British Journal of Pharmacology (1998) 124, 1321–1327
http://www.stockton-press.co.uk/bjp

In vitro characterization of tachykinin NK_2-receptors modulating motor responses of human colonic muscle strips

[1,6]Tiziano Croci, [1]Giulio Aureggi, [1]Luciano Manara, [2]Xavier Emonds-Alt, [3]Gerard Le Fur, [4]Jean-Pierre Maffrand, [5]Sylvain Mukenge and [5]Gianfranco Ferla

[1]Research Center Sanofi Midy, Via G.B. Piranesi 38, 20137 Milan, Italy; [2]Sanofi Recherche, 371 rue du Pr. J. Blayac, 34184 Montpellier; [3]Sanofi Recherche, 32/34 rue Marbeuf, 75008 Paris, France; [4]Sanofi Recherche, 195 Route d'Espagne, 31036 Toulouse, France; and [5]Ospedale San Raffaele, Via Olgettina 60, 20132 Milan, Italy

1 Human *in vitro* preparations of transverse or distal colonic circular smooth muscle were potently and dose-dependently contracted by neurokinin A (EC_{50}, 4.9 nM), the tachykinin NK_2-receptor selective agonist [β-Ala^8]neurokinin A (4–10) ([β-Ala^8]NKA (4–10)) (EC_{50}, 5.0 nM), neurokinin B (EC_{50}, 5.3 nM) and substance P (EC_{50}, 160 nM), but not by the tachykinin NK_1-receptor selective agonist [Sar^{9}Met(O_2)^{11}] substance P, or the NK_3-receptor selective agonists, senktide and [MePhe^7] neurokinin B. No regional differences between transverse and distal colon were observed in response to [β-Ala^8]NKA (4–10).

2 Atropine (1 μM) and tetrodotoxin (1 μM) did not significantly inhibit responses to [β-Ala^8]NKA (4–10), neurokinin A, substance P or neurokinin B.

3 The newly developed non-peptide antagonists for tachykinin NK_2-receptors SR 48968, SR 144190 and its N-demethyl (SR 144743) and N,N-demethyl (SR 144782) metabolites, were used to challenge agonist responses, as appropriate. SR 144190 and the metabolites all potently and competitively antagonized the response to [β-Ala^8]NKA (4–10), with similar potency (Schild plot pA_2 values 9.4, 9.4 and 9.3, slope = 1). SR 48968 antagonism was not competitive: the Schild plot slope was biphasic with a high (X intercept ~ 9.3) and a low (X intercept 8.4, slope 1.6) affinity site. Co-incubation of SR 48968 (10, 100 nM) and SR 144782 (10 nM) produced additive effects; in this experimental condition, SR 48968 apparent affinity (pK_B) was 8.2. In addition, SR 144782 (0.1 μM) antagonized responses to neurokinin A, substance P and neurokinin B, with pK_B consistent with its affinity for tachykinin NK_2-receptors. The potent and selective NK_1 and NK_3-receptor antagonists, SR 140333 and SR 142801 (both 0.1 μM), failed to inhibit contractions induced by SP or NKB.

4 In conclusion, the *in vitro* mechanical responses of circular smooth muscle preparations from human colon are strongly consistent with the presence of non-neuronal tachykinin NK_2-receptors, but not tachykinin NK_1- or NK_3-receptors. Our findings with SR 48968 suggest the existence of two tachykinin NK_2-receptor subtypes, that it seems to distinguish, unlike SR 144190 and its metabolites. However, the precise nature of SR 48968 allotopic antagonism remains to be elucidated, since allosteric effects at the tachykinin NK_2-receptor might well account for the complexity of the observed interaction.

Keywords: Tachykinins; NK_2-receptors; human colon; SR 48968; SR 144190; SR 144782; SR 144743; [β-Ala^8]NKA (4–10)

Introduction

The family of neuropeptides called tachykinins, including their endogenous mediators, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), induces marked contractile effects through activation of specific receptors in several human smooth muscle preparations obtained from the respiratory, genito-urinary and gastrointestinal tract (Maggi *et al*., 1990, 1992; Giuliani *et al*., 1991; Huber *et al*., 1993; Croci *et al*., 1995; Holzer-Petsche, 1995; Holzer & Holzer-Petsche, 1997a, b). Different tachykinin receptors, NK_1, NK_2 and NK_3, have been functionally defined using peptide-specific agonists and antagonists, and were cloned in several animal species including man (Maggi *et al*., 1993; Maggi, 1995; Holtzer-Petsche, 1995). Neurokinin A preferentially stimulates the tachykinin NK_2-receptor while substance P and neurokinin B are the preferred endogenous ligands of the tachykinin NK_1- and NK_3-receptors respectively (Maggi, 1995; Patacchini & Maggi, 1995; Regoli *et al*., 1995). The pharmacological characteristics and pathophysiological role of tachykinin receptors in the gut have been discussed in recent reviews (Holzer and Holzer-Petsche, 1997a,b).

Species differences have been noted for tachykinin receptors (Holtzer-Petsche, 1995), so functional studies are needed to assess the affinity for native human receptors of the novel non-peptide specific antagonists, with a view to their clinical therapeutic potential.

We used the mechanical responses of human colonic circular smooth muscle preparations elicited *in vitro* by selected agonists, to investigate the pharmacological properties of tachykinin receptors therein, including their susceptibility to appropriate antagonists. A preliminary account of this work was presented at the DDW, May 11–14, 1997, Washington DC, U.S.A. (Croci *et al*., 1997a). This study was approved by the ethics committee of the San Raffaele Hospital, Milan.

[6] Author for correspondence.

Methods

Tissue preparation

Specimens of human transverse or distal colon, taken from macroscopically normal regions, were obtained from patients (10 males and 12 females, aged 55–76) undergoing surgery for colonic cancer (80% rectum, 20% transverse) at the San Raffaele Hospital, Milan. Patients did not receive radiotherapy and were not previously treated chronically with steroids or chemotherapics. However, two subjects were under anti-hypertensive therapy with enapril, one received a calcium antagonist and two the H_2-receptor antagonist, ranitidine. Uncontrolled patient medication history, standard anaesthesia and other possible variations between samples in this study, apparently had no influence on tachykinin agonist responses obtained from the colon of different patients. Specimens were available at the operating theatre, each consisting of a whole colon segment; they were washed in saline and immediately placed in a cold (4°C) pre-aerated (95% O_2, 5% CO_2) Krebs' solution (composition mM: Na Cl 118.4, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.7) and transported to Sanofi laboratories within about 30 min. Mucosa and sub-mucosa were gently removed and muscular regions between the taenia coli were cut into strips approximately 3 mm wide along the circular axis (total length of each preparation 2 cm).

Smooth muscle strips stored overnight (16–18 h) in cold (4°C) pre-aerated Krebs' solution to reduce spontaneous phasic contractions and tonus, maintained their full sensitivity to different peptide and non-peptide stimulants consistent with a previous report (Couture *et al*., 1981). Under our experimental conditions, the pattern of spontaneous activity was reduced but still present in some tissue and strips obtained from the same colon specimen. However, the low degree of phasic activity did not hamper tonic contraction assessment.

Experimental conditions

Eighteen to twenty-four strips were dissected from each specimen, allowing for a direct comparison of agonist and antagonist activities. A few responses were performed in duplicate, in which case the results were always averaged and considered as a single one from the same specimen.

Colonic strips were mounted in a 20-ml organ bath containing warm (37°C) aerated (95% O_2, 5% CO_2) Krebs' solution and stretched with 1–1.5 g; they were washed and allowed to equilibrate for 1 h, then challenged with a primer concentration (1 μM) of agonist (i.e. [β-Ala^8]NKA (4–10), neurokinin B, neurokinin A, substance P or carbachol) and washed five times. Contractions were recorded isotonically. About 2 h later, a cumulative agonist concentration response curve (contact time 5–15 min) was plotted, followed – after a similar interval – by a second one; results were always expressed as a percentage of the maximal contraction given by the first reference curve. Five washout procedures were performed immediately after the first cumulative agonist concentration response curve. The response to carbachol (0.1 mM) was determined at the end of the experiment. For each specimen, at least one preparation that had given two reproducible cumulative concentration response curves was used as control; this confirmed that desensitization did not occur under our experimental conditions. Only one tachykinin receptor agonist or antagonist was tested on each strip.

Antagonists incubation times were: 2 h for SR 48968, SR 144190 and its metabolites SR 144743 and SR 144782; 30 min for atropine and 15 min for tetrodotoxin. Control tissues were incubated only with the drug vehicles (tachykinin antagonists were dissolved in 2% dimethylsulfoxide; NKB, [β-Ala^8]NKA (4–10) and senktide: 0.2% ammonium hydroxide; [Sar^{9}Met(O$_2$)^{11}]: 0.03% acetic acid; SP, NKA and other drugs: distilled water). Four to twelve different colonic preparations were used for each cumulative concentration response curve. Figure 1 shows a representative tracing of human colonic circular smooth muscle contracted by [β-Ala^8]NKA (4–10) in the absence and presence of the NK_2 receptor antagonist, SR 144190.

Calculations and statistical analysis

The agonist concentration producing 50% maximal response (EC_{50}) was calculated using a four-parameter logistic model according to Ratkovsky & Reedy (1986), with adjustment made by non-linear regression using the Levenberg-Marquard algorithm in RS/1 software.

The pA_2 value for antagonists, as defined by Arunlakshana & Schild (1959), was obtained from linear regression of mean values of the log (Dr-1) *vs* the negative log of the antagonist concentration. When the Schild plot slope was not significantly different from 1, it was constrained to unity. Computer analysis was done as described by Tallarida & Murray (1987).

Chemicals

SR 48968, (S)-(−) N-methyl N-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)-butyl]-benzamide hydrochloride and its R-enantiomer SR 48965; SR 144190 (R)-(+)3-{1-[2-(4-benzoyl-2-(3,4-difluorophenyl)-morpholin-2-yl)-ethyl]-4-phenylpiperidin-4-yl}-1,1-dimethylurea, hydrochloride) (Croci *et al*., 1995); SR 144743 (R)-(+)3-{1-[2-(4-benzoyl-2-(3,4-difluorophenyl)-morpholin-2-yl)-ethyl]-4-phenylpiperidin-4-yl}-1-urea, hydrochloride; SR 144782 (R)-(+)-3-{1-[2-(4-benzoyl-2-(3,4-difluorophenyl)-morpholin-2-yl)-ethyl]-4-phenylpiperidin-4-yl}-1-methylurea, hydrochloride; SR 140333 (S)1-{2-[3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl] ethyl}-4-phenyl-1-azoniabicyclo [2,2,2]octane chloride (Croci *et al*., 1995); SR 142801 (R)-N-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)-piperidin-3-yl)propyl)-4-phenyl-piperidin-4-yl)-N-methylacetamide, hydrochloride (Emonds-Alt *et al*., 1995b), were synthesized at Sanofi Recherche, Montpellier, France. The following chemicals were purchased from the commercial sources indicated: Sigma-Aldrich Corp. (St Louis, Mo, U.S.A.): tetrodotoxin, carbachol, atropine sulphate; Novabiochem (Laufelfingen, Switzer-

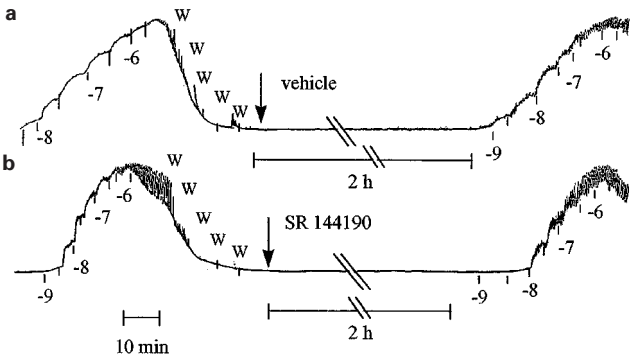

Figure 1 Representative tracings of the contractile response of human colon circular smooth muscle strips to [β-Ala^8]NKA (4–10) in absence (control-vehicle) or presence of the tachykinin NK_2-receptor antagonist (10^{-8}M SR 144190).

land): neurokinin A, neurokinin B, substance P, [Sar^9, $Met(O_2)^{11}$]substance P, [β-Ala^8]NKA (4–10), senktide and [$MePhe^7$]neurokinin B.

Results

Tachykinin receptor agonists caused concentration-dependent contractions of human colonic circular smooth muscle: their EC_{50}, nM (in parentheses 95% confidence limits) were [β-Ala^8]NKA (4–10), 5.0 (4.4–5.7); neurokinin A, 4.9 (3.9–6.1), neurokinin B; 5.3 (3.9–7.1); substance P, 160 (133–191) (see Figure 2); their maximal response was 88±5% (mean± s.e.mean) of the maximal effect of 0.1 mM carbachol. The tachykinin NK_1-receptor selective agonist, [Sar^9,$Met(O_2)^{11}$] substance P, or the NK_3-receptor selective agonists, [$MePhe^7$]NKB and senktide had no effect up to 1 μM ($n=5$, strips from different patients). Atropine (1 μM) and tetrodotoxin (1 μM) did not inhibit the response elicited by tachykinin receptor agonists (Figure 2). A similar response to [β-Ala^8]NKA (4–10) was obtained in transverse and distal colon: EC_{50}, nM, 4.8 (3.9–5.9) and 5.9 (5.3–6.8) respectively. The selective tachykinin NK_1 and NK_3-receptor antagonists, SR 140333 and SR 142801, both 0.1 μM, did not significantly inhibit SP or NKB responses; agonists' EC_{50} ($n=3$) were 200 (140–320) and 10 (6–15) nM, as listed.

As shown in Figure 3, the tachykinin NK_2-receptor antagonist SR 144190 (1–100 nM) produced a concentration-dependent rightward shift of the concentration response curves to [β-Ala^8]NKA (4–10). This potent antagonism (pA_2 9.25) appeared to be competitive, as shown by Schild-regression analysis that gave a slope of 1.13, not significantly different from unity; after constraining the slope to unity, the pA_2 value was 9.42 (see Table 1). The N-demethyl and N,N-demethyl metabolites of SR 144190, SR 144743 and 144782 (1–30 nM), both antagonized [β-Ala^8]NKA (4–10) responses competitively (Figures 4 and 5); their calculated pA_2 were 9.34 (slope 1.06) and 9.43 (slope 0.92) and, after constraining the slopes to unity, 9.41 and 9.33 (see Table 1).

As shown in Figure 6 and Table 1, the tachykinin NK_2-receptor antagonist SR 48968 (0.3 nM to 300 nM) also produced a concentration-dependent rightward shift of the concentration-response curves to [β-Ala^8]NKA (4–10). However this antagonism appeared to be non-competitive, as

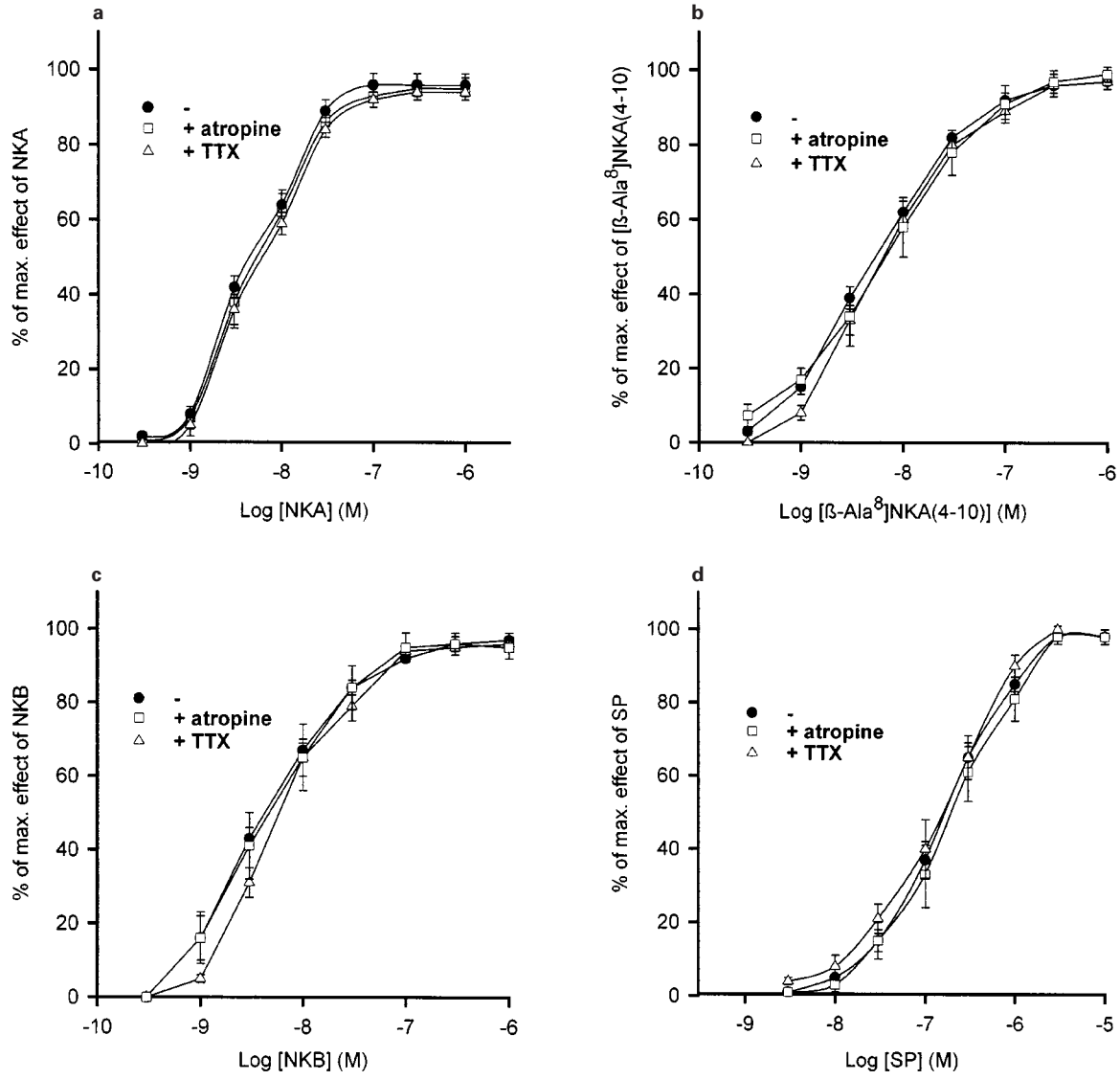

Figure 2 Contractions induced by tachykinin receptor agonists in circular smooth muscle from human colon *in vitro*. Concentration-response curves to NKA (a), [β-Ala^8]NKA (4–10) (b), NKB (c) and SP (d) in the absence or presence of atropine (1 μM) or tetrodotoxin (TTX, 1 μM). Results are mean±s.e.mean of 4–7 colonic preparations from different patients.

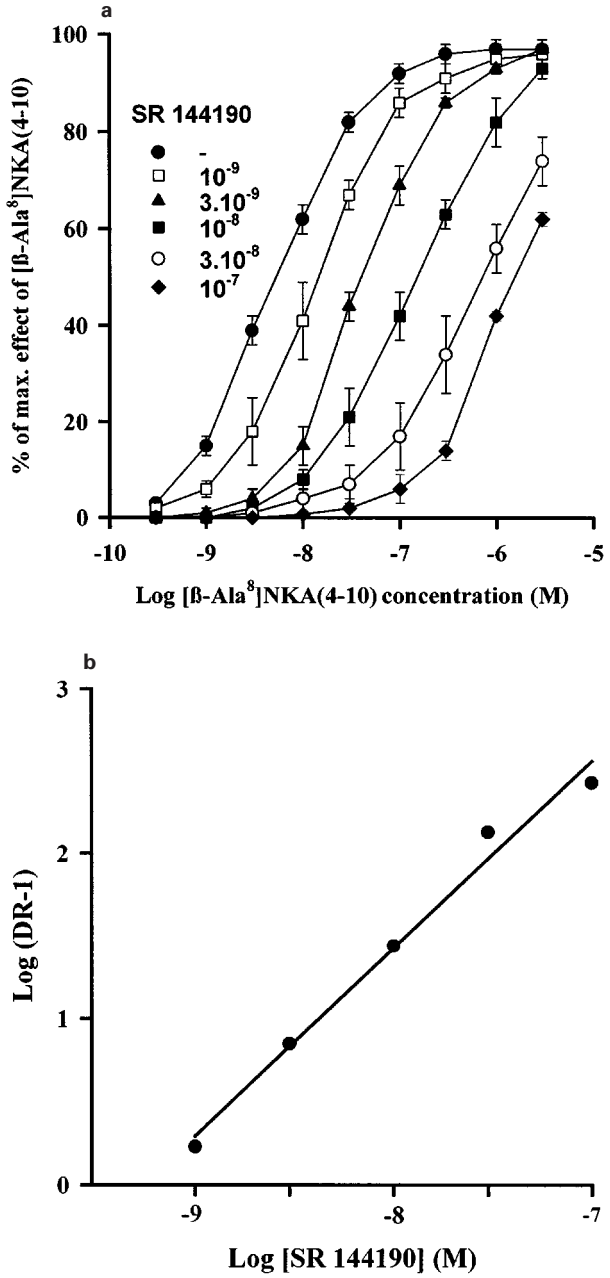

Figure 3 Effect of the tachykinin NK_2-receptor antagonist SR 144190 on contractions induced by [β-Ala^8]NKA (4–10) in circular smooth muscle from human colon *in vitro*. Concentration-response curves to [β-Ala^8]NKA (4–10) in the absence or presence of SR 144190 (1–100 nM) (a) and the corresponding Schild plot (b). Results are mean±s.e.mean of 4–12 colonic preparations from different patients.

shown by Schild regression analysis that gave a pronounced deviation of the curve from a straight line. SR 48968 slope was biphasic with apparent high (X intercept ~9.3) and low (X intercept = 8.4, slope 1.6) affinity sites (Table 1 and Figure 6); comparable results were obtained when we replaced [β-Ala^8]NKA (4–10) with NKA as agonist (data not shown). The R-enantiomer of SR 48968, SR 48965 (0.1, 0.3 μM) antagonized the [β-Ala^8]NKA (4–10) response with a pK_B of 6.3±0.3 (n = 3).

In order to investigate the nature of SR 48968's inhibition of the [β-Ala^8]NKA (4–10) response, SR 48968 (1–100 nM) was co-incubated with the competitive tachykinin NK_2-receptor antagonist, SR 144782 (10 nM) and the inhibition was compared with that produced by SR 144782 alone. As shown in Figure 7, the concentration response curves to [β-Ala^8]NKA (4–10) were shifted rightward only by 10 and 100 nM SR 48968, producing additive effects with SR 144282: the mean apparent pK_B was 8.2.

Neither SR 144190 nor its metabolites SR 144782 or SR 144743, nor SR 48968, up to the highest concentration tested, inhibited carbachol (0.5 μM) contractions or showed any agonist activity (data not shown).

The competitive NK_2-receptor antagonist SR 144782 (0.1 μM) antagonized responses to NKA, SP and NKB with similar pK_B (mean±s.e.mean, 9.4±0.2, 9.3±0.2, 9.5±0.3 respectively).

Discussion

The results of the present study are indicative of the presence in the human colon of functional tachykinin NK_2-receptors, but not tachykinin NK_1- or NK_3-receptors, located on circular smooth muscle; no regional differences in response to [β-Ala^8]NKA (4–10) were observed between transverse and distal colon. Unlike the tachykinin NK_2-receptor agonist [β-Ala^8]NKA (4–10), the selective tachykinin NK_1- and NK_3-receptor agonists [Sar^9,Met(O$_2$)^{11}]SP and senktide or [Me-Phe^7]NKB all failed to elicit any response in our colonic preparation. The natural peptides NKA, NKB and, to a lesser extent SP, potently contracted the colonic muscle strips but their action appeared to be mediated only by the tachykinin NK_2-receptor; thus the potent tachykinin NK_2-receptor antagonist SR 144782 inhibited the agonist response, with pK_B values consistent with its affinity for the tachykinin NK_2-receptor and the selective NK_1- and NK_3-receptor antagonists, SR 140333 and SR 142801, both failed to inhibit contractions induced by SP or NKB.

NKB was a powerful stimulant of the human colonic tachykinin NK_2-receptor and the agonists' rank order of potency we scored, i.e. [β-Ala^8]NKA (4–10) = NKA = NKB > SP, was not the same as that reported for

Table 1 Quantitative antagonism of [β-Ala^8]NKA (4–10) responses in human colonic circular smooth muscle preparations by their non-peptide antagonists

Antagonists	*Concentration range,* nM	*pA_2*	*Slope*	*Schild-regression*	*pA_2* (slope = 1)
SR 144190	1–100	9.25±0.10	1.13±0.08	linear	9.42±0.07
SR 144743	1–30	9.34±0.07	1.06±0.07	linear	9.41±0.04
SR 144782	1–30	9.43±0.10	0.92±0.07	linear	9.33±0.04
SR 48968	0.3–300	9.18±0.17	1.00±0.13	non linear	–
	10–300	8.41±0.04	1.65±0.07**	linear	–

Values are mean±s.e.mean. The pA_2 was obtained from the concentration response curves shown in Figures 2 and 3. **P<0.01 significantly different from unity.

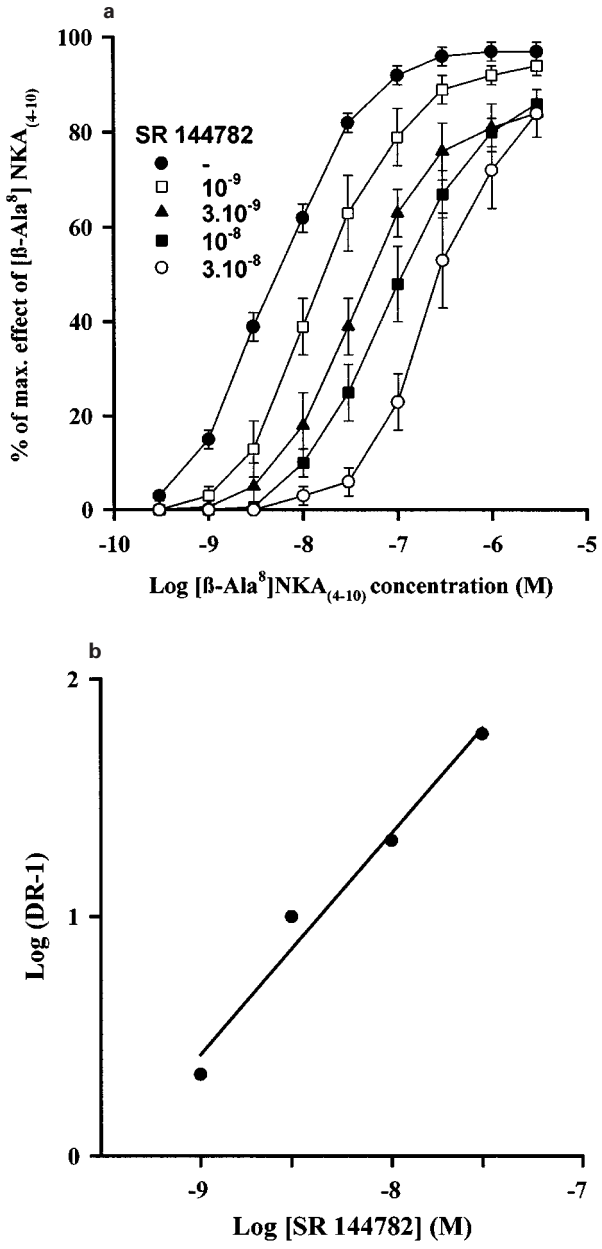

Figure 4 Effect of the tachykinin NK_2-receptor antagonist SR 144782 on contractions induced by [β-Ala^8]NKA (4–10) in circular smooth muscle from human colon *in vitro*. Concentration-response curves to [β-Ala^8]NKA (4–10) in the absence or presence of SR 144782 (1–30 nM) (a) and the corresponding Schild plot (b). Results are mean ± s.e.mean of 4–12 colonic preparations from different patients.

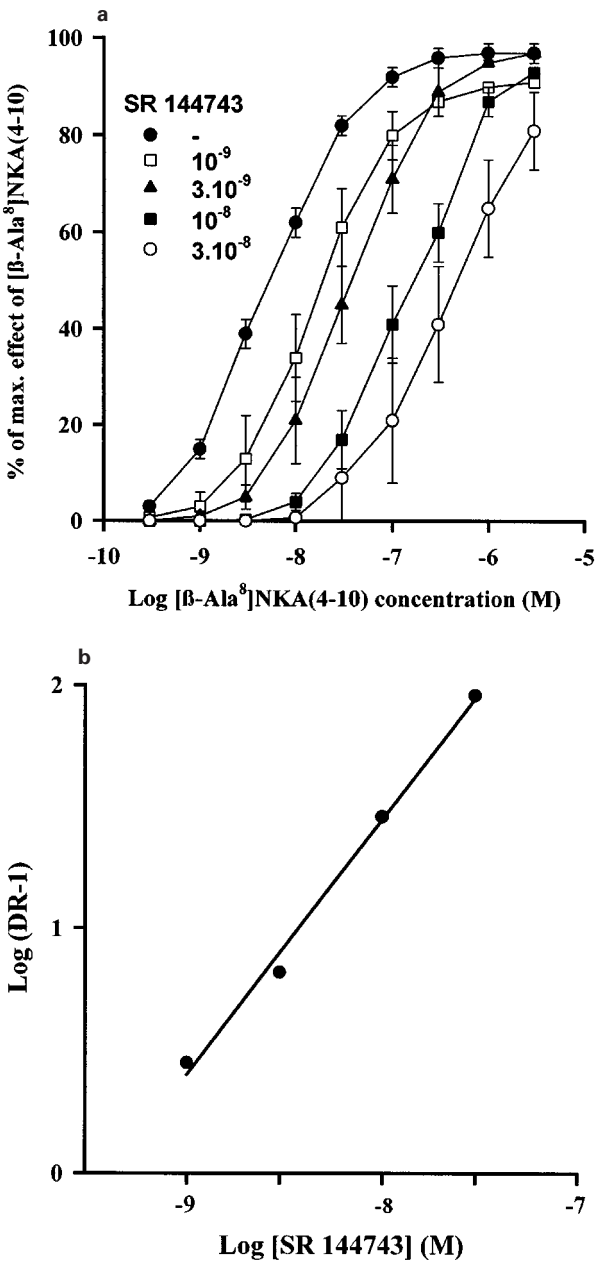

Figure 5 Effect of the tachykinin NK_2-receptor antagonist SR 144743 on contractions induced by [β-Ala^8]NKA (4–10) in circular smooth muscle from human colon *in vitro*. Concentration-response curves to [β-Ala^8]NKA (4–10) in the absence or presence of SR 144743 (1–30 nM) (a) and the corresponding Schild plot (b). Results are mean ± s.e.mean of 4–12 colonic preparations from different patients.

NK_2-receptors ([β-Ala^8]NKA (4–10) = NKA > NKB > SP) according to the nomenclature of Montreal (Henry, 1987).

Our finding of atropine- and tetrodotoxin-insensitive agonist responses indicates that neuronal receptors are not involved. However, modest but significant tetrodotoxin-sensitive neurogenic responses to NKA and SP have been reported in the human taenia coli (Kolbel *et al*., 1994).

On the basis of the above results, we moved on to test the novel non-peptide tachykinin receptor antagonists for their ability to prevent mechanical responses elicited by the selective tachykinin NK_2 receptor agonist [β-Ala^8]NKA (4–10) in human colonic muscle strips. SR 48968 and SR 144190 have been reported to be selective and potent antagonists of the tachykinin NK_2-receptor in several *in vitro* and *in vivo* tests (Croci *et al*., 1995, 1997a,b; Emonds-Alt *et al*., 1995a, 1997). The two antagonists have similar subnanomolar potency in all species studied, except the hamster. In binding studies, in membrane preparations from CHO cells transfected with the human tachykinin NK_2-receptor, the K_i were 0.04 and 0.03 nM for SR 48968 and SR 144190 in the given order (Emonds-Alt *et al*., 1992; 1993; 1997). In human bronchus, SR 48968 and SR 144190 affinities for tachykinin NK_2-receptors (pA_2) were 9.4 and 9.8 respectively (Emonds-Alt *et al*., 1992; 1993; 1997), however SR 144190 appears to be more selective than SR 48968 (Emonds-Alt *et al*., 1997). SR 48968, but not SR 144190, has also been shown to interact *in vitro* with μ-opioid receptors, at quite high concentrations (μ molar range) (Boyle *et al*., 1993; Emonds-Alt *et al*., 1997; Martin *et al*., 1993). Yet

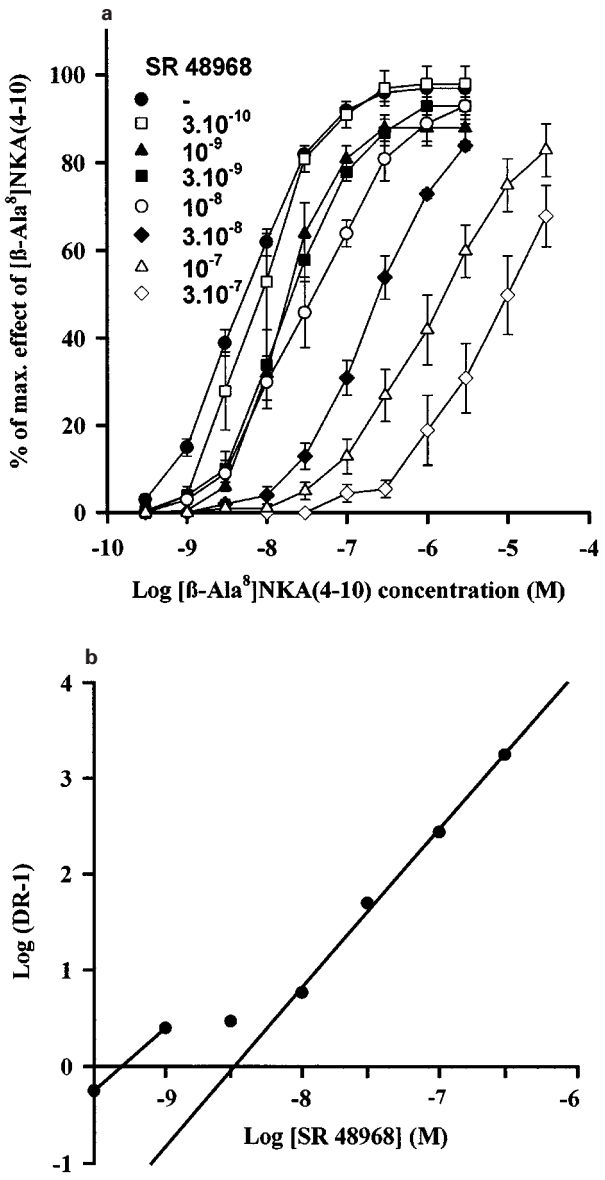

Figure 6 Effect of the tachykinin NK_2-receptor antagonist SR 48968 on contractions induced by [β-Ala^8]NKA (4–10) in human colon *in vitro*. Concentration-response curves to [β-Ala^8]NKA (4–10) in the absence or presence of SR 48968 (0.3–300 nM) (a) and the corresponding Schild plot (b). Results are mean ± s.e.mean of 4–12 colonic preparations from different patients.

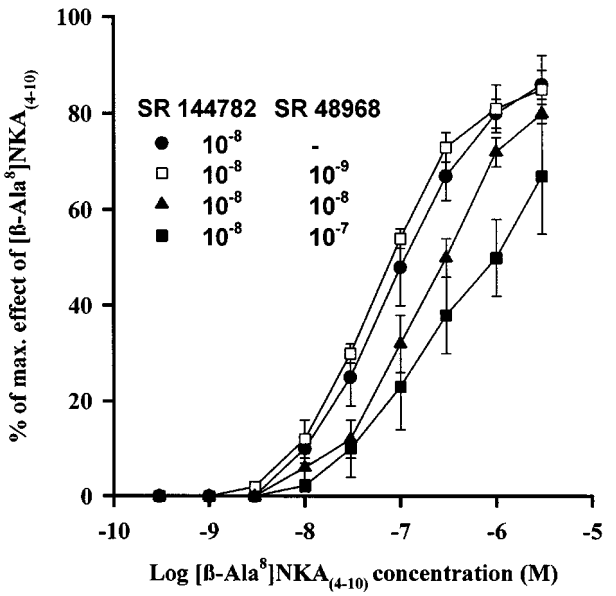

Figure 7 Effect of co-incubation of the tachykinin NK_2-antagonists SR 48698 and SR 144782 on contractions induced by [β-Ala^8]NKA (4–10) in circular smooth muscle from human colon *in vitro*. Concentration-response curves to [β-Ala^8]NKA (4–10) in the presence of SR 144782 (10 nM) alone or SR 144782 (10 nM) plus SR 48968 (1–100 nM). Results are mean ± s.e.mean of four colonic preparations from different patients.

these *in vitro* findings are of questionable functional significance, since in rats SR 48968, at otherwise fully effective doses (naloxone-insensitive antagonism of castor oil diarrhea) did not slow gastrointestinal transit or cause constipation, unlike the opioid loperamide whose antidiarrheal action was prevented by naloxone (Croci *et al*., 1997b)

In isolated human colonic muscle strips, SR 144190 was a potent and competitive antagonist of tachykinin NK_2-receptors with a pA_2 value of 9.42. SR 48968 antagonism was also potent but not competitive, with a biphasic slope and a high and a low-affinity site, apparently suggesting two distinct tachykinin NK_2-receptor subtypes.

Heterogeneity of NK_2-receptors has already been offered to explain differences in antagonist affinity in several *in vitro* functional preparations (Giuliani *et al*., 1991; Maggi *et al*., 1993; Croci *et al*., 1995; Maggi, 1995). On these grounds, SR 48968 was claimed to have at least ten times higher affinity for NK_{2A}- than for NK_{2B}-receptors. However some of these differences are possibly species- rather than receptor subtype-related. The mechanism of the interaction of SR 48968 with tachykinin NK_2-receptors may be complex and allosteric in nature (Advenier *et al*., 1992; Croci *et al*., 1995); it was suggested that SR 48968 behaves as an allosteric inhibitor bound to a crucial region, thereby preventing the formation of the high-affinity state of the receptor for agonist binding (Gether *et al*., 1993). SR 48968 was also considered an irreversible antagonist of tachykinin NK_2-receptors in isolated guinea-pig gallbladder and proximal colon (Patacchini *et al*., 1994), or a competitive one in human bronchus and other *in vitro* functional preparations (Emonds-Alt *et al*., 1992; 1993; Croci *et al*., 1995).

We investigated the nature of SR 48968 antagonism with the tachykinin NK_2-receptor in the human colon, by co-incubating it with the competitive tachykinin NK_2-receptor antagonist, SR 144782: the combination of antagonists produced an additive inhibitory effect on [β-Ala^8]NKA (4–10) responses; this suggests the existence of two tachykinin NK_2-receptor subtypes that SR 48968 seems to distinguish, whereas SR 144190 and its metabolites do not.

However, the precise nature of SR 48968 antagonism still remains to be elucidated; non-competitive antagonism may be due to allosteric effects (Kenakin, 1997), mediated by negative and positive cooperativity occurring at low and high antagonist concentrations. Interestingly, the N and N,N demethyl metabolites of SR 144190 were likewise potent and competitive antagonists of the colonic contractions elicited by the selective tachykinin NK_2-receptor agonist [β-Ala^8]NKA (4–10). Finally we obtained evidence for the stereoselectivity of SR 48968 antagonism, as shown by the substantially lower potecy of its R-enantiomer, SR 48965 in preventing [β-Ala^8]NKA (4–10) responses.

The present findings attest to the potency and selectivity of these novel non-peptide antagonists for tachykinin NK_2-receptor naturally expressed in the circular smooth muscle of human colon and are consistent with those obtained in the

human bronchus (Emonds-Alt *et al*., 1992; 1993; 1997); thus the use of SR 48968 and SR 144190 as tools for clarifying the underlying receptor mechanism in normal and altered gut function, is warranted.

Tachykinin NK_2- receptor antagonists might offer a new therapeutic approach to the treatment of several intestinal motor disturbances, including diarrhoea, irritable bowel syndrome and inflammatory bowel diseases. In addition, endogenous tachykinins have been suggested to play a role in gut dysmotility disorders associated with infection, stress and pain (Croci *et al*., 1997b; Holzer & Holzer-Petsche, 1997a).

The authors would like to thank Mrs M. Bonalume, M.G. Dozio and E. Mazzoni for skillful technical assistance.

References

ADVENIER, C., ROUISSI, N., NGUYEN, Q.T., EMONDS-ALT, X., BRELIERE, J.C., NELIAT, G., NALINE, E. & REGOLI, D. (1992). Neurokinin A (NK2) receptor revisited with SR 48968, a potent, non-peptide antagonist. *Biochem. Biophys. Res. Commun.,* **184,** 1418–1424

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemoter.,* **14,** 48–57.

BOYLE, S.J., MANLEY, S., TANG, K.W., MEECHAN, K., GUARD, S., WATLING, K.J., WOODRUFF, G.N. & McNIGHT, A.T. (1993). Affinity of the NK_2 antagonist SR 48968 at NK_3-tachykinin and μ-opioid receptors. *Br. J. Pharmacol.,* **108,** 24P.

COUTURE, R., MIZRAHI, J., REGOLI, D. & DEVROEDE, G. (1981). Peptides and the human colon: an *in vitro* pharmacological study. *Can. J. Physiol. Pharmacol.,* **59,** 957–964.

CROCI, T., EMONDS-ALT, X., LE FUR, G. & MANARA, L. (1995). *In vitro* characterization of the non-peptide tachykinin NK_1 and NK_2 receptor antagonists, SR 140333 and SR 48968 in different rat and guinea-pig intestinal segments. *Life Sci.,* **56,** 267–275.

CROCI, T., AUREGGI, G., MANARA, L., EMONDS-ALT, X., GULLY, D., MAFFRAND, J.P., LE FUR, G., MUKENGE, S. & FERLA, G. (1997a). Functional neurotensin and tachykinin NK_2-receptors in human colon strips. *Gastroenterol.,* **112,** A716.

CROCI, T., LANDI, M., EMONDS-ALT, X., LE FUR, G., MAFFRAND, J.P. & MANARA, L. (1997b). Role of tachykinins in castor oil diarrhoea in rats. *Br. J. Pharmacol.,* **121,** 375–380.

EMONDS-ALT, X., ADVENIER, C., COGNON, C., CROCI, T., DAOUI, S., DUCOUX, J.P., LANDI, M., NALINE, E., NELIAT, G., PONCELET, M., PROIETTO, V., VAN BROECK, D., VILAIN, P., SOUBRIE, P., LE FUR, G., MAFFRAND, J.P. & BRELIERE, J.C. (1997). Biochemical and pharmacological activities of SR 144190, a new potent non-peptide tachykinin NK_2 receptor antagonist. *Neuropeptides.,* **31,** 449–458.

EMONDS-ALT, X., ADVENIER, C., CROCI, T., MANARA, L., NELIAT, G., PONCELET, M., PROIETTO, V., SANTUCCI, V., SOUBRIE, P., VAN BROECK, D., VILAIN, P., LE FUR, G. & BRELIERE, J.C. (1993). SR 48968, a neurokinin A (NK_2) receptor antagonist. *Reg. Pept.,* **46,** 31–36.

EMONDS-ALT, X., ADVENIER, C., SOUBRIE, P., LE FUR, G. & BRELIERE, J.C. (1995a). SR 48968: nonpeptide antagonist of the tachykinin NK2 receptor. *Drugs of the Future,* **20,** 701–707.

EMONDS-ALT, X., BICHON, D., DUCOUX, J.P., HEAULME, M., MILOUX, B., PONCELET, M., PROIETTO, V., VAN BROECK, D., VILAIN, P., NELIAT, G., SOUBRIE, P., G. LE FUR & BRELIERE, J. C. (1995b). SR 142801, the first potent non-peptide antagonist of the tachykinin NK_3 receptor. *Life Sci.,* **56,** PL27–32.

EMONDS-ALT, X., VILAIN, P., GOULAOIUC, P., PROIETTO, V., VAN BROECK, D., ADVENIER, C., NALINE, E., NELIAT, G., LE FUR, G. & BRELIERE, J.C. (1992). A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor. *Life Sci.,* **50,** PL101–PL106.

GETHER, U., YOKOTA, Y., EMONDS-ALT, X., BRELIERE, J.C., LOWE, J.A., III, SNIDER, R.M., NAKANISHI, S. & SCHWARTZ, T.W. (1993). Two non-peptide tachykinin antagonists act through epitopes on corresponding segments of the NK1 and NK2 receptors. *Proc. Natl. Acad. Sci. U.S.A.,* **90,** 6194–6198.

GIULIANI, S., BARBANTI, G., TURINI, D., QUARTARA, L., ROVERO, P., GIACHETTI, A. & MAGGI, C.A. (1991). NK_2 tachykinin receptors and contraction of circular muscle of the human colon: characterization of the NK_2 receptor subtype. *Eur. J. Pharmacol.,* **203,** 365–370.

HENRY, J.L. (1987). Discussion of nomenclature for tachykinins and tachykinin receptors. In: Henry J.L., Couture R., Cuello A.C., Pelletier, G., Quiron, R., Regoli, D. (eds) *Substance P and Neurokinins. Proceedings of 1986 Symposium*, Montreal. New York: Springer Verlag, pp 16–7.

HOLZER, P. & HOLZER-PETSCHE, U. (1997a). Tachykinins in the Gut. Part I. Expression, Release and Motor Function. *Pharmacol. Ther.,* **73,** 173–217.

HOLZER, P. & HOLZER-PETSCHE, U. (1997b). Tachykinins in the Gut. Part II. Roles in neural Excitation, Secretion and Inflammation. *Pharmacol. Ther.,* **73,** 219–263.

HOLZER-PETSCHE, U. (1995). Tachykinin receptors in gastrointestinal motility. *Regulatory Peptides,* **57,** 19–42.

HUBER, O., BERTRAND, C., BUNNETT, N.W., PELLEGRINI, C.A., NADEL, J.A., NAKAZATO, P., DEBAS, T.H. & GEPPETTI, P. (1993). Tachykinins mediate contraction of the human lower esophageal sphincter in vitro via activation of NK_2 receptors. *Eur. J. Pharmacol.,* **239,** 103–109.

KENAKIN, T.P. (1997). Allotopic, Non-competitive and Irreversible Antagonism. In: *Pharmacologic Analysis of Drug-Receptor Interaction*. In: 3rd edn., pp. 275–395. Lippincott-Raven, Philadelphia-New York.

KOLBEL, C.B., MAYER, E.A., HOLTMANN, G., LAYER, P., PETSCHULAT, W., ERHARD, J. & GOEBELL, H. (1994). Effects of neurokinins on human colonic motility. *Neurogastroenterol. Mot.,* **6,** 119–127.

MAGGI, C.A. (1995). Tachykinin Receptors. In: Ruffolo R.R., Hollinger M.A. (eds.) *G-protein coupled transmembrane signaling mechanisms*. CRC Press: Boca Raton, pp 95–151.

MAGGI, C.A., GIULIANI, S.; PATACCHINI, R., SANTICIOLI, P., THEODORSSON, E., BARBANTI, G., TURINI, D. & GIACHETTI, A. (1992). Tachykinin antagonists inhibit nerve-mediated contractions in the circular muscle of the human colon. *Gastroenterol.* **102,** 88–96.

MAGGI, C.A., PATACCHINI, R., ROVERO, P. & GIACHETTI, A. (1993). Tachykinin receptors and tachykinin receptor antagonists. *J. Auton. Pharmacol.,* **13,** 23–93.

MAGGI, C.A., PATACCHINI, R., SANTICIOLI, P., GIULIANI, S., TURINI, D., BARBANTI, G., GIACHETTI, A. & MELI, A. (1990). Human isolated ileum: motor responses of the circular smooth muscle to electrical field stimulation and exogenous neuropeptides. *Naunyn. Schmiedeberg's Arch. Pharmacol.,* **341,** 256–261.

MARTIN, C.A.E., EMONDS-ALT, X. & ADVENIER, C. (1993). Inhibition of cholinergic neurotransmission in isolated guinea-pig main bronchi by SR 48968. *Eur. J. Pharmacol.,* **243,** 309–312.

PATACCHINI R., DE GIORGIO, R., GIACHETTI, A. & MAGGI, C.A. (1994). Different mechanism of tachykinin NK_2 receptor blockade by SR 48968 and MEN 10,267 in the guinea-pig isolated gallbladder and colon. *Eur. J. Pharmacol.,* **271,** 111–119.

PATACCHINI R. & MAGGI, C.A. (1995). Tachykinin receptors and receptor subtypes. *Arch. Int. Pharmacodyn.,* **329:** 161–184.

RATKOVSKY, D.A. & REEDY, T.J. (1986). Choosing near-linear parameters in four parameter logisitic model for radioligand and related assay. *Biometrics,* **42,** 575–582.

REGOLI, D., BOUDON, A., FAUCHERE, J.L. (1995). Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.,* **46,** 551–599.

TALLARIDA, R.J. & MURRAY, R.B. (1987). Manual of Pharmacologic Calculations with Computer Programs. New York: Springer-Verlag, 58–61.

(Received March 12, 1998
Revised April 20, 1998
Accepted April 23, 1998)