ELSEVIER

European Journal of Pharmacology 278 (1995) 17–25

ejp

Activity of SR 142801 at peripheral tachykinin receptors

Riccardo Patacchini [a,*], Lorand Barthò [b], Peter Holzer [c], Carlo Alberto Maggi [a]

[a] *Pharmacology Department, A. Menarini Pharmaceuticals, Florence, Italy*
[b] *Pharmacology Department, University Medical School of Pécs, Pécs, Hungary*
[c] *Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria*

Received 17 November 1994; revised 31 January 1995; accepted 7 February 1995

Abstract

The pharmacological profile of the novel tachykinin NK_3 receptor antagonist SR 142801, ((*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl) propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide), was studied at tachykinin NK_1, NK_2 and NK_3 receptors, in several in vitro bioassays. In the guinea-pig isolated ileum longitudinal muscle preparation, SR 142801 (10 nM–1 μM) caused an insurmountable antagonism of tachykinin NK_3 receptor-mediated contractions produced by senktide (apparent $pK_B = 9.27$). The blockade induced by SR 142801 was essentially irreversible, since it was not removed by washout (up to 2 h) and was increased by prolonging the incubation from 15 to 120 min. SR 142801 showed similar antagonist potency at rat tachykinin NK_3 receptors (portal vein) and rabbit tachykinin NK_2 receptors (pulmonary artery) ($pK_B = 7.49$ and 7.66, respectively), whereas it was distinctly less potent at hamster tachykinin NK_2 receptors (trachea; $pK_B = 6.84$) and inactive at guinea-pig tachykinin NK_1 receptors (ileum, longitudinal muscle). In the guinea-pig whole ileum SR 142801 (100 nM) did not affect the contraction produced by capsaicin (1 μM). The combined SR 142801 pretreatment and tachyphylaxis of neuronal CGRP (calcitonin gene-related peptide) receptors produced a slight (about 25%), but significant reduction of the response to capsaicin, suggesting that tachykinin NK_3 receptors play a minor role in capsaicin-induced neuronal excitation of afferent nerves in the guinea-pig ileum.

Keywords: Tachykinin; Tachykinin NK_3 receptor; Capsaicin; Tachykinin receptor antagonist; SR 142801

1. Introduction

Since the discovery of the quinuclidine derivative, CP 96,345, as the first non-peptide substance P receptor antagonist (Snider et al., 1991) a number of potent and selective compounds have been described which produce a powerful blockade of either tachykinin NK_1 or NK_2 receptors (Maggi et al., 1993c for review). The availability of these ligands has been instrumental to start elucidating the pathophysiological roles of tachykinins in the central and peripheral nervous system and some of these compounds are also likely candidates for testing in humans.

The tachykinin NK_3 receptor was originally defined on the basis of the rank order of potency of natural tachykinins, neurokinin B > neurokinin A > substance P. Selective agonists such as senktide (Laufer et al., 1986; Wormser et al., 1986) and [MePhe^7]neurokinin B (Drapeau et al., 1987) have been developed, which are useful tools to probe the distribution and function of tachykinin NK_3 receptors. Following its isolation and molecular cloning from various species (Maggi et al., 1993c for review), the distribution of the tachykinin NK_3 receptor has also been probed at the level of mRNA expression of the receptor protein (Tsuchida et al., 1990). The outcome of these studies indicates that the tachykinin NK_3 receptor is especially abundant in the central nervous system while its expression in peripheral organs is much more limited, the guinea-pig ileum (Laufer et al., 1986) and rat portal vein (Mastrangelo et al., 1986) being notable exceptions in this respect.

However, the definition of the exact importance of tachykinin NK_3 receptors in regulating bodily functions has remained largely speculative, since no potent

* Corresponding author. Pharmacology Department, Research Laboratories, A. Menarini Pharmaceuticals, Via Sette Santi 3, 50131 Florence, Italy. Tel. 39-55-5680350, fax 39-55-5680419.

SSDI 0014-2999(95)00090-9

and selective antagonists have been described for quite a long time. The recent discovery of SR 142801 as a potent and selective tachykinin NK_3 receptor antagonist (Emonds-Alt et al., 1995) is therefore particularly welcome in the tachykinin field. The aim of this study was to determine the profile of action of SR 142801 at tachykinin NK_1, NK_2 and NK_3 receptors by using a number of bioassays which are currently used to explore the pharmacology of this transmitter family. Special attention was devoted to assess the competitivity, reversibility and time dependency of receptor blockade exerted by SR 142801 at tachykinin NK_3 receptors.

Since the late 1970s, it was recognized that a large fraction of the contraction produced by selective chemical stimulation (capsaicin) of afferent nerves in the guinea-pig ileum is indirectly mediated through acetylcholine release from enteric motorneurones (Barthò and Szolcsányi, 1978; Barthò et al., 1982). The involvement of endogenous tachykinins and of neurally located tachykinin receptors in this response has long been suspected (see Barthò and Holzer, 1985 for review). A role of substance P has been indicated by the inhibitory effect of substance P desensitization (Barthò et al., 1982), but the type of tachykinin receptor involved has not yet been explored. The second aim of this study was therefore to ascertain whether SR 142801 may affect this response in a way indicative of tachykinin NK_3 receptor involvement.

2. Materials and methods

2.1. Effect of SR 142801 and SR 142806 at tachykinin NK_1, NK_2 and NK_3 receptors

Male albino New Zealand rabbits (2.5–3.0 kg), male Syrian golden hamsters (100–120 g), male albino guinea-pigs (250–300 g) and male albino rats (Wistar strain, 300–350 g) were stunned and bled. Endothelium-denuded strips of rabbit pulmonary artery, rings of hamster trachea, strips of guinea-pig ileum longitudinal muscle and longitudinal segments of rat portal vein were prepared and placed in 5 ml organ baths filled with oxygenated (96% O_2 and 4% CO_2) Krebs solution at 37° C, having the following composition: NaCl, 119 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.5 mM; KCl, 2.5 mM; and glucose, 11 mM. Longitudinal muscle strips of guinea-pig ileum and segments of rat portal vein were connected to isotonic force transducers (load 3 mN), while the other preparations were fixed to isometric force transducers, as described previously (Maggi et al., 1990).

The antagonist activity of SR 142801 was studied at tachykinin NK_1 receptors in the guinea-pig ileum (atropine and chlorpheniramine 1 μM, indomethacin 3 μM in the bath) against the direct contractile effects produced by the tachykinin NK_1 selective agonists [Sar^9]substance P sulfone and septide. The activity of SR 142801 at the tachykinin NK_2 receptor was studied in the rabbit pulmonary artery and hamster trachea, against neurokinin A as the agonist. The activity of SR 142801 at tachykinin NK_3 receptors was evaluated in the guinea-pig ileum (incubated with drug-free Krebs solution), and in the rat portal vein, using senktide as the tachykinin NK_3 selective agonist. In a limited number of experiments with the guinea-pig ileum SR 142801 was tested against scyliorhinin II, a dogfish tachykinin peptide (Conlon et al., 1986) reportedly selective for tachykinin NK_3 receptors (Buck and Krstenansky, 1987; Beaujouan et al., 1988). Cumulative dose-response curves to the agonists were obtained in all preparations, each concentration being added when the effect of the preceding one had reached a steady state. Preliminary experiments indicated lack of desensitization of the above tissues to the cumulative administration of the selective agonists used.

All the experiments performed in the guinea-pig ileum, using senktide as the agonist, were conducted in the presence of the tachykinin NK_1 receptor selective antagonist SR 140333 (0.1 μM; 30 min before each agonist concentration-response curve) to avoid a possible activation of tachykinin NK_1 receptors by senktide at high concentrations. Furthermore, the contractile response to carbachol (10 μM) was used as the internal standard for the former experiments.

In all preparations SR 142801 was evaluated after a long (120 min) incubation period with the tissues, as suggested by Emonds-Alt et al. (1995). However, a number of experiments were performed in which SR 142801 was left in contact with the guinea-pig ileum for 15, 60 or 120 min, to explore the time dependency of the tachykinin NK_3 receptor blockade produced by this ligand. In a series of experiments with the guinea-pig ileum preparation, SR 142801 was tested on contractions evoked by electrical field stimulation (single pulses of 60 V, 0.5 ms duration, every 10 s).

In another series of experiments, the reversibility of SR 142801-induced tachykinin NK_3 receptor blockade was evaluated as follows: senktide (1 nM) was administered to the guinea-pig ileum at 30-min intervals, until reproducible contractile responses were obtained (generally 3–4 administrations). At this time SR 142801 (30 nM) was added to the bath solution, 60 min before repeating the agonist challenge. Tissues were then thoroughly washed with fresh Krebs solution, which was renewed every 5 min. Administration of senktide was repeated again 30, 60, 90 and 120 min after removal of the antagonist, and the responses obtained were compared to the control one.

SR 142806, the (*R*)-enantiomer of SR 142801, was tested at rat and guinea-pig tachykinin NK_3 receptors after a 120-min incubation period with tissues.

2.2. Effect of SR 142801 on the capsaicin-induced contractions in the guinea-pig ileum

About 1.5 cm long segments of guinea-pig whole ileum were suspended in 7 ml organ baths containing Tyrode's solution of the following composition (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.6, aereated with 95% O_2 and 5% CO_2. Mechanical activity was recorded isotonically along the longitudinal axis (load 55 mN). After an equilibration of 15–20 min the tissues were challenged with senktide (2 nM for 2 min) and in some preparations also with cholecystokinin octapeptide (CCK-8, 2 nM). Pairs of tissues were then exposed to SR 142801 or solvent. Senktide or CCK-8 administrations were then repeated once or twice at 5–60 min from the administration of SR 142801. Capsaicin (1 μM) was administered 60–70 min after SR 142801 (100 nM) had been added to the bath.

In some experiments L-nitroarginine (30 μM) was present in the bath fluid of both control and SR 142-801-treated ileal segment throughout the experiments, to prevent a putative tachykinin NK_3 receptor-mediated release of nitric oxide (Maggi et al., 1993b). In these experiments we studied the combined effect of SR 142801 and CGRP (calcitonin gene-related peptide) receptor tachyphylaxis on the response to capsaicin. The $CGRP_1$ receptor antagonist CGRP-(8–37) (3 μM) was added to block the smooth muscle relaxant action of CGRP. In addition, rat α-CGRP (200 nM; 20 min before the challenge with capsaicin) was added, 10 min after administration of CGRP-(8–37), to produce tachyphylaxis of neuronal $CGRP_2$ receptors mediating acetylcholine release (cf. Barthò et al., 1993).

The preparations were standardized by determining the maximal contraction due to 40 mM KCl at the end of the experiments.

2.3. Evaluation of data

Agonist activity of each test compound was expressed as EC_{50} or molar concentration of peptide producing 50% of maximal effect. Antagonists producing parallel rightward shifts of concentration-response curves to the agonist, without depression of the maximal response, were checked for competitivity by the Schild plot method (Arunlakshana and Schild, 1959). Antagonists providing plots with linear regression lines and slopes not significantly different from unity were considered competitive. The affinity of competitive an-

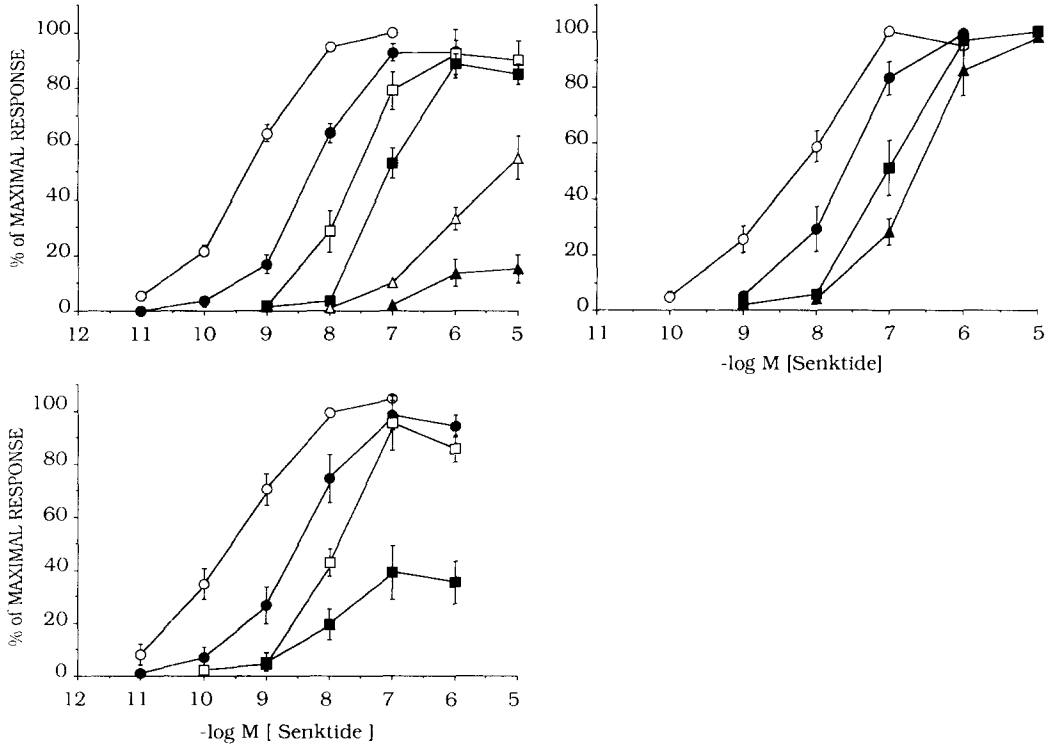

Fig. 1. Upper left panel: Concentration-response curves for senktide in the guinea-pig isolated ileum, in the absence (○) and after a 120-min incubation with SR 142801 10 nM (●), 30 nM (□), 100 nM (■), 300 nM (△) and 1 μM (▲), in the presence of SR 140333 (0.1 μM; 30 min before each concentration-response curve to the agonist). Upper right panel: Concentration-response curves for senktide in the rat isolated portal vein, in the absence (○) and after a 120-min incubation with SR 142801 0.1 μM (●), 0.3 μM (■) and 1 μM (▲). Lower panel: Concentration-response curves for senktide in the guinea-pig isolated ileum, in the absence (○) and after a 120-min incubation with SR 142806 100 nM (●), 300 nM (□) and 1 μM (■), in the presence of SR 140333 (0.1 μM; 30 min before each concentration-response curve to the agonist). Each value in the figure is the mean ± S.E.M. of 4–12 experiments.

tagonists was expressed in terms of pK_B (negative logarithm of the antagonist dissociation constant), and, assuming a slope of -1, it was estimated as the mean of the individual values obtained with the equation: pK_B = log[dose ratio − 1] − log[antagonist concentration] (Kenakin, 1993; Jenkinson, 1991). In some experiments, the extent of the rightward shift of the agonist concentration-response curve produced by a given antagonist was expressed as A′/A, or ratio between two agonist concentrations producing 50% of the control maximal response, in the presence and in the absence of the antagonist, respectively.

2.4. Statistical analysis

The values in the text, tables or figures are expressed as means ± 95% confidence limits, or ±S.E.M. Statistical analysis was performed by means of Student's *t*-test for paired or unpaired data or by means of two-way analysis of variance (ANOVA), followed by Tukey's test, when applicable. Regression analysis of log concentration-effect curves was performed by the least-squares method, considering the curves linear between 20 and 80% of the maximal response.

2.5. Drugs

Neurokinin A was synthesized at Menarini Laboratories (Florence, Italy), by conventional solid-phase methods. SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-*n*-3yl]ethyl}-4-phenyl-1-azoniabi-cyclo[2,2,2]octane chloride), SR 142801 ((*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl) propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide) and SR 142806 ((*R*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl) propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide), were kindly provided by Drs. X. Emonds-Alt and G. Le Fur, Sanofi (Montpellier, France). Other drugs used were: indomethacin, chlorpheniramine, L-nitroarginine and capsaicin (Sigma, St. Louis, MO, USA); atropine (Serva, Heidelberg, Germany); rat α-CGRP, hCGRP-(8–37), CCK-8, senktide, septide and [Sar^9]substance P sulfone (Peninsula, St. Helens, UK); carbachol HCl (Merck, Darmstadt, Germany).

3. Results

3.1. Activity of SR 142801 and SR 142806 at guinea-pig and rat tachykinin NK_3 receptors

In the guinea-pig ileum longitudinal muscle myenteric plexus preparation, the tachykinin NK_3 receptor-selective agonist senktide induced reproducible concentration-dependent contractile responses (EC_{50} = 0.88 nM; 95% confidence limits: 0.45–1.31 nM; n = 16) (Fig. 1). SR 142801 (10–30 nM), after a 120-min incubation period, produced an apparent competitive antagonism of senktide-mediated effects (Fig. 1). From the shifts of the curves to senktide produced by SR 142801 at these concentrations, an apparent pK_B value of 9.27 (95% confidence limits: 8.96–9.57) was calculated, as mean of individual values obtained by the Schild equation (see Materials and methods).

Scyliorhinin II, a dogfish tachykinin peptide showing selectivity for tachykinin NK_3 receptors (Buck and Krstenansky, 1987; Beaujouan et al., 1988), produced concentration-dependent contractions (EC_{50} = 0.82 nM, 95% confidence limits: 0.24–1.40 nM; n = 10) of the guinea-pig ileum, whose maximum averaged 100 ± 4% (n = 6) of the maximal response to senktide. SR 142801 (10 nM; 120 min before), produced an apparent competitive antagonism of scyliorhinin II-mediated effects, showing similar potency (A′/A = 21.1 ± 5; n = 4) to that shown against senktide (A′/A = 11.9 ± 2; n = 4) under similar conditions.

At 100, 300 nM and 1 μM SR 142801 induced non-parallel rightward shifts of the control curves to senktide, and a depression of the maximal response attainable; the maximal responses to senktide averaged 88.4 ± 3% (n = 5), 54.9 ± 7% (n = 5) and 15.4 ± 5% (n = 5) of control maximum, respectively (Fig. 1).

SR 142806, the (*R*)-enantiomer of SR 142801, was at least 10-fold weaker antagonist than SR 142801 at guinea-pig tachykinin NK_3 receptors (Fig. 1). SR 142806 (1 μM) produced a non-parallel rightward shift of the control curve to senktide, and a depression of the maximal response attainable (38 ± 8%, n = 5, of control). In the guinea-pig ileum, neither SR 142801 nor SR 142806, up to 300 nM, inhibited the contractile response to carbachol (10 μM), nor the twitch contractions induced by electrical field stimulation. However, at 1 μM, both SR 142801 and SR 142806 reduced the electrically induced contractions (−32 ± 4%, n = 5 and −18 ± 8%, n = 5, as compared to control twitch, respectively), and slightly inhibited the response to carbachol (−6.4 ± 3%, n = 5 and −7.0 ± 5%, n = 5, as compared to control response, respectively).

In the rat isolated portal vein senktide induced concentration-dependent contractile responses (EC_{50} = 7.76 nM; 95% confidence limits: 3.1–12.4 nM; n = 12) (Fig. 1). SR 142801 (0.1–1 μM; 120-min incubation period) antagonized the senktide-induced contractions with apparently competitive kinetics (Fig. 1). The estimated affinity of SR 142801 for rat tachykinin NK_3 receptors was: pK_B = 7.49 (95% confidence limits: 7.2–7.8). In contrast, SR 142806 (120-min incubation period) did not affect the control agonist-response curve up to 1 μM, a concentration at which it produced a slight antagonist effect (A′/A = 5.1 ± 2; n = 4) toward senktide-induced contractions (not shown).

Concentrations of SR 142806 higher than 1 μM were not further assayed, because they depressed smooth muscle contractility (inhibition of the response to KCl 80 mM).

3.2. Time dependency and reversibility of the blockade induced by SR 142801 at tachykinin NK_3 receptors

The time dependency of tachykinin NK_3 receptor blockade induced by SR 142801 was evaluated in the guinea-pig ileum, by incubating the tissue with a 30 nM concentration of the antagonist for 15, 60 or 120 min. As shown in Fig. 2, the extent of the rightward shift of the curve to senktide produced by SR 142801 was significantly increased by prolonging the incubation time from 15 min ($A'/A = 13.3 \pm 4$, $n = 4$) to 60 min ($A'/A = 34.0 \pm 7$, $n = 4$, $P < 0.05$) and 120 min ($A'/A = 130.1 \pm 35$, $n = 4$, $P < 0.05$).

The reversibility of receptor blockade induced by SR 142801 was evaluated, in the guinea-pig ileum, as the capacity of washout to restore the contraction produced by senktide (1 nM), after a 60-min contact period with the antagonist (30 nM). As shown in Fig. 3, no recovery of the response to the agonist occurred up to 120 min from the washout of SR 142801 from the bath.

3.3. Activity of SR 142801 at tachykinin NK_1 and NK_2 receptors

SR 142801, up to 1 μM, was inactive as agonist or antagonist at tachykinin NK_1 receptors of the guinea-pig ileum (atropine 1 μM in the bath), either against [Sar^9]substance P sulfone or septide as agonists (Table 1). In contrast, SR 142801 showed appreciable affinity for tachykinin NK_2 receptors. In the rabbit isolated pulmonary artery SR 142801 (0.1–1 μM) competitively antagonized the neurokinin A-mediated contractions ($pK_B = 7.66$, Table 1). SR 142801 (1–10 μM) also antagonized neurokinin A-mediated contractions in the hamster trachea with competitive kinetics, but with lower potency ($pK_B = 6.84$, Table 1).

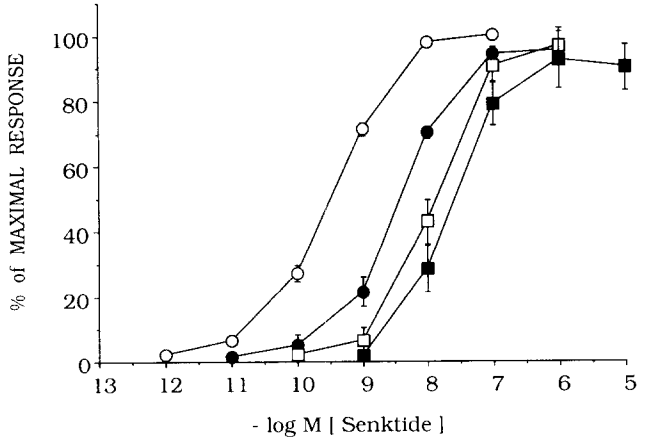

Fig. 2. Time dependency of tachykinin NK_3 receptor blockade induced by SR 142801. Concentration-response curves for senktide are shown in the guinea-pig isolated ileum, in the absence (○) and after 15-min (●), 60-min (□) and 120-min (■) incubation with SR 142801 30 nM. Each value in the figure is the mean ± S.E.M. of 4–12 experiments.

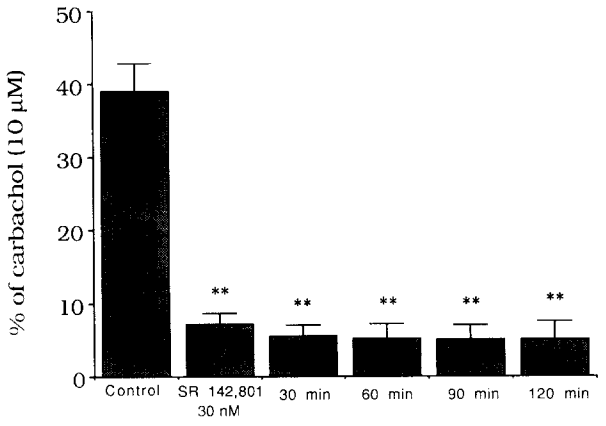

Fig. 3. Irreversibility of tachykinin NK_3 receptor blockade induced by SR 142801. The contractile response to senktide (1 nM) is shown in the guinea-pig ileum: in control experiments, in the presence of SR 142801 (30 nM; 60-min incubation), and after 30, 60, 90 and 120 min from washout of SR 142801. Each column is the mean ± S.E.M. of 5 determinations. ** Significantly different from control response, $P < 0.01$.

3.4. Effect of SR 142801 on the capsaicin-induced contractions in the guinea-pig ileum

Senktide (2 nM, $n = 18$) and CCK-8 (2 nM, $n = 6$) produced submaximal contractions of the guinea-pig whole ileum, averaging 54.7 ± 3.7 and 35.3 ± 3.1% of the maximal response to 40 mM KCl, respectively. SR 142801 (100 nM) produced a time-dependent reduction of the response to senktide, causing an over 90% depression at 50–60 min (Fig. 4). In contrast, the

Table 1
Affinity of SR 142801 at tachykinin NK_1 and NK_2 receptors

Antagonist	NK_1		NK_2	
	GPI [a]	GPI [b]	RPA	HT
SR 142801	IN	IN	7.66 (7.42–7.90)	6.84 (6.61–7.07)

The values in the table are mean pK_B (−log of the antagonist dissociation constant) of 6 (GPI) to 12 (RPA, HT) determinations. In brackets are 95% confidence limits. GPI = guinea-pig ileum longitudinal muscle (atropine and chlorpheniramine 1 μM, indomethacin 3 μM in the bath); agonists: [a] [Sar^9]substance P sulfone, [b] septide. RPA = endothelium-deprived rabbit pulmonary artery; agonist: neurokinin A. HT = hamster isolated trachea; agonist: neurokinin A. IN = inactive up to 1 μM.

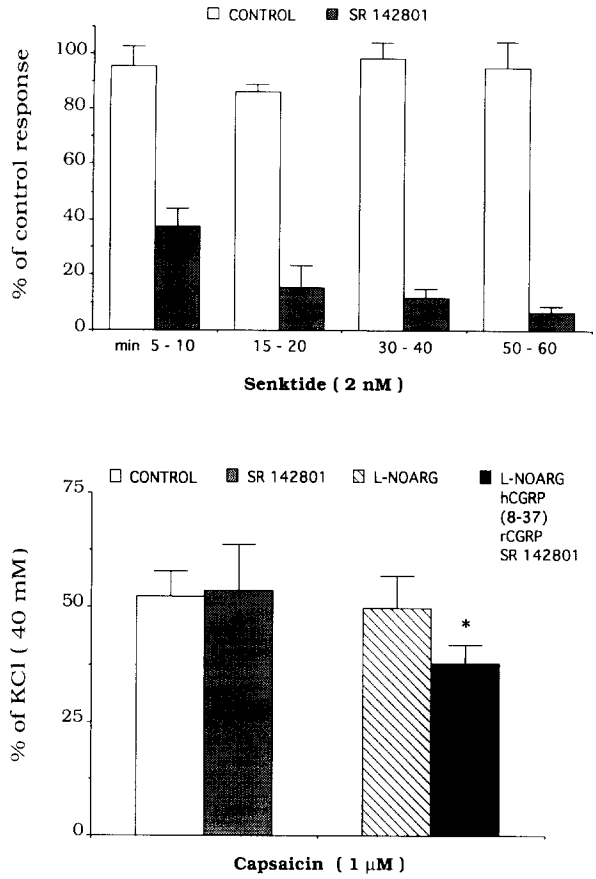

Fig. 4. Upper panel: Contractile responses to senktide (2 nM) in the guinea-pig ileum, alone and after 5–10, 15–20, 30–40 and 50–60 min incubation with SR 142801 (100 nM). Each column is the mean ± S.E.M. of 4–6 determinations. Lower panel: Contractile responses to capsaicin (1 μM) in the guinea-pig ileum, alone (first column), in the presence of SR 142801 (100 nM; 60 min before) (second column), in the presence of L-nitroarginine (30 μM) (third column) and in the presence of L-nitroarginine (30 μM), hCGRP-(8–37) (3 μM; 30 min before), rat α-CGRP (200 nM; 20 min before) and SR 142801 (100 nM; 60 min before) (fourth column). *Significantly different from control response to capsaicin in the presence of L-nitroarginine, $P < 0.05$. Each column is the mean ± S.E.M. of 4–6 determinations.

cholinergically mediated response to CCK-8 (Vizi et al., 1973) remained unaffected by either SR 142801 (100 nM, $n = 5$) or by SR 142801 plus tachyphylaxis (rat α-CGRP 200 nM, 20 min before) of neuronal CGRP receptors ($n = 5$, data not shown), indicating that either treatment did not unspecifically depress acetylcholine release from cholinergic neurons.

Capsaicin (1 μM) produced submaximal contractions (range 34–70%) of the guinea-pig ileum, averaging 52.3 ± 5.6 and 49.8 ± 7.1% of the response to KCl in the absence and presence of L-nitroarginine, respectively ($n = 5$ for each group, n.s.). Blockade of tachykinin NK_3 receptors by SR 142801 (100 nM) did not affect the response to capsaicin (Fig. 4). When evoked in the presence of SR 142801 plus tachyphylaxis of neuronal CGRP receptors, the response to capsaicin was slightly (about 25% reduction) but significantly reduced as compared to controls ($P < 0.05$, Fig. 4).

4. Discussion

In keeping with the results presented by Emonds-Alt et al. (1995), the present findings indicate that: (1) SR 142801 is a potent and selective antagonist at tachykinin NK_3 receptors of the guinea-pig; (2) the blocking action of SR 142801 at guinea-pig and rat tachykinin NK_3 receptors is stereoselective, since the (*R*)-enantiomer (SR 142806) is at least 10-fold weaker; and (3) SR 142801 is consistently less potent at rat than guinea-pig tachykinin NK_3 receptors. The latter is further evidence for heterogeneity between the tachykinin NK_3 receptors expressed by these two species, as previously shown by binding studies using SR 48968, [Pro^7]neurokinin B (Petitet et al., 1993) and SR 142801 itself (Emonds-Alt et al., 1995). SR 142801 also exerts a sizeable antagonism at tachykinin NK_2 receptors (e.g. $pK_B = 7.66$ in the rabbit pulmonary artery) although its affinity for tachykinin NK_2 receptors is markedly lower than that for guinea-pig tachykinin NK_3 receptors (cf. Emonds-Alt et al., 1995).

By extending the analysis to the mechanism of tachykinin NK_3 receptor blockade produced by SR 142801, we have shown that SR 142801 produces an essentially irreversible blockade of tachykinin NK_3 receptors in the guinea-pig ileum. This conclusion is supported by the following observations: (a) the antagonism produced by SR 142801 is not reversed by washout (up to 2 h from the administration), (b) the extent of the antagonism is time-dependent, and (c) the concentration-response curves to the tachykinin NK_3 receptor-selective agonist senktide undergo a non-parallel rightward shift with reduction of the maximum, starting from 100 nM concentration of antagonist. In keeping with our conclusion is the observation made by Emonds-Alt et al. (1995), who noted that a long incubation period (140 min) is needed to allow SR 142801 to display a 'full activity'. However, part of the inhibition of the maximal response to senktide exerted by SR 142801 at the highest concentration tested (1 μM) may be due to non-specific depression of smooth muscle contractility caused by this compound (and also by its (*R*)-enantiomer). Concerning the latter effect, it may be argued that it is related to the binding affinity shown by SR 142801 for verapamil-sensitive Ca^{2+} channels (Emonds-Alt et al., 1995). It should be noted also that, at concentrations lower than 100 nM, SR 142801 produced a block much like what would be expected for a competitive antagonist. This is not necessarily at variance with an irreversible mechanism of interaction of SR 142801 with guinea-pig tachykinin

NK_3 receptors, since even irreversible and non-competitive antagonists may produce competitive-like effects at low concentrations, depending on the receptor reserve present in the preparation employed (Kenakin, 1993). The affinity of SR 142801 for tachykinin NK_3 receptors of the guinea-pig ileum was tentatively estimated (apparent $pK_B = 9.27$; senktide as agonist), which is in good agreement with that ($pK_B = 9.15$; [MePhe^7]neurokinin B as agonist) reported by Emonds-Alt et al. (1995). However, owing to the irreversible nature of this antagonist at guinea-pig tachykinin NK_3 receptors, such an affinity estimate has the important limitation to be dependent on the incubation time (Kenakin, 1993, for discussion), which, in our experiments, was 120 min.

It is noteworthy that SR 140333 and SR 48968, two non-peptide antagonists bearing a chemical structure similar to SR 142801 (cf. Emonds-Alt et al., 1992, 1993, 1995), exert an insurmountable, time-dependent, and slowly reversible antagonism at certain tachykinin NK_1 and NK_2 receptors (Emonds-Alt et al., 1992, 1993; Advenier et al., 1992, Maggi et al., 1993a; Patacchini et al., 1994), similar to that observed with SR 142801 at guinea-pig tachykinin NK_3 receptors (present results). Several explanations have been proposed, alternative to a slowly reversible/irreversible interaction with the target receptors, to account for the slow kinetics of action of both SR 140333 and SR 48968, including slow diffusion (Martin et al., 1992) and interaction with non-receptor binding sites close to the receptor region (Advenier et al., 1992). Although these hypotheses have not yet been proven or disproven, none of them can account for the overall pharmacological profile of SR 142801 at guinea-pig tachykinin NK_3 receptors. Not even the very high affinity for tachykinin receptors shown by the former SR compounds may explain in itself their slow reversibility from the receptor. In fact, we have recently shown that MEN 10,627, a novel tachykinin NK_2 receptor-selective antagonist endowed with an antagonist potency at least comparable to that of SR 48968 (Maggi et al., 1994b), displays simple competitive and reversible antagonism at tachykinin NK_2 receptors in the guinea-pig gallbladder and colon, two preparations in which SR 48968 behaves as an essentially irreversible antagonist (Patacchini et al., 1994). Rather, we propose that certain chemical groups present in the structure of SR 140333, SR 48968 and SR 142801 might be responsible for a tight and slowly reversible interaction with putatively conserved residues in the sequence of tachykinin NK_1, NK_2 and NK_3 receptor proteins.

The capsaicin-induced contraction of the longitudinal muscle of the guinea-pig ileum represents a 'classical' test object for studying the role of the 'efferent' function of sensory neurons at the visceral level. Earlier studies had established that a large fraction (about 70%) of this response is tetrodotoxin- and atropine-sensitive, implying that mediators released from peripheral endings of afferent neurons produce their effect largely by stimulating cholinergic motoneurons in the myenteric plexus (Barthò and Szolcsányi, 1978; Barthò et al., 1982; Barthò and Vizi, 1985; Takaki and Nakayama, 1989). The tetrodotoxin-resistant component of the response to capsaicin is thought to be mediated by endogenous tachykinins, probably acting via tachykinin NK_1 receptors on longitudinal muscle cells (see Barthò and Holzer, 1985, for review). Since the contractile effect of capsaicin was depressed by substance P tachyphylaxis (Barthò et al., 1982), and stimulation of tachykinin NK_3 receptors (by e.g. senktide) produces release of acetylcholine in the ileum (Guard and Watson, 1987; Fox and Morton, 1991) and depolarizes myenteric neurons (Hanani et al., 1988), it was speculated that endogenous tachykinins, probably substance P, released from afferent nerves by capsaicin, produce acetylcholine release via tachykinin NK_3 receptors on cholinergic neurons (see Barthò and Holzer, 1985). The present findings demonstrate that if such a contribution exists, it is far less than it was hypothesized until now, since SR 140801 at a concentration producing $> 90\%$ inhibition of the response to senktide did not affect the response to an equieffective concentration of capsaicin. Thus, a selective blockade of neuronal tachykinin NK_3 receptors does not prevent neuronal excitation following application of capsaicin in the guinea-pig ileum. In addition to tachykinins, a number of other mediators are known to be stored in capsaicin-sensitive primary afferent neurons: CGRP is an important mediator of responses to capsaicin in various preparations. In the guinea-pig ileum, CGRP produces two distinct and opposite effects on contractility: it relaxes the longitudinal muscle via $CGRP_1$ receptors, sensitive to blockade by the C-terminal fragment CGRP-(8–37), and produces neuronal excitation and acetylcholine release via $CGRP_2$ receptors, insensitive to CGRP-(8–37) (Barthò et al., 1987a,b, 1991, 1993). We have shown previously that CGRP tachyphylaxis on neuronal CGRP receptors (in the presence of CGRP-(8–37) to block the muscle relaxant action of CGRP) is not sufficient to prevent the effect of capsaicin in the longitudinal muscle of the ileum (Barthò et al., 1993). In the present experiments the combined administration of SR 142801 and $CGRP_2$ receptor tachyphylaxis produced a quite limited but significant reduction of the response to capsaicin. As the neurogenic response to CCK-8 was not affected, this small inhibitory effect appears selective and may indicate that both tachykinin NK_3 and $CGRP_2$ receptors contribute to capsaicin-induced contraction. However, a large part of the response to capsaicin remained unchanged, and this negative finding suggests that some other, thus far unknown, mediators are involved in the

response to capsaicin. As tachykinin NK_3 receptor stimulation is known to produce nitric oxide release in the enteric nervous system (Maggi et al., 1993b, 1994a), which may in turn affect neuromuscular excitability, these experiments were performed in the presence of L-nitroarginine which, however, did not affect the response to capsaicin.

In conclusion, the present findings demonstrate that SR 142801 is a potent and selective antagonist for the guinea-pig tachykinin NK_3 receptor. At this site we found that SR 142801 exerts an essentially irreversible antagonism, similary to that observed with the chemically related compounds SR 140333 and SR 48968 at certain tachykinin NK_1 and NK_2 receptors. Confirming the findings of Emonds-Alt et al. (1995) we found that the affinity of SR 142801 is markedly species-dependent, a finding common to many tachykinin NK_1 and NK_2 receptor-selective antagonists. Furthermore, the use of SR 142801 has provided evidence that, in the guinea-pig ileum, tachykinin NK_3 receptors play a minor role in the capsaicin-induced neuronal excitation of primary afferent nerves.

Acknowledgements

We wish to thank Drs. X. Emonds-Alt and G. Le Fur, Sanofi, Montpellier, France, for the generous gift of SR 140333, SR 142801 and SR 142806, and Mr. Wolfgang Schluet for excellent tecnical assistance. This study was supported in part by the Austrian-Hungarian Foundation, by the Austrian Research Foundation (grant No. 9473) and by the Hungarian grant No. ETT T-04 739/93 and OTKA T-013045.

References

Advenier, C., N. Rouissi, Q.T. Nguyen, X. Emonds-Alt, J.C. Breliere, G. Neliat, E. Naline and D. Regoli, 1992, Neurokinin A (NK_2) receptor revisited with SR 48968, a potent, non-peptide antagonist, Biochem. Biophys. Res. Commun. 184, 1418.

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. Chemother. 14, 48.

Barthò, L. and P. Holzer, 1985, Search for a physiological role of SP in gastrointestinal motility, Neuroscience 16, 1.

Barthò, L. and J. Szolcsányi, 1978, The site of action of capsaicin on the guinea-pig isolated ileum, Naunyn-Schmied. Arch. Pharmacol. 305, 75.

Barthò, L. and E.S. Vizi, 1985, Neurochemical evidence for the release of acetylcholine from the guinea-pig ileum myenteric plexus by capsaicin, Eur. J. Pharmacol. 110, 125.

Barthò, L., P. Holzer, F. Lembeck and J. Szolcsányi, 1982, Evidence that the contractile response of the guinea-pig ileum is due to release of SP, J. Physiol. (London) 332, 157.

Barthò, L., F. Lembeck and P. Holzer, 1987a, CGRP is a potent relaxant of intestinal muscle, Eur. J. Pharmacol. 135, 449.

Barthò, L., G. Pethö, A. Antal, P. Holzer and J. Szolcsányi, 1987b, Two types of relaxation due to capsaicin in the guinea-pig isolated ileum, Neurosci. Lett. 81, 146.

Barthò, L., G. Koczan, P. Holzer, C.A. Maggi and J. Szolcsányi, 1991, Antagonism of the effects of CGRP and of capsaicin on the guinea-pig isolated ileum by human αCGRP-(8–37), Neurosci. Lett. 129, 156.

Barthò, L., G. Koczan and C.A. Maggi, 1993, Studies on the mechanisms of the contractile action of rat CGRP and capsaicin on the guinea-pig ileum: effect of hCGRP-(8–37) and CGRP tachyphylaxis, Neuropeptides 25, 325.

Beaujouan, J.C., M. Saffroy, F. Petitet, Y. Torrens and J. Glowinski, 1988, Neuropeptide K, scyliorhinin I and II: new tools in the tachykinin receptor field, Eur. J. Pharmacol. 151, 353.

Buck, S.H. and J.L. Krstenansky, 1987, The dogfish peptides scyliorhinin I and scyliorhinin II bind with differential selectivity to mammalian tachykinin receptors, Eur. J. Pharmacol. 144, 109.

Conlon, J.M., C.F. Deacon, L. O'Toole and L. Thim, 1986, Scyliorhinin I and II: two novel tachykinins from dogfish gut, FEBS Lett. 200, 111.

Drapeau, G., P. D'Orleans-Juste, S. Dion, N.-E. Rhaleb, N.-E. Rouissi and D. Regoli, 1987, Selective agonists for substance P and neurokinin receptors, Neuropeptides 10, 43.

Emonds-Alt, X., P. Vilain, P. Goulaoic, D. Van Broeck, C. Advenier, E. Naline, G. Neliat, G. Le Fur and J.C. Breliere, 1992, A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor, Life Sci. 50, 101.

Emonds-Alt, X., J.D. Doutremepuich, M. Heaulme, G. Neliat, V. Santucci, R. Steinberg, P. Vilain, D. Bichon, J.P. Ducoux, E. Proietto, D. Van Broeck, P. Soubrie, G. Le Fur and J.C. Breliere, 1993, In vitro and in vivo biological activities of SR 140,333, a novel potent nonpeptide tachykinin NK_1 receptor antagonist, Eur. J. Pharmacol. 250, 403.

Emonds-Alt, X., D. Bichon, J.P. Ducoux, M. Heaulme, B. Miloux, M. Poncelet, E. Proietto, D. Van Broeck, P. Vilain, G. Neliat, P. Soubrie, G. Le Fur and J.C. Breliere, 1995, SR 142801, the first potent nonpeptide antagonist of the tachykinin NK_3 receptor, Life Sci. 56, PL 27.

Fox, A.J. and I.K.M. Morton, 1991, Mu and kappa opioid receptor modulation of 5-HT_3 and NK_3 receptor-evoked release of acetylcholine from the guinea-pig ileum myenteric plexus, Naunyn-Schmied. Arch. Pharmacol. 344, 8.

Guard, S. and S.P. Watson, 1987, Evidence for NK_3 receptor-mediated tachykinin release in the guinea-pig ileum, Eur. J. Pharmacol. 144, 409.

Hanani, M., M. Chorev, C. Gilon and Z. Selinger, 1988, The actions of receptor-selective substance P analogs on myenteric neurons: an electrophysiological investigation, Eur. J. Pharmacol. 153, 247.

Jenkinson, D.H., 1991, How we describe competitive antagonists: three questions of usage, Trends Pharmacol. Sci. 12, 53.

Kenakin, T.P., 1993, Pharmacologic Analysis of Drug-Receptor Interaction, 2nd edn. (Raven Press, New York, NY).

Laufer, R., U. Wormser, Z.Y. Friedman, C. Gilon, M. Chorev and Z. Selinger, 1986, Neurokinin B is a preferred agonist for a neuronal substance P receptor and its action is antagonized by enkephalin, Proc. Natl. Acad. Sci. USA 82, 7444.

Maggi, C.A., R. Patacchini, S. Giuliani, P. Rovero, S. Dion, D. Regoli, A. Giachetti and A. Meli, 1990, Competitive antagonists discriminate between NK_2 tachykinin receptor subtypes, Br. J. Pharmacol. 100, 588.

Maggi, C.A., R. Patacchini, S. Giuliani and A. Giachetti, 1993a, In vivo and in vitro pharmacology of SR 48968, a non-peptide tachykinin NK_2 receptor antagonist, Eur. J. Pharmacol. 234, 83.

Maggi, C.A., R. Patacchini, S. Meini and S. Giuliani, 1993b, Nitric oxide is the mediator of tachykinin NK_3 receptor-induced relaxation in the circular muscle of the guinea-pig ileum, Eur. J. Pharmacol. 240, 45.

Maggi, C.A., R. Patacchini, P. Rovero and A. Giachetti, 1993c, Tachykinin receptors and tachykinin receptor antagonists, J. Autonom. Pharmacol. 13, 23.

Maggi, C.A., V. Zagorodnyuk and S. Giuliani, 1994a, Tachykinin NK_3 receptor mediates NANC hyperpolarization and relaxation via nitric oxide release in the circular muscle of the guinea-pig colon, Regul. Pept. 53, 259.

Maggi, C.A., M. Astolfi, S. Giuliani, C. Goso, S. Manzini, S. Meini, R. Patacchini, V. Pavone, C. Pedone, L. Quartara, A.R. Renzetti and A. Giachetti, 1994b, MEN 10,627 a novel polycyclic peptide antagonist of tachykinin NK_2 receptors, J. Pharmacol. Exp. Ther. 271, 1489.

Martin, C.A.E., E. Naline, X. Emonds-Alt and C. Advenier, 1992, Influence of (±)-CP-96,345 and SR 48968 on electrical field stimulation of the isolated guinea-pig main bronchus, Eur. J. Pharmacol. 224, 137.

Mastrangelo, D., R. Mathison, H.J. Huggel, S. Dion, P. D'Orleans-Juste, N.E. Rhaleb, G. Drapeau, P. Rovero and D. Regoli, 1986, The rat isolated portal vein: a preparation sensitive to neurokinins, particularly neurokinin B, Eur. J. Pharmacol. 134, 321.

Patacchini, R., R. De Giorgio, A. Giachetti and C.A. Maggi, 1994, Different mechanism of tachykinin NK_2 receptor blockade by SR 48968 and MEN 10,627 in the guinea-pig isolated gallbladder and colon, Eur. J. Pharmacol. 271, 111.

Petitet, F., J.C. Beaujoan, M. Saffroy, Y. Torrens and J. Glowinski, 1993, The nonpeptide NK_2 antagonist SR 48,968 is also a NK_3 antagonist in the guinea-pig but not in the rat, Biochem. Biophys. Res. Commun. 191, 180.

Snider, M.R., J.W. Constantine, J.A. Lowe, III, K.P. Longo, W.S. Lebel, H.A. Woody, S.E. Drozda, M.C. Desai, F.J. Vinick, R.W. Spencer and H.J. Hess, 1991, A potent nonpeptide antagonist of the substance P (NK_1) receptor, Science 251, 435.

Takaki, M. and S. Nakayama, 1989, Effects of capsaicin on myenteric neurons of the guinea-pig ileum, Neurosci. Lett. 105, 125.

Tsuchida, K., R. Shigemoto, Y. Yokota and S. Nakanishi, 1990, Tissue distribution and quantitation of the mRNA for three rat tachykinin receptors, Eur. J. Biochem. 193, 751.

Vizi, E.S., G. Bertaccini, M. Impicciatore and J. Knoll, 1973, Evidence that acetylcholine released by gastrin and related polypeptides contributes to their effect on gastrointestinal motility, Gastroenterology 64, 268.

Wormser, U., R. Laufer, Y. Hart, M. Chorev, C. Gilon and Z. Selinger, 1986, Highly selective agonists for substance P receptor subtypes, EMBO J. 5, 2805.