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Potency and selectivity of the tachykinin NK_3 receptor antagonist SR 142801

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Abstract

Binding studies indicated that tachykinin NK_3 binding sites in peripheral (ileum) and central (cerebral cortex) tissues of the guinea pig exhibit similar pharmacological properties. They also confirmed that the tachykinin NK_3 receptor antagonist (*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide (SR 142801) has a higher affinity for tachykinin NK_3 binding sites in the guinea pig than in the rat. SR 142801 exhibited a much lower affinity for tachykinin NK_2 and NK_1 binding sites. SR 142801 was shown to be a potent uncompetitive antagonist of the senktide-induced formation of [^{3}H]inositol monophosphate in slices from the guinea-pig ileum (apparent $K_B = 3.2$ nM, 51% reduction of the maximal response), a functional test for tachykinin NK_3 receptors. In agreement with results of binding studies, the effect of SR 142801 was stereoselective since its enantiomer SR 142806 was much less potent. In the rat urinary bladder, a tissue devoid of tachykinin NK_3 receptors, SR 142801 was without effect on the [Pro^9]substance P- or the septide-induced formation of [^{3}H]inositol monophosphate but it slightly reduced the response of the tachykinin NK_2 receptor agonist [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) ($K_B = 339$ nM). Altogether, these data indicate that SR 142801 is a highly selective tachykinin NK_3 receptor antagonist which is more potent in the guinea pig than in the rat.

Keywords: Tachykinin; Tachykinin NK_3 receptor; Inositol monophosphate accumulation; Ileum, guinea pig; Nonpeptide tachykinin NK_3 receptor antagonist; Species difference

1. Introduction

Extensive progress has been made during the last few years in the development of new tachykinin receptor agonists and antagonists. Selective peptide receptor agonists are available for the tachykinin NK_1, NK_2 and NK_3 receptors whose endogenous ligands are substance P, neurokinin A and neurokinin B, respectively. [Pro^9]Substance P and [Sar^9,Met(O$_2$)^{11}]substance P are highly specific agonists for tachykinin NK_1 receptors, [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) is a selective tachykinin NK_2 receptor agonist, and senktide (succinyl-[Asp^6,MePhe^8]substance P-(6–11)) is a selective and highly potent tachykinin NK_3 receptor agonist (Torrens et al., 1991; Regoli et al., 1994; Patacchini and Maggi, 1995). Furthermore, some pharmacological evidence indicates that septide, an analogue of substance P-(6–11), acts either on specific tachykinin receptors or on a particular site of tachykinin NK_1 receptors (Glowinski, 1995; Maggi, 1995). Recently, selective potent nonpeptide and biologically stable tachykinin NK_1 and NK_2 receptor antagonists have been discovered. This has facilitated the pharmacological characterization of these receptors as well as the investigation of their functional properties (Regoli et al., 1994; Patacchini and Maggi, 1995). These receptor antagonists have also made it possible to reveal species differences in the properties of the tachykinin NK_1 and NK_2 receptors, differences which were then confirmed by molecular cDNA cloning, determination of their molecular structure and mutagenesis experiments (see review by Hall et al., 1993).

The existence of species differences for tachykinin NK_3 receptors was first suggested when we observed that SR 48968, a potent nonpeptide tachykinin NK_2 receptor antagonist, interferes with the binding of [^{3}H]senktide to cortical tachykinin NK_3 binding sites from the guinea pig but not from those of the rat (Petitet et al., 1993a). Moreover, SR 48968, which is devoid of tachykinin NK_3 receptor antagonist properties in the rat, was shown to inhibit the senktide-evoked formation of inositol phosphates in the guinea-pig ileum ($pK_B = 7.1$) (Petitet et al.,

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1993a). Other authors have indicated that SR 48968 antagonizes the release of Ca^{2+} from inositol trisphosphate-sensitive intracellular stores evoked by tachykinin NK_3 receptor agonists in Chinese hamster ovary cells tranfected with human tachykinin NK_3 receptor cDNA (Pinnock et al., 1994). Nevertheless, as underlined by Advenier et al. (1992), in spite of its lack of specificity in the guinea pig or the human, SR 48968 is still much more potent on tachykinin NK_2 receptors than on tachykinin NK_3 receptors in these two species.

The observation in the guinea pig of an interaction of the tachykinin NK_2 receptor antagonist SR 48968 (in high concentration) with tachykinin NK_3 receptors accelerated the discovery of the first potent and selective nonpeptide tachykinin NK_3 receptor antagonist, SR 142801 (Emonds-Alt et al., 1995). The present investigation performed in vitro was undertaken to determine: (1) the potency of SR 142801 in inhibiting the binding of selective tachykinin NK_3 receptor radioligands and in antagonizing the senktide-induced accumulation of [^{3}H]inositol monophosphate in the guinea-pig ileum because we have previously demonstrated that, under appropriate conditions, this preparation is particularly suitable for the analysis of the properties of tachykinin NK_3 receptors coupled to a phospolipase C (Petitet et al., 1993c); (2) the selectivity of this new tachykinin NK_3 receptor antagonist; and finally (3) some additional information on species differences in the pharmacological properties of the tachykinin NK_3 receptors.

2. Materials and methods

2.1. Biological preparations

2.1.1. Guinea-pig ileum membranes

2.1.1.1. Guinea-pig ileum membranes for tachykinin NK_1 and NK_2 binding studies. Membranes from the ileum of male Hartley guinea pigs (300–350 g; Charles River, Cléon, France) were prepared as described by Morimoto et al. (1992) with the following modifications. The mucosa was scraped off and smooth muscle tissues were cut with scissors and homogenised at 4°C with a polytron apparatus (Kinematica) for 30 s in Tris-HCl buffer, 50 mM, pH 7.4, containing sucrose (0.25 M) and EDTA (0.1 mM). The homogenate was then centrifuged ($800 \times g$, 10 min, 4°C) to remove tissue clumps. The supernatant was centrifuged at high speed ($14\,000 \times g$, 20 min, 4°C) and the resulting pellet was washed in Tris-HCl (50 mM, pH 7.4). Finally, the crude membrane fraction was obtained by further centrifugation of the suspended pellet ($14\,000 \times g$, 20 min, 4°C).

2.1.1.2. Guinea-pig ileum membranes for tachykinin NK_3 binding studies. Membranes from longitudinal muscle-myenteric plexus and circular muscle from the ileum of male Hartley guinea pigs were prepared essentially as previously described for longitudinal muscle-myenteric plexus membranes (Guard et al., 1990). The final pellets were resuspended in HEPES (20 mM, pH 7.4) to a protein concentration of 1.7–3.4 mg ml^{-1} (longitudinal muscle-myenteric plexus) and 1.7–2.5 mg ml^{-1} (circular muscle). Membranes were immediately used or stored in aliquots at −80°C.

2.1.2. Rat duodenal membranes

Male Sprague-Dawley rats (200–250 g; Charles River) were used and membranes from smooth muscles of the duodenum were prepared as previously described (Bergström et al., 1987).

2.1.3. Hamster, rat and guinea-pig urinary bladder membranes

Male Golden Syrian hamsters (100–120 g; R. Janvier, Le Genest-Saint Isle, France) were killed with a lethal intraperitoneal injection of pentobarbital (0.8 g/kg). Male Sprague-Dawley rats (200–250 g) and male Hartley guinea pigs (300–350 g) were killed by decapitation. Urinary bladders were rapidly removed and chopped in three directions ($200 \times 200 \times 200$ μm), using a MacIlwain apparatus. Tissues were homogenised with a polytron apparatus for 30 s in Tris-HCl buffer, 50 mM, pH 7.4 at 4°C and passed through a gauze filter. The homogenate was centrifuged at $14\,000 \times g$ for 20 min at 4°C, the supernatant was discarded and the pellet was then resuspended in the same Tris buffer. The final pellet was obtained by centrifugation of the suspension at $14\,000 \times g$ for 20 min at 4°C.

2.1.4. Rat and guinea-pig brain membranes

Rats and guinea pigs were killed by decapitation and brains were rapidly removed. Membrane suspensions of either the whole brain (except the cerebral cortex and the cerebellum) or the cerebral cortex were prepared as previously described (Petitet et al., 1993b).

2.1.5. Synaptosomal fraction from the rat brain

The crude synaptosomal fraction from the rat brain (minus the cerebral cortex and the cerebellum) was prepared as previously described (Viger et al., 1983).

2.1.6. Cultures of Chinese hamster ovary (CHO) cells expressing tachykinin NK_1 receptors

CHO cells transfected with the rat or the human tachykinin NK_1 receptor cDNA were kindly provided by Dr. L. Pradier and were cultured in Ham F-12 medium supplemented with 10% foetal calf serum, 100 IU/ml penicillin, 100 IU/ml streptomycin, 25 mM HEPES, 0.12% $NaHCO_3$ on NUNC (Denmark) culture dishes (16-mm diameter wells; 10^4 cells/well). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2/95% air for 24 h before performing the binding assays.

2.2. Binding studies

2.2.1. Membrane preparations

The various membrane pellets were resuspended in an incubation medium consisting of Tris-HCl buffer, 50 mM, pH 7.4, 4°C, enriched with $MnCl_2$ (3 mM), bovine serum albumin (0.4 mg/ml), bacitracin (40 μg/ml), leupeptin (4 μg/ml) and thiorphan (1 μM). In [^{3}H]neurokinin A binding assays, kelatorphan (1 μM) was used instead of thiorphan.

2.2.2. [^{3}H][Pro^9]Substance P binding

Experiments with brain, urinary bladder and ileum membranes were performed as previously described (Petitet et al., 1992, 1993b; Torrens et al., 1995). When CHO cells were used, cells were first washed three times for 15 min with 0.5 ml of Krebs phosphate buffer (in mM: NaCl 120, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4$ 1.2 and NaH_2PO_4 15.6, pH 7.2) containing bovine serum albumin (0.4 mg/ml), bacitracin (0.03 mg/ml) and glucose (6 mg/ml). They were finally incubated for 60 min at 20°C in a final volume of 200 μl with [^{3}H][Pro^9]substance P (0.5 nM).

2.2.3. [^{3}H]Neurokinin A binding

For binding experiments with the rat or the hamster urinary bladder with the guinea-pig ileum, kinetic and pharmacological studies were first performed in order to confirm that [^{3}H]neurokinin A binding corresponded indeed to the labeling of tachykinin NK_2 binding sites. Membranes from the rat or the hamster urinary bladder (60 μl, 300 μg protein) were incubated at equilibrium (25 min at 20°C) in a final volume of 200 μl with [^{3}H]neurokinin A (0.5 nM). Experiments with guinea-pig ileum membranes were done under the same conditions except that the equilibrium time was 30 min at 20°C. Binding assays with rat duodenal membranes were performed as previously described (25 min at 20°C) (Bergström et al., 1987).

2.2.4. [^{3}H]Senktide and [^{125}I][MePhe^7]neurokinin B binding

Kinetic and pharmacological experiments were first performed in order to confirm that the sites labeled with [^{3}H]senktide or [^{125}I][MePhe^7]neurokinin B on membranes from the rat or guinea-pig cerebral cortex and from the guinea-pig ileal longitudinal muscle-myenteric plexus corresponded indeed to tachykinin NK_3 binding sites (Suman-Chauhan et al., 1994; Guard et al., 1990). Experiments were performed with either [^{3}H]senktide (2 nM) or [^{125}I][MePhe^7]neurokinin B (20 pM) in a final volume of 200 μl, the incubation (at 20°C) lasting 60 or 45 min, respectively; ileal membranes were incubated with [^{125}I][MePhe^7]neurokinin B for 60 min at 20°C. [^{125}I]Bolton-Hunter eledoisin (40 pM) binding experiments in which the crude synaptosomal fraction from the rat cerebral cortex was used were performed as previously described (Beaujouan et al., 1984).

In all binding assays performed with membranes, incubations were stopped by filtration through Skatron filters pretreated for 3–4 h with polyethylenimine (0.1%) at 4°C, using a Skatron cell harvester apparatus. Filters were washed with 9 ml of the incubation medium (without peptidase inhibitors) at 4°C. The pellet-bound radioactivity was counted in an LKB-Wallace liquid spectrometer, using 4 ml of Aquasol-2. In the case of CHO cells, the incubation was stopped by discarding the supernatant and rapidly washing the cells three times with 0.5 ml of cold incubation medium. Cells were detached with 0.5 ml of Triton X-100 (0.1%) containing bovine serum albumin (1 mg/ml) and the cell-bound radioactivity was counted.

2.3. Estimation of the accumulation of [^{3}H]inositol monophosphate in slices from the guinea-pig ileum and the rat urinary bladder

Guinea pigs were decapited and the ileum was rapidly removed. Similarly, rats were killed by decapitation and the urinary bladder was rapidly removed. In both cases, the estimation of the formation of [^{3}H]inositol monophosphate from [^{3}H]myo-inositol was achieved in the presence of lithium chloride (10 mM final concentration), using a 30-min incubation with the agonist at 37°C, as previously described (Petitet et al., 1993a; Torrens et al., 1995). When used, antagonists were added 15 or 60 min before the agonists.

2.4. Ligands and drugs

[^{125}I]Bolton-Hunter eledoisin was obtained by coupling the [^{125}I]Bolton-Hunter reagent (Amersham: monoiodo-derivative (spec. act. 2000 Ci/mmol) to eledoisin as previously described (Beaujouan et al., 1984). [^{3}H]Neurokinin A was synthesized by reacting [^{3}H]methyl-4-tolyl sulphonate (spec. act. 75 Ci/mmol) with [HCy^{10}]neurokinin A thiolactone according to a procedure previously described (Chassaing et al., 1985). [^{3}H][Pro^9]Substance P (spec. act. 75 Ci/mmol) was synthesized as described elsewhere (Petitet et al., 1991). Aquasol-2, [^{125}I]iodohistidyl, [MePhe^7]neurokinin B (spec. act. 2200 Ci/mmol) and [^{3}H]senktide (spec. act. 74 Ci/mmol) were purchased from NEN, while myo-[2-^{3}H]inositol (spec. act. 19 Ci/mmol) was from Amersham, France. [Pro^9]Substance P and [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10) were synthesized and kindly provided by Drs S. Lavielle and G. Chassaing (University, Paris VI). Senktide was purchased from Peninsula Laboratories (Belmont CA, USA). [MePhe^7]Neurokinin B was obtained from Bachem (Switzerland). (*S*)-(*N*)-(1-(3-(1-Benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl) propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide (SR 142801) and its (*R*)-enantiomer (SR 142806) were kindly provided by Dr G. Le Fur (Sanofi Recherche).

3. Results

3.1. Higher affinity of the tachykinin NK_3 receptor antagonist SR 142801 for tachykinin NK_3 binding sites in the guinea pig than in the rat

Binding studies were mainly performed with [^{125}I][MePhe^7]neurokinin B and [^{3}H]senktide but [^{125}I]Bolton-Hunter eledoisin was also used in some experiments. As expected, similar results were obtained whatever ligand was used in competition experiments performed with selective tachykinin receptor agonists.

In the guinea pig, tachykinin NK_3 binding sites were only observed in longitudinal muscle-myenteric plexus membranes but not on circular muscle membranes. Senktide and [MePhe^7]neurokinin B exhibited nanomolar affinities for ileal tachykinin NK_3 binding sites while the selective tachykinin NK_1 and NK_2 receptor agonists [Pro^9]substance P and [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10), respectively, were completely devoid of affinity (Table 1). The tachykinin NK_3 receptor antagonist SR 142801 exhibited a very high affinity (in a range similar to that of [MePhe^7]neurokinin B) for tachykinin NK_3 binding sites. Similar results were obtained with the two ligands, [^{125}I][MePhe^7]neurokinin B and [^{3}H]senktide (IC_{50} = 2.1 ± 0 7 nM and 6.2 ± 2.9 nM, respectively) (Table 1). In both cases, the competitive curve was monophasic and the Hill coefficient (n_H) was close to unity ($n_H = 1.2 \pm 0.2$ and 0.8 ± 0.2, respectively).

As indicated in Table 1, the affinity of tachykinin NK_3 agonists for tachykinin NK_3 binding sites on cortical membranes from the guinea pig and the rat was similar to that found in the guinea-pig ileum (longitudinal muscle-myenteric plexus). The selective tachykinin NK_1 and NK_2 receptor agonists were also devoid of affinity. As in the guinea-pig ileum, SR 142801 had a high affinity for the tachychinin NK_3 binding sites in the cerebral cortex of the guinea pig, similar results being observed with the two ligands, [^{125}I][MePhe^7]neurokinin B and [^{3}H]senktide ($IC_{50} = 2.1 \pm 0.7$ and 3.7 ± 0.3 nM, respectively) (Table 1). In both cases, the competitive curve was monophasic and the Hill coefficient was close to unity ($n_H = 0.9 \pm 0.2$ and 1.2 ± 0.1, respectively) (Fig. 1).

Confirming the existence of species differences in the properties of tachykinin NK_3 binding sites, SR 142801 had a weaker affinity for cortical tachykinin NK_3 binding sites in the rat than in the guinea-pig since the IC_{50} values of the receptor antagonist for inhibiting [^{125}I][MePhe^7]neurokinin B and [^{3}H]senktide binding were about 10- to 20-fold higher (37 ± 4 and 34 ± 11 nM) in the rat (Table 1). SR 142801 also competitively inhibited, in a concentration-dependent manner, the high-affinity specific binding of the two radioligands to rat cortical membranes. Hill coefficients were close to unity ($n_H = 0.9 \pm 0.1$ and 0.8 ± 0.2, respectively) (Fig. 1, Table 1). Similar results were obtained with the [^{125}I]Bolton-Hunter eledoisin binding assay since SR 142801 competitively inhibited the high-affinity specific binding of [^{125}I]Bolton-Hunter eledoisin to rat cortical synaptosomes with an IC_{50} of 56 ± 15 nM (results not shown).

The inhibitory effect of SR 142801 was stereoselective since in the guinea pig, whatever the ligand used, the (*R*)-enantiomer SR 142806 was about 60-fold less efficient. Nevertheless, differences in the affinity of SR 142806 for tachykinin NK_3 binding sites of the guinea pig and of the rat could still be observed (Table 1).

3.2. Respective affinities of SR 142801 and SR 142806 for tachykinin NK_1 and NK_2 binding sites in various species

There are species differences in the affinity of SR 142801 and the tachykinin NK_2 receptor antagonist SR

Table 1
Affinities of selective tachykinin receptor agonists SR 142801 and SR 142806 for tachykinin NK_3 binding sites in the guinea pig and the rat

Compound	Guinea pig				Rat	
	[^{125}I][MePhe^7]NKB IC_{50} (nM)		[^{3}H]Senktide IC_{50} (nM)		[^{125}I][MePhe^7]NKB IC_{50} (nM)	[^{3}H]Senktide IC_{50} (nM)
	c. cortex	ileum	c. cortex	ileum	c. cortex	c. cortex
Senktide	15.2	8.6	6.7	4.6	23.3	6.3
[MePhe^7]Neurokinin B	6.3	3.9	4.0	2.4	4.8	6.7
[Pro^9]Substance P	> 10 000	> 10 000	> 10 000	> 10 000	> 10 000	> 10 000
[Lys^5,MeLeu^9,Nle^{10}]-Neurokinin A-(4–10)	> 10 000					
> 10 000	> 10 000	> 10 000	> 10 000	> 10 000		
SR 142801	2.1	2.1	3.7	6.2	37	34
SR 142806	184	123	151	272	1 760	3 430

Binding experiments were performed as described in Section Section 2 with membranes from the guinea pig or the rat using [^{125}I][MePhe^7]neurokinin B ([^{125}I][MePhe^7]NKB) and [^{3}H]senktide as ligands. Specific binding of [^{125}I][MePhe^7]neurokinin B to guinea pig and rat cortical membranes represented 81% and 66% of total binding, respectively, while the specific binding of [^{3}H]senktide to guinea pig and rat cortical membranes represented 74% and 47% of total binding, respectively. Specific binding of [^{125}I][MePhe^7]NKB and [^{3}H]senktide to guinea-pig ileum represented 83% and 62% of total binding, respectively. IC_{50} values (nM) are the mean of data obtained in two to five independent experiments, each value being determinated in triplicate. The standard deviation did not exceed 44% of the mean.

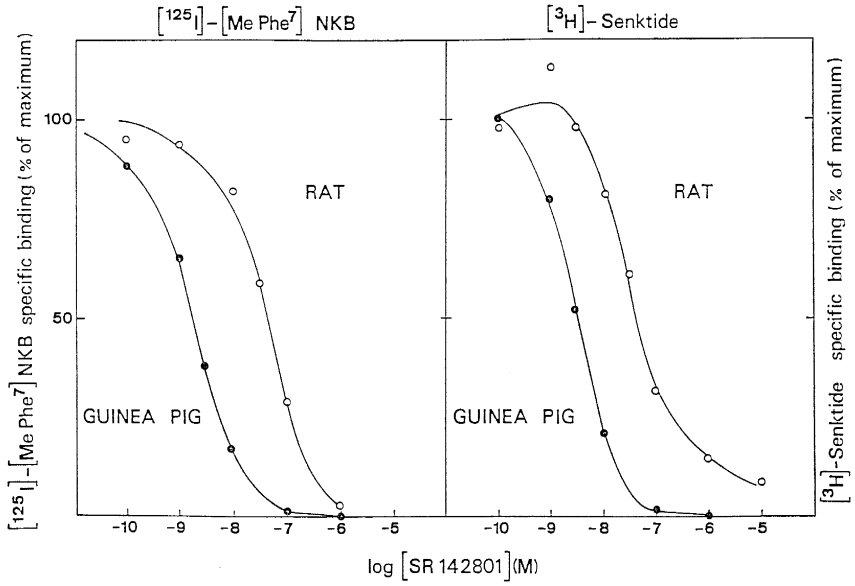

Fig. 1. Competitive inhibition by the nonpeptide tachykinin NK_3 receptor antagonist SR 142801 of [^{125}I][MePhe^7]neurokinin B and [^{3}H]senktide binding to membranes from the guinea pig and rat cerebral cortex. Cortical membranes were incubated as described in Section Section 2 with [^{125}I][MePhe^7]neurokinin B ([^{125}I][MePhe^7]NKB) (20 pM) (left panel) or [^{3}H]senktide (2 nM) (right panel) and increasing concentrations of the nonpeptide antagonist SR 142801. Nonspecific binding was determined in the presence of either [MePhe^7]neurokinin B (1 μM) or senktide (1 μM). Each point is the mean of data obtained in three or four experiments, each value being determined in triplicate. Results are expressed as a percentage of the control [^{125}I][MePhe^7]neurokinin B or [^{3}H]senktide specific binding.

48968 for cortical tachykinin NK_3 binding sites (Petitet et al., 1993a). Indeed, the tachykinin NK_3 receptor antagonist SR 142801 could eventually interfere with tachykinin NK_1 and NK_2 binding sites in some species since, as already indicated, there are species differences in the pharmacological properties of these binding sites.

Tachykinin NK_1 binding assays were performed with the highly selective tachykinin NK_1 radioligand, [^{3}H][Pro^9]substance P (Petitet et al., 1991). As shown in Table 2, in the rat, whatever the tissue used, for example the brain or the urinary bladder, and even in CHO cells transfected with the rat tachykinin NK_1 receptor cDNA, the tachykinin NK_3 receptor antagonist SR 142801 was completely devoid of affinity for tachykinin NK_1 binding sites. However, compared with its effect in the guinea pig, SR 142801 slightly inhibited the binding of [^{3}H][Pro^9]substance P to tachykinin NK_1 binding sites in membranes of the brain or the ileum (IC_{50} = 2000 nM). This was even more evident with CHO cells transfected with the human tachykinin NK_1 receptor cDNA (IC_{50} = 600 nM) (Table 2). Although the efficiency of the (*R*)-enantiomer SR 142806 was lower than that of SR 142801, species differences could still be observed (Table 2).

Binding assays performed with the tachykinin NK_2 ligand [^{3}H]neurokinin A indicated first that SR 142801 was much more potent in displacing ligand binding to tachykinin NK_2 binding sites than that of [^{3}H][Pro^9]substance P binding to tachykinin NK_1 binding sites, and secondly that there were species differences in the efficiency of SR 142801 to displace [^{3}H]neurokinin A binding (Table 2). Indeed, SR 142801 affected the binding of [^{3}H]neurokinin A to tachykinin NK_2 binding sites on membranes of the rat urinary bladder and duodenum or on membranes of the hamster urinary bladder, a tissue which possesses tachykinin NK_2 binding sites only (IC_{50} = 350–1000 nM) (Table 2). In addition, SR 142801 inhibited the

Table 2
Affinities of SR 142801 and SR 142806 for tachykinin NK_1 and NK_2 binding sites from various species

	Preparations	SR 142801 IC_{50} (nM)	SR 142806 IC_{50} (nM)
[^{3}H][Pro^9]Substance P	Rat brain	> 10000	> 10000
	Rat urinary bladder	25000	10000
	NK_1 rat CHO	> 10000	> 10000
NK_1	Guinea-pig brain	2110	9270
	Guinea-pig ileum	2340	5080
	NK_1 human CHO	600	2600
[^{3}H]Neurokinin A	Rat urinary bladder	348	460
	Rat duodenum	1000	1300
NK_2	Hamster urinary bladder	739	790
	Guinea-pig ileum	38	132

The different preparations (membranes, intact Chinese hamster ovary (CHO) cells) were incubated with either [^{3}H][Pro^9]substance P or [^{3}H]neurokinin A and increasing concentrations of either the nonpeptide tachykinin NK_3 receptor antagonist SR 142801 or its (*R*)-eniantomer SR 142806. Tissue preparations and binding assay conditions were as described in Section Section 2. Each IC_{50} value (nM) is the mean of data obtained in two to four experiments, each value being determinated in triplicate. Standard deviations in tachykinin NK_1 and NK_2 binding assays did not exceed 28% and 38% of the mean, respectively.

Table 3
Inhibition by the tachykinin NK_3 receptor antagonist SR 142801 of the senktide-, [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10)-, [Pro^9]substance P- and septide-induced accumulation of [^{3}H]inositol monophosphate

Antagonist	Agonist	K_B (nM)	Inhibition type	Maximal response (% of change)
SR 142801 preincub. 15 min	Senktide	6.0[a] (4.0–9.1)	Uncompetitive	−47% (0.1 μM)
SR 142801 preincub. 60 min	Senktide	3.2[a] (2.3–4.5)	Uncompetitive	−51% (0.1 μM)
SR 142806 preincub. 60 min	Senktide	191[a] (129–282)	Uncompetitive	−12% (0.1 μM)
SR 142801 preincub. 60 min	[Lys^5,MeLeu^9,Nle^{10}]Neurokinin A-(4–10)	339 (257–447)	Competitive	−2% (1 μM)
SR 142801 preincub. 60 min	[Pro^9]Substance P	> 1000	No effect	−4% (1 μM)
SR 142801 preincub. 60 min	Septide	> 1000	No effect	−13% (1 μM)

Experiments were conducted as described in Section 2. SR 142801 or SR 142806 was added to the incubation medium 60 min before the tachykinin receptor agonists used at various concentrations, except in one series of experiments in which a 15-min preincubation was used. Experiments with senktide were performed with slices from the guinea-pig ileum while those with other receptor agonists were performed with slices from the rat urinary bladder. For surmountable antagonism, K_B values were calculated from the equation $K_B = [\text{antagonist}]/(DR - 1)$, where DR is the dose ratio of the agonist half-maximal concentration in the presence and absence of the antagonist. When insurmountable antagonism was observed, an apparent K_B^a for the antagonist was derived by the use of a double reciprocal regression plot, according to the method of Kenakin (1993). A graph of $1/[A]$ versus $1/[A']$ was plotted, where [A] and [A′] were the equieffective concentrations of the agonist in the absence or presence of antagonist, respectively. Thus $K_B^a = [B]/(\text{slope}/\text{intercept} - 1)$, where [B], the maximal response to the agonist, was depressed to less than 50% of the maximal response by the uncompetitive antagonist. The effects of SR 142801 (0.1 or 1 μM) and SR 142806 (0.1 μM) on maximal responses induced by receptor agonists are expressed as a percentage of the reduction of the maximal response of the receptor agonist obtained in the absence of the receptor antagonist (100%). Each value is the mean of data obtained in two to five independent experiments, each point being determinated in triplicate.

binding of [^{3}H]neurokinin A to membranes from the guinea-pig ileum with a much higher efficacy (38 ± 10 nM) than to membranes from other species (Table 2). In the guinea pig, the affinity of SR 142801 for tachykinin NK_2 binding sites was only 6- to 18-fold lower than its affinity for tachykinin NK_3 binding sites. Interestingly, no marked differences in the IC_{50} values were observed when experiments were performed with SR 142806 or SR 142801, indicating that the stereoselectivity of SR 142801 for tachykinin NK_2 binding sites is not as pronounced as that for tachykinin NK_1 or NK_3 binding sites (Tables 1 and 2).

When substantial affinities could be demonstrated, in all cases SR 142801 and SR 142806 competitively inhibited

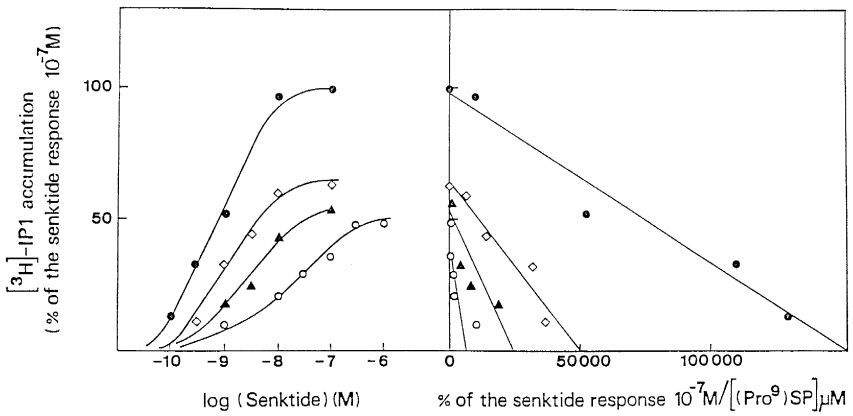

Fig. 2. Inhibition by the tachykinin NK_3 receptor antagonist SR 142801 of the senktide-induced formation of [^{3}H]inositol monophosphate in slices from the guinea-pig ileum. Concentration-response curves of senktide without (●) or in the presence of various concentrations of SR 142801: (◇, 0.01 μM; ▲, 0.03 μM; ○, 0.1 μM). Left panel: SR 142801 was added to the incubation medium 60 min before the selective tachykinin NK_3 receptor agonist (senktide) used at various concentrations, the accumulation of [^{3}H]inositol monophosphate ([^{3}H]-IP1) being determined at the end of a 30-min incubation. In each case, results corresponding to the accumulation of [^{3}H]inositol monophosphate are expressed as a percentage of the maximal senktide response (0.1 μM). The mean value for the maximal accumulation of [^{3}H]inositol monophosphate induced by senktide was 203% of the control value (obtained in the absence of senktide). Right panel: Linear plots of the concentration-response curves showing (% maximal response) = f (% maximal response/[agonist] (μM)). Lines represent the best fit of the data calculated by linear regression analysis, correlation coefficient values (r) extend from 0.82 to 0.98.

in a concentration-dependent manner the specific binding of [^{3}H][Pro^9]substance P and [^{3}H]neurokinin A to the various membrane preparations and Hill coefficients were found to be close to unity (results not shown).

3.3. Blocking effect of SR 142801 on the senktide-evoked accumulation of [^{3}H]inositol monophosphate in the guinea-pig ileum

The potency of SR 142801 as a tachykinin NK_3 receptor antagonist was verified by measuring its capacity to inhibit the senktide-induced accumulation of [^{3}H]inositol monophosphate in guinea-pig ileum slices. In this tissue, the selective tachykinin NK_3 receptor agonist senktide stimulated with great potency the formation of [^{3}H]inositol monophosphate ($EC_{50} = 0.56 \pm 0.11$ nM, $n = 10$) (result not shown). When used alone, SR 142801 was devoid of activity. SR 142801 antagonized with great efficiency the senktide-evoked response. The blockade of the senktide response was slightly more pronounced when tissues were exposed for a longer time to the antagonist (60-min preincubation), but the difference between results obtained with either a 15-min or a 60-min preincubation with the antagonist was not significant (Table 3).

As particularly shown in experiments performed with a 60-min preincubation with the antagonist, SR 142801 induced a concentration-dependent non-parallel rightward shift of the senktide concentration-response curve (apparent inhibition constant for insurmountable antagonism, $K_B^a = 3.2$ nM), with a significant reduction of the maximal response (51% at 0.1 μM). Therefore, the inhibitory effect of SR 142801 appears to be uncompetitive (insurmountable, EC_{50} modified) (Fig. 2 and Table 3). The (*R*)-enantiomer SR 142806 exhibited the same profile of antagonist activity but, as expected, was much less efficient ($K_B^a =$ 191 nM) than SR 142801 (Table 3 and results not shown).

3.4. Selectivity of the tachykinin NK_3 receptor antagonist SR 142801

Unfortunately, the selective tachykinin NK_1 receptor agonist [Pro^9]substance P and septide have limited stimulatory effects on the formation of [^{3}H]inositol monophosphate in the guinea-pig ileum. In addition, selective tachykinin NK_2 receptor agonists have also a very low efficacy for mediating this response in this tissue (Petitet et al., 1993c). Therefore, it was not possible to determine whether SR 142801 could inhibit (even with a low potency) responses mediated through tachykinin NK_1, 'septide-sensitive' or NK_2 receptors in this tissue.

However, the rat urinary bladder seems to be particularly appropriate to look for the selectivity of SR 142801 since this tissue is devoid of tachykinin NK_3 receptors (senktide has no spasmogenic activity and does not stimulate the formation of [^{3}H]inositol monophosphate) but possesses tachykinin NK_1, NK_2 and 'septide-sensitive' receptors coupled to a phospholipase C (Burcher and Buck, 1986; Meini et al., 1994; Torrens et al., 1995).

As expected from binding studies, following a 60-min preincubation SR 142801 (1 μM) affected neither the [Pro^9]substance P nor the septide-evoked formation of [^{3}H]inositol monophosphate (Table 3). Under identical conditions, SR 142801 slightly reduced the potent stimulation of [^{3}H]inositol monophosphate formation induced by the selective tachykinin NK_2 receptor agonist [Lys^5,MeLeu^9,Nle^{10}]neurokinin A-(4–10), the K_B being 339 nM. This slight inhibition was competitive since a parallel rightward displacement of the agonist dose-response curve was observed (Table 3 and results not shown).

4. Discussion

Tachykinin NK_3 receptors are present in the brain and the spinal cord of different mammals and in some peripheral tissues, the guinea-pig ileum in particular (Beaujouan et al., 1984; Laufer et al., 1985; Tsuchida et al., 1990; Dietl and Palacios, 1991; Buell et al., 1992). SR 142801 is the first potent and selective nonpeptide antagonist which has been described (Emonds-Alt et al., 1995). In the guinea-pig ileum, SR 142801 has been shown to be a competitive antagonist of the [MePhe^7]neurokinin B-induced contraction and to inhibit the release of acetylcholine evoked by senktide or [MePhe^7]neurokinin B. Moreover, in gerbils, this tachykinin NK_3 receptor antagonist potently inhibits the turning behaviour induced by the intrastriatal injection of senktide (Emonds-Alt et al., 1995). In CHO cells expressing human tachykinin NK_3 receptors, SR 142801 antagonizes with a great efficiency the increase in inositol monophosphate formation, arachidonic acid release, cyclic AMP accumulation and intracellular Ca^{2+} concentration induced by selective tachykinin NK_3 receptor agonists (Oury-Donat et al., 1995). However, to our knowledge, the efficiency of this receptor antagonist toward a tachykinin NK_3 receptor agonist-evoked activation of a transducing system coupled to the tachykinin NK_3 receptors has not been examined in normal tissues.

In the present study, the efficiency of SR 142801 in antagonizing the formation of [^{3}H]inositol monophosphate evoked by senktide was investigated in slices from the guinea-pig ileum, since tachykinin NK_3 receptor agonists induce a marked and selective accumulation of [^{3}H]inositol monophosphate in this tissue (Petitet et al., 1993c). Tachykinin NK_3 receptors seem to be directly coupled to a phospholipase C, because in the guinea pig ileum the potent stimulatory effect of senktide on phosphoinositide breakdown persists in the presence of either atropine or tetrodotoxin (Guard et al., 1988).

Our results confirm the high potency of senktide (EC_{50} = 0.56 nM) in stimulating the formation of [^{3}H]inositol monophosphate in the guinea-pig ileum and demonstrate that SR 142801, which is devoid of agonist properties, acts

as an uncompetitive and potent tachykinin NK_3 receptor antagonist (apparent inhibition constant $K_B^a = 3.2$ nM and depression of the maximal senktide response: 51% at 0.1 μM). This latter observation differs from the competitive inhibitory effect of SR 142801 on the [MePhe^7]neurokinin B-induced contraction of the guinea-pig ileum (Emonds-Alt et al., 1995) but can be compared to the uncompetitive inhibition of the senktide-induced contraction of the guinea-pig ileum longitudinal muscle induced by a high concentration of either SR 142801 or the tachykinin NK_2 receptor antagonist SR 48968 (Maggi et al., 1994; Patacchini et al., 1995). Difficulties in the interpretation of such results are underlined by a recent study indicating that responses to tachykinin NK_1 receptor agonists in CHO cells expressing tachykinin NK_1 receptors can be either competitively or uncompetitively blocked by an antagonist, depending on the identity of the transducing system activated (Sagan et al., 1996).

The guinea-pig ileum is a complex tissue, and the action of senktide is considered to be exclusively neuronal (Wormser et al., 1986; Guard et al., 1988). Confirming and extending previous results (Guard et al., 1990), tachychinin NK_3 binding sites were exclusively found in membranes from the longitudinal muscle-myenteric plexus but not in those of the circular muscle. Therefore, it can be assumed that the stimulatory effect of senkide on [^{3}H]inositol monophosphate formation is linked to the stimulation of the neuronal tachykinin NK_3 receptors implicated in the release of acetylcholine and tachykinins.

In some in vitro assays, tissues must be preincubated for a long time in the presence of the antagonist before the addition of the receptor agonist in order to demonstrate the full blocking action of some nonpeptide tachykinin NK_1 or NK_3 receptor antagonists. According to Patacchini et al. (1995), the blockade by SR 142801 of the senktide-evoked contraction of the guinea-pig ileum longitudinal muscle is essentially irreversible and increased by prolonging the incubation from 15 to 120 min. In our study, the efficacy of SR 142801 for blocking the senktide-evoked formation of [^{3}H]inositol monophosphate was slightly higher following a 60- than a 15-min preincubation with the antagonist, but the difference was not significant. Differences in the rapidity of action of SR 142801 could be dependent on the signal measured ([^{3}H]inositol monophosphate formation versus ileum contraction) or on the type of preparation used (prisms in our experiments versus strips of ileum in contraction experiments). In fact, SR 142801 can block the release of acetylcholine evoked by senktide following a 10-min application (Emonds-Alt et al., 1995). Furthermore, in our experiments the apparent K_B value (3.2 nM) of the tachykinin NK_3 receptor antagonist closely agreed with its IC_{50} values in binding studies performed with longitudinal muscle-myenteric plexus membranes from the guinea-pig ileum using either [^{125}I][MePhe^7]neurokinin B or [^{3}H]senktide (2.1 and 6.2 nM, respectively) as ligand. Finally, as in other assays (Emonds-Alt et al., 1995; Oury-Donat et al., 1995; Patacchini et al., 1995), the antagonist effect of SR 142801 toward the senktide-evoked accumulation of [^{3}H]inositol monophosphate was stereospecific. Indeed, the (*R*)-enantiomer SR 142806 was much less potent in inhibiting uncompetitively the senktide-evoked response (apparent $K_B = 191$ nM), and this difference was comparable to that observed in binding studies performed with the two ligands ($IC_{50} = 123$ and 272 nM).

Species differences in the pharmacological properties and in the molecular structure of tachykinin NK_1 and NK_2 receptors have been demonstrated thanks to the development of nonpeptide receptor antagonists (see reviews by Hall et al., 1993; Regoli et al., 1994; Patacchini and Maggi, 1995). Species differences were also observed for tachykinin NK_3 receptors when the tachychinin NK_3 receptor agonist [Pro^7]neurokinin B was used and then more convincingly so when the tachykinin NK_2 receptor antagonist SR 48968 was used (Petitet et al., 1993a). Indeed, this potent tachykinin NK_2 receptor antagonist, highly selective in the rat, also blocks tachykinin NK_3 receptors in the guinea-pig or the human when used in a relatively high concentration (Petitet et al., 1993a; Chung et al., 1994; Nguyen et al., 1994; Suman-Chauhan et al., 1994). More recently, species differences in tachykinin NK_3 receptors have directly been demonstrated thanks to the tachykinin NK_3 receptor antagonist SR 142801. Indeed, according to Emonds-Alt et al. (1995) the K_i of SR 142801 for high-affinity tachykinin NK_3 binding sites is between 0.10–0.40 nM in cerebral cortices from the guinea pig and the gerbil as well as in CHO cells expressing the human tachykinin NK_3 receptor (ligand [^{125}I]iodohistidyl-[MePhe^7]neurokinin B) while it is about 100-fold higher (15 nM) (ligand [^{125}I]eledoisin) in the rat brain. Confirming the existence of species differences, our binding studies performed with cortical membranes with the two selective tachykinin NK_3 ligands [^{125}I]iodohistidyl-[MePhe^7]neurokinin B and [^{3}H]senktide indicated that IC_{50} values for SR 142801 were 2.1 and 3.7 nM, respectively, in the guinea pig and 37 and 34 nM, respectively, in the rat. Presently, we have no explanation for the slight difference in the affinity of SR 142801 for guinea-pig cortical (or ileal) tachykinin NK_3 binding sites observed in our study and in that of Emonds-Alt et al. (1995). Although the affinity for tachykinin NK_3 binding sites of the (*R*)-enantiomer SR 142806 was much lower than that of SR 142801, a similar discrepancy was observed with this compound, its affinity being also 10-fold higher in the guinea pig than in the rat. Beside SR 142801, two other recently developed and structurally different nonpeptide tachykinin NK_3 receptor antagonists, PD 154740 and PD 157672, have been shown to be more potent in the human than in the rat, and it has been indicated that only two amino acid residues are responsible for the species selectivity of these three tachykinin NK_3 receptor antagonists (Chung et al., 1995).

Information on the selectivity of SR 142801 is already

available. According to Emonds-Alt et al. (1995), although this compound was devoid of affinity for tachykinin NK_1 and NK_2 binding sites in the rat brain cortex and the hamster urinary bladder, respectively, an affinity in the range of 60–100 nM was found for tachykinin NK_1 and NK_2 binding sites in human lymphoblast cells (IM9) and in the rat urinary bladder or the guinea-pig ileum, respectively. In addition, SR 142801 does not significantly antagonize the [Sar^9,$Me(O_2)^{11}$]substance P-induced relaxation of the rabbit pulmonary artery previously contracted with noradrenaline (a tachykinin NK_1 receptor functional assay), or the [βAla^8]neurokinin A-(4–10)-induced contraction of the endothelium-denuded rabbit pulmonary artery (a tachykinin NK_2 receptor functional assay). However, although SR 142801 is a more potent tachykinin NK_3 receptor antagonist in the guinea pig than in the rat, it shows similar antagonist potency at rat tachykinin NK_3 receptors (portal vein) and rabbit tachykinin NK_2 receptors (pulmonary artery) but is less potent at hamster tachykinin NK_2 receptors (trachea) (Patacchini et al., 1995). Finally, SR 142801 has been shown to exhibit some affinity (in the micromolar range) for opiate receptors as well as for Ca^{2+} and Na^+ channels (Emonds-Alt et al., 1995).

Our study indicates that SR 142801 is devoid of affinity for NK_1 binding sites ([^{3}H][Pro^9]substance P) in several preparations from the rat. However, some affinity of this tachykinin NK_3 antagonist for these sites was observed in the brain and the ileum of the guinea pig and in CHO cells expressing human tachykinin NK_1 receptors, revealing once more the existence of species differences. SR 142801 was also found to have some affinity for [^{3}H]neurokinin A binding sites in the rat duodenum and the hamster urinary bladder, tissues which possess mainly, if not exclusively, tachykinin NK_2 receptors (Bergström et al., 1987; Bristow et al., 1987). In addition, a much higher affinity for these [^{3}H]neurokinin A binding sites was observed in the guinea-pig ileum (38 nM), which could be attributed both to an interaction of SR 142801 with tachykinin NK_2 receptors in this tissue and possibly to nonspecific labeling of tachykinin NK_3 receptors by [^{3}H]neurokinin A. Altogether, our binding data indicate that in spite of marked differences in the affinity of SR 142801 for tachykinin NK_3 and NK_2 or NK_1 binding sites in the guinea pig, the respective affinities of this compound for these binding sites are always lower in the rat.

Unfortunately, due to the weak or lack of effect of the tachykinin NK_1 receptor agonist, septide, and selective tachykinin NK_2 receptor agonists on the formation of [^{3}H]inositol monophosphate in the guinea-pig ileum, the functional selectivity of SR 142801 could not be tested in this tissue. This functional selectivity was investigated in the rat urinary bladder, a tissue which possesses tachykinin NK_1, NK_2 and 'septide-sensitive' receptors coupled to a phospholipase C but which is devoid of tachykinin NK_3 receptors (Burcher and Buck, 1986; Meini et al., 1994; Torrens et al., 1995). In this preparation, SR 142801 antagonized neither the [Pro^9]substance P- nor the septide-evoked formation of [^{3}H]inositol monophosphate but it moderately and competitively blocked the large response evoked by the selective tachykinin NK_2 receptor agonist [Lys^5,$MeLeu^9$,Nle^{10}]neurokinin A-(4–10) with a K_B value of 340 nM, which is in agreement with binding data (IC_{50} = 348 nM).

Altogether, our results show that peripheral and central NK_3 binding sites in the guinea pig exhibit similar pharmacological properties and further indicate that SR 142801 is a highly potent and stereospecific tachykinin NK_3 receptor antagonist in the guinea pig. They also confirm that this compound has a lower affinity for tachykinin NK_3 receptors in the rat. In high concentrations, it can interfere with tachykinin NK_2 receptors in the guinea pig and to a lesser extent in the rat. Moreover, according to our binding data, in the rat, SR 142801 seems still to be very selective for tachykinin NK_3 receptors since interference with other tachykinin receptors appears only to occur with much higher concentrations of the antagonist than those required to block tachykinin NK_3 receptors. This may be of interest, since to our knowledge a nonpeptide selective tachykinin NK_3 receptor antagonist exhibiting a higher efficiency in the rat than in the guinea pig is not yet available.

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