td>21 ± 2</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Gerbil brain cortex</td>
<td>0.16 ± 0.03</td>
<td>544 ± 77</td>
<td>544 ± 77</td>
</tr>
<tr>
<td>Rat brain cortex</td>
<td>0.053 ± 0.001</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>

*Note: Values are means ± S.D. obtained from at least three independent experiments performed in triplicate.*

### Citric Acid-Induced Cough in Guinea Pigs

Tricolored, awake, unrestrained male or female guinea pigs (250–400 g) were placed in a body plethysmograph. They were then exposed for 10 min to an aerosol of either an aqueous solution of citric acid (0.4 M) or saline as a control. SSR240600 was administered by the intraperitoneal route at various times before the aerosol challenge. Coughs were counted by a trained observer, and recognized by the characteristic animal posture and the pressure variation in the body plethysmograph (Advenier et al., 1993; Girard et al., 1995; Daoui et al., 1998).

### Results

#### Binding Studies

The inhibition constants ($K_i$) for SSR240600 obtained in the different binding assays for tachykinin receptors are shown in Table 1. SSR240600 inhibited the binding of radioactive substance P to tachykinin NK1 receptors with subnanomolar $K_i$ values, using established human cell lines as well as human brain membranes. SSR240600 also displayed a high affinity for tachykinin NK1 receptors from various animal species, especially guinea pigs. In binding assays for tachykinin NK2 and NK3 receptors, SSR240600 slightly interfered with the binding of their respective ligands, with $K_i$ values always above 10 nM in all species studied, including human. Finally, SSR240600 was assayed in 100 (mainly human) receptor-binding, ion channel-binding, and enzyme assays including adenosine (A1, A2A, A2B), adrenergic ($\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$) dopamine (D1, D2), nicotinic, muscarinic (M1, M2, M3, M4, M5), opiate ($\mu$, $\kappa$, $\delta$, opioid receptor-like receptor 1) serotonin (5-HT1A, 5-HT2, 5-HT3, 5-HT4, 5-HT5A, 5-HT6, 5-HT7), angiotensin (AT1, AT2), bradykinin (B1, B2), calcitonin gene-related peptide, cholecystokinin (CCK1, CCK2), corticotropin-releasing factor (CRF1, 2).
CRF₂), galanin (GAL₁, GAL₂), neurotensin (NT₁), vasopres-

sin (V₁A), hormones (glucocorticoid, estrogen, progesterone,
testosterone), ion channels (sodium, calcium, potassium and
chloride), cyclooxygenases (COX₁, COX₂), phosphodiester-
ases (III and IV), acetylcholinesterase. SSR240600, at con-
centrations up to 1 μM, was inactive (inhibition less than
50%), except in receptor (IC₅₀ = 0.21 μM) and sodium
channel site 2 (IC₅₀ = 0.18 μM) assays (data not shown).

**In Vitro Functional Studies.** Antagonistic activity of
SSR240600 at human tachykinin NK₁ receptors was studied
by measuring inhibition of inositol phosphate-1 formation
provoked by NK₁ receptor activation in human astrocytoma
U373MG cells. Activation of tachykinin NK₁ receptors in
U373MG cells by three different specific agonists

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Agonists (Concentration)</th>
<th>IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK₁</td>
<td>[Sar⁹, Met(O₂)¹¹]Substance P (0.1 μM)</td>
<td>0.66 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>GR73632 (0.1 μM)</td>
<td>0.57 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Septide (0.1 μM)</td>
<td>0.45 ± 0.07</td>
</tr>
<tr>
<td>NK₂</td>
<td>[βAla⁸]Neurokinin A (4-10) (10 nM)</td>
<td>140 ± 7</td>
</tr>
<tr>
<td>NK₃</td>
<td>[MePhe⁷]Neurokinin B (10 nM)</td>
<td>1760 ± 100</td>
</tr>
</tbody>
</table>

**TABLE 2**

Inhibition by SSR240600 of tachykinin receptor-mediated inositol
phosphate-1 formation in human astrocytoma U373MG cells (NK₁
receptors) and in CHO cells expressing either human NK₁ or NK₃
receptors

Results are given as IC₅₀ values. Values are means ± S.E.M. from at least three
independent experiments performed in triplicate.

![Graph A](image1)

**Fig. 2.** Concentration-response curves for [Sar⁹, Met(O₂)¹¹]Substance P-induced endothelium-dependent relaxation of rabbit pulmonary artery precontracted with 100 nM norepinephrine (A), [βAla⁸]neurokinin A-induced contractions of endothelium-deprived rabbit pulmonary artery (B), and [MePhe⁷]neurokinin B-induced contractions of guinea pig ileum (C) in the absence and in the presence of SSR240600. Values are means ± S.E.M. (n = 6).
([Sar³, Met(O²)¹¹]substance P, septide, and GR73632) provoked the formation of inositol phosphate-1, which was concentration dependently inhibited by SSR240600 regardless of the agonist used. In contrast, SSR240600 showed a much lower potency to block inositol phosphate-1 formation following the activation of human tachykinin NK₂ and NK₃ receptors stably expressed in CHO cells. The IC₅₀ values are given in Table 2.

In a classical tachykinin NK₁ receptor assay using isolated tissues, ([Sar³, Met(O²)¹¹]substance P-induced endothelium-dependent relaxation of rabbit pulmonary artery previously contracted by norepinephrine, SSR240600 produced a concentration-dependent rightward shift of the concentration-response curves of ([Sar³, Met(O²)¹¹]substance P (Fig. 2A). However, SSR240600 also induced a reduction of the maximal response to the agonist, suggesting that SSR240600 antagonism was not purely competitive. The slope of the Schild plot (0.65) was significantly different from unity and the apparent affinity of SSR240600 was thus calculated in terms of pD₂ (negative logarithm of the molar concentration of antagonist that produces a 50% reduction of the maximal response to the agonist). The pD₂ value was 8.67 ± 0.08 (n = 18). The activity of SSR240600 was then examined on tissue preparations containing tachykinin NK₁ and NK₂ receptors. At concentrations up to 0.1 μM, it had no effect in bioassays for NK₁ ([βAla³]nerokinin A-induced contractions of endothelium-deprived rabbit pulmonary artery) (Fig. 2B) or NK₂ (MePhe³)nerokinin B-induced contractions of guinea pig ileum) (Fig. 2C) receptors. At a concentration of 1 μM, SSR240600 produced a rightward shift of the agonist concentration-response curve in the two bioassays, with a reduction of maximal response to the agonist in bioassay for NK₂ receptors. Finally, in an assay using an isolated human tissue, SSR240600 potently inhibited contractions of human isolated small bronchi (diameter <1 mm) induced by [Sar³, Met(O²)¹¹]substance P (100 nM) with a pIC₅₀ of 8.6 (Fig. 3).

In Vivo Studies. The in vivo activity of SSR240600 was first investigated using typical pharmacological responses to tachykinin NK₁ receptor agonists ([Sar³, Met(O²)¹¹]substance P, GR73632, or substance P). In anesthetized dogs, intravenous injection of [Sar³, Met(O²)¹¹]substance P (5 ng/kg) provoked a reproducible hypotension of 30 to 40 mm Hg. SSR240600 administered either intravenously (3–10 μg/kg) or intraduodenally (30–300 μg/kg) produced a dose- and time-dependent inhibition of this [Sar³, Met(O²)¹¹]substance P-induced hypotension (Fig. 4). At the doses tested, SSR240600 itself had no effect on mean blood pressure. SSR240600 also potently antagonized GR73632-induced bronchoconstriction in anesthetized guinea pigs (Fig. 5). Intravenous injection of GR73632 (0.5 ng/kg) produced a reproducible bronchoconstriction that was dose dependently inhibited by pretreatment with intravenously administered SSR240600 (50 and 100 μg/kg). Furthermore, 3 h after a single oral administration, SSR240600 (3 mg/kg) inhibited GR73632-induced bronchoconstriction by 83 ± 5% (n = 5), indicating both oral bioavailability and a long-lasting effect of the compound. SSR240600 itself did not modify the resting bronchial tone at the doses tested. Finally, intravenous administration of substance P (0.3 μg/kg) induced plasma extravasation in different guinea pig tissues. After intraperitoneal administration, 30 min before substance P, SSR240600 at doses equal to or greater than 1 mg/kg inhibited the plasma extravasation (Fig. 6).

SSR240600 was then studied on citric acid-induced cough in awake guinea pigs, a model in which endogenous tachykinins and their receptors are suspected to play an important role. Cough was provoked by exposure of animals to an aerosol of aqueous citric acid solution (0.4 M) for 10 min. Intraperitoneal administration of SSR240600 (1–10 mg/kg), 30 min before the citric acid challenge, resulted in a dose-dependent inhibition of cough (Fig. 7A). This inhibition was highly time dependent (Fig. 7B), increasing with the length of the pretreatment. The cough inhibition following 120 min pretreatment with 1 mg/kg i.p. SSR240600 was comparable with that observed after 30 min pretreatment with 10 mg/kg i.p.

Discussion

This paper describes biochemical and pharmacological activities of SSR240600, a new, selective and highly potent nonpeptide antagonist of the tachykinin NK₁ receptor. In binding experiments, SSR240600 potently inhibited binding of radioactive substance P to human tachykinin NK₁ receptors with inhibition constants (Kᵢ) in the subnanomolar range. Of particular interest is its very high affinity for the native tachykinin NK₁ receptor present in human brain cortex membrane preparation. The potency of SSR240600 was comparable with that previously observed with another chemically related tachykinin NK₁ receptor antagonist, SR140333 (Emonds-Alt et al., 1993), except that its affinity was typically species-dependent. Contrary to SR140333, it was more active on tachykinin NK₁ receptors of guinea pigs than of rats and gerbils. Such species-dependent affinities have been observed for other nonpeptide and peptidomimetic NK₁ receptor antagonists (McLean et al., 1993, 1996; Aramori et al., 1994; Beattie et al., 1995; Cellier et al., 1996; Quartara and Maggi, 1997). The potent antagonism of SSR240600 at tachykinin NK₁ receptors has been further demonstrated in different in vitro functional assays for tachykinin receptors. First, like SR140333, it blocked with high efficacy both tachykinin NK₁ receptor-mediated inositol phosphate-1 formation in human astrocytoma U373MG cells.
(Oury-Donat et al., 1994) as well as contractions of isolated human small bronchi (Naline et al., 1996). Second, it also potently antagonized [(Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>] substance P-induced endothelium-dependent relaxation of rabbit pulmonary artery precontracted by norepinephrine, a typical tachykinin NK<sub>1</sub> receptor assay (Regoli et al., 1994). Like SR140333 (Emonds-Alt et al., 1993), SSR240600 antagonism was not purely competitive. A similar profile was reported with other peptidomimetic or nonpeptide tachykinin NK<sub>1</sub> receptor antagonists (Beattie et al., 1995; Cirillo et al., 1998).

The selectivity of SSR240600 for tachykinin NK<sub>1</sub> receptors has also been clearly demonstrated in our binding and in vitro functional studies. Indeed, the affinities measured in binding assays for tachykinin NK<sub>2</sub> and NK<sub>3</sub> receptors remained very low compared with tachykinin activities or activities displayed by specific antagonists of tachykinin NK<sub>2</sub> (SR48968, SR144190) (Emonds-Alt et al., 1992, 1997) or NK<sub>3</sub> (SR142801, SSR146977) (Emonds-Alt et al., 1995, 2002) receptors at these receptors. The selectivity of SSR240600 for tachykinin NK<sub>1</sub> receptors was further evidenced in different in vitro functional assays for these tachykinin receptors. In assays using isolated organ preparations typical for tachyki-

**Fig. 4.** Inhibition by SSR240600 of [(Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>] substance P-induced hypotension in anesthetized dogs. SSR240600 was administered by the intravenous (A) or intraduodenal (B) route. [(Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>] Substance P (5 ng/kg) was injected intravenously at various times after SSR240600 administration. Results are expressed as percentage inhibition of the reduction of mean blood pressure (about 30–40 mm Hg) induced by [(Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>] substance P before SSR240600 administration. Values are means ± S.E.M. (n = 3–5). Significant variations from control are shown as * for P < 0.05 and ** for P < 0.01 (ANOVA for repeated measures followed by Dunnett’s t test).
nin NK₂ and NK₃ receptors (Regoli et al., 1994), it did not interact with these receptors, at concentrations up to 0.1 μM. Its antagonist activity at these receptors at a concentration of 1 μM remained limited compared with the activities of specific antagonists of these receptors in the same assays (Emonds-Alt et al., 1992, 1995, 1997, 2002). This limited effect of SSR240600 on tachykinin receptors other than NK₁ receptors was confirmed in functional assays using CHO cells stably expressing human tachykinin NK₂ and NK₃ receptors. In these assays, the inhibition of tachykinin NK₁ or NK₃ receptor-mediated inositol phosphate-1 formation by SSR240600 was only observed at IC₅₀ values about 200-fold higher than those obtained in similar experimental conditions for specific NK₂ (SR 48968, SR144190) (F. Oury-Donat, O. Thurneyssen, and P. F. Oury-Donat et al., 1992, 1995, 1997, 2002).

Fig. 5. Inhibition by SSR240600 of GR73632-induced bronchoconstriction in anesthetized guinea pigs. SSR240600 was administered by the intravenous route at various doses, 30 min before administration of SSR240600. Values are expressed as percentage inhibition of bronchoconstriction induced by GR73632 before administration of SSR240600. Values are means ± S.E.M. (n = 5). Significant variations from control are shown as ** for P < 0.01 (ANOVA followed by Dunnett’s t test).

Fig. 6. Inhibition by SSR240600 of substance P-induced microvascular leakage in anesthetized guinea pigs. Saline (control) or SSR240600 was administered by the intraperitoneal route at various doses, 30 min before Evans blue dye (30 mg/kg i.v.). One minute after dye administration, plasma extravasation was provoked by substance P (0.3 μg/kg i.v.). Basal level was determined in the absence of substance P. Results are expressed as tissue content of Evans blue dye. Values are means ± S.E.M. (n = 4–6). Significant variations from control are shown as ** for P < 0.01 (ANOVA followed by Dunnett’s t test).

Fig. 7. Inhibition by SSR240600 of cough provoked by exposure of conscious guinea pigs to an aerosol of citric acid solution (0.4 M) for 10 min. A, SSR240600 was administered at different doses by the intraperitoneal route, 30 min before the citric acid challenge. B, SSR240600 (1 mg/kg i.p.) was administered at different times before the citric acid challenge. Results are expressed as percentage inhibition of control and are means ± S.E.M. (n = 4–10). Significant variations from control are shown as * for P < 0.05 and ** for P < 0.01 (ANOVA for repeated measures followed by Dunnett’s t test).
On the contrary, GR205171 was shown to have a potent antitussive effect was reported for a peptidomimetic antagonist (FK888) (Yasumitsu et al., 1996). However, another potent and selective non-peptide tachykinin NK1 receptor antagonist (Emonds-Alt et al., 1993; Hirayama et al., 1993; Cellier et al., 1996; McLean et al., 1996; Cirillo et al., 1998), SSR240600 very potently inhibited tachykinin NK1 receptor-mediated hypotension, bronchoconstriction, and plasma extravasation, three typical effects of substance P and its analogs (Regoli et al., 1994; Quartara and Maggi, 1998). It was active by the oral route and had long-lasting effects.

SSR240600 was then studied in an animal model where endogenous tachykinins through their respective receptors are suspected to play a major role: citric acid-induced cough in unanesthetized guinea pigs (Widdicombe, 1995; Advenier and Emonds-Alt, 1996; Advenier et al., 1997). Either tachykinin NK1 (Advenier et al., 1993; Girard et al., 1995; Yasumitsu et al., 1996; Emonds-Alt et al., 1997) or NK3 (Daoui et al., 1998; Emonds-Alt et al., 2002) receptor antagonists have been reported to have potent antitussive activity in this model. Regarding the effect of nonpeptide tachykinin NK1 receptor antagonists in this model, the results are controversial. No inhibitory activity was observed for a nonpeptide antagonist such as SR143333 (Girard et al., 1995), whereas an antitussive effect was reported for a peptidomimetic antagonist (FK888) (Yasumitsu et al., 1996). However, another nonpeptide tachykinin NK1 receptor antagonist, CP-99,994 (McLean et al., 1993), was shown to block cough induced by capsaicin in unanesthetized guinea pigs as well as by mechanical stimulation of trachea in anesthetized cats (Bolsér, 1996; Bolsér et al., 1997). In the present study, SSR240600 was clearly shown to potentially inhibit citric acid-induced cough in unanesthetized guinea pigs. Moreover, in the same model and under the same experimental conditions used for SR143333 and SSR240600, another tachykinin NK1 receptor antagonist, GR205171 (Gardner et al., 1996), also displayed potent antitussive activity (C. Advenier, E. Naline, and S. Daoui, unpublished results).

The antitussive activity of SSR240600 in guinea pigs may be related to its ability to readily penetrate into the brain. Indeed, the antitussive activity of CP-99,994 was reported as probably mainly mediated by a central action (Bolsér, 1996; Bolsér et al., 1997). On the other hand, there is some parallelism between antitussive activity and other centrally mediated activities of the tachykinin NK1 receptor antagonists. SR143333 was shown to have several activities in the rat central nervous system (Jung et al., 1994), but it was also reported to lack activity in some models, in particular, models for emesis, in which brain penetration of the compound is essential (Rupniak et al., 1997; Diemunsch and Grélot, 2000). On the contrary, GR205171 was shown to have a potent broad-spectrum anti-emetic activity (Gardner et al., 1996; Diemunsch and Grélot, 2000). Similarly, CP-99,994 also showed potent anti-emetic activity as well as other typical centrally mediated effects (Rupniak et al., 1997). Moreover, a potent antidepressant-like activity of SSR240600 in guinea pigs was clearly demonstrated (Steinberg et al., 2002) as for centrally active tachykinin NK1 receptor antagonists (Rupniak and Kramer, 1999). A preliminary pharmacokinetic study (C. Briot, unpublished results) also showed an efficient brain penetration of the compound in guinea pigs with slow kinetics (peak plasma level of 650 ng/ml obtained at 1 h, brain tissue level of 70 ng/g reached at 4 h, and stable between 4 and 8 h after oral administration at 10 mg/kg), explaining its time-dependent antitussive activity. However, the results reported for the evaluation of FK888 brain penetration are opposite (Yasumitsu et al., 1996; Rupniak et al., 1997), but together, they suggest a low brain penetration, if any. The antitussive activity of FK888 in guinea pigs could be due to a peripheral effect related to its peptidomimetic structure since a low brain-penetrant nonpeptide antagonist, SR143333, was completely inactive.

In conclusion, SSR240600 is a novel, highly potent nonpeptide antagonist of the tachykinin NK1 receptor. It is active by the oral route with long-lasting effects and can penetrate into the brain.

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References


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