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Comparison of tachykinin NK_1 and NK_2 receptors in the circular muscle of the guinea-pig ileum and proximal colon

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1 The aim of this study was the pharmacological characterization of tachykinin NK_1 and NK_2 receptors mediating contraction in the circular muscle of the guinea-pig ileum and proximal colon. The action of substance P (SP), neurokinin A (NKA) and of the synthetic agonists [Sar^9]SP sulphone, [Glp^6,Pro^9]SP(6-11) (septide) and [βAla^8]NKA(4-10) was investigated. The affinities of various peptide and nonpeptide antagonists for the NK_1 and NK_2 receptor was estimated by use of receptor selective agonists.

2 The natural agonists, SP and NKA, produced concentration-dependent contraction in both preparations. EC_{50} values were 100 pM and 5 nM for SP, 1.2 nM and 19 nM for NKA in the ileum and colon, respectively. The action of SP and NKA was not significantly modified by peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each).

3 Synthetic NK_1 and NK_2 receptor agonists produced concentration-dependent contraction of the circular muscle of the ileum and proximal colon. EC_{50} values were 83 pM, 36 pM and 10 nM in the ileum, 8 nM, 0.7 nM and 12 nM in the colon for [Sar^9]SP sulphone, septide and [βAla^8]NKA(4-10), respectively. The pseudopeptide derivative of NKA(4-10), MDL 28,564 behaved as a full or near-to-full agonist in both preparations, its EC_{50}s being 474 nM and 55 nM in the ileum and colon, respectively.

4 Nifedipine (1 μM) abolished the response to septide and [Sar^9]SP sulphone in the ileum and produced a rightward shift and large depression of the response in the colon. The response to [βAla^8]NKA(4-10) was abolished in the ileum and largely unaffected in the colon.

5 The NK_1 receptor antagonists, (±)-CP 96,34, FK 888 and GR 82,334 competitively antagonized the response to septide and [Sar^9]SP sulphone in both preparations without affecting that to [βAla^8]NKA(4-10). In general, the NK_1 receptor antagonists were significantly more potent toward septide than [Sar^9]SP sulphone in both preparations.

6 The NK_2 receptor antagonists, GR 94,800 and SR 48,968 selectively antagonized the response to [βAla^8]NKA(4-10) without affecting that to [Sar^9]SP sulphone or septide in the ileum and colon. SR 48,968 produced noncompetitive antagonism of the response to the NK_2 receptor agonist in the ileum and competitive antagonism in the colon.

7 MEN 10,376 and the cyclic pseudopeptide MEN 10,573 antagonized in a competitive manner the response to [βAla^8]NKA(4-10) in the ileum and colon. While MEN 10,573 was equipotent in both preparations, MEN 10,376 was significantly more potent in the colon than in the ileum. MEN 10,376 was also effective against septide in both preparations, without affecting the response to [Sar^9]SP sulphone. MEN 10,573 antagonized the response to [Sar^9]SP sulphone and septide in both preparations, pK_B values against septide being intermediate, and significantly different from, those measured against [βAla^8]NKA(4-10) and [Sar^9]SP sulphone.

8 These findings show that tachykinin NK_1 and NK_2 receptors mediate contraction of the circular muscle of the guinea-pig ileum and colon. In both preparations NK_1 receptor antagonists display higher apparent affinity when tested against septide than [Sar^9]SP sulphone. These findings are compatible with the proposed existence of NK_1 receptor subtypes in guinea-pig, although alternative explanations (e.g. agonist binding to different epitopes of the same receptor protein) cannot be excluded at present. Furthermore, an intraspecies heterogeneity of the NK_2 receptor in the circular muscle of the guinea-pig ileum and colon is suggested.

Keywords: Tachykinins; NK_1 receptor; NK_2 receptor; guinea-pig ileum; guinea-pig colon; circular muscle; tachykinin receptor antagonist

Introduction

Three main types of receptors, NK_1, NK_2 and NK_3, mediate the actions of tachykinins which are encoded by their common, C-terminal sequence (Maggi *et al.*, 1993a for review). In the circular muscle of the guinea-pig ileum, the contractile response to NK_1 or NK_2 receptor stimulation occurs through a direct excitation of smooth muscle cells, while the NK_3 receptor-mediated contraction is indirect, being produced through activation of intramural neurones/nerves (Maggi *et al.*, 1990a).

The lack of a potent and selective NK_3 antagonist still hampers the full pharmacological and physiological characterization of this receptor in the intestine. On the other hand, the use of selective antagonists has delineated the hierarchical role of substance P (SP) and neurokinin A (NKA), via NK_1 and NK_2 receptors, respectively, as enteric excitatory transmitters in the guinea-pig ileum and colon (Bartho *et al.*, 1992; Zagorodnyuk *et al.*, 1993a). The recent developments in the pharmacology of tachykinin receptors, suggesting the possible existence of receptor subtypes (Maggi *et al.*, 1993a for review), make it important to perform a systematic inves-

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tigation of the apparent affinities of the various antagonists toward various tachykinin receptor agonists.

Species-related pharmacological differences, detected by variable affinities of competitive receptor antagonists, have emerged for both NK_1 and NK_2 receptors (Maggi *et al.*, 1993a for review). The nonpeptide antagonist, (±)-CP 96,345 (Snider *et al.*, 1991) is about 100 times more potent at NK_1 receptor expressed in e.g. man and guinea-pigs than rats or mice (Gitter *et al.*, 1991; Patacchini *et al.*, 1992; Barr & Watson, 1993), while the nonpeptide antagonist, RP 67,580 (Garret *et al.*, 1991) is more potent at rat than guinea-pig or human NK_1 receptors. For NK_2-receptors, certain peptide (e.g. MEN 10,376, Maggi *et al.*, 1991a) and nonpeptide antagonists (SR 48,968; Emonds-Alt *et al.*, 1992; Maggi *et al.*, 1993b) are more potent at human, bovine, guinea-pig and rabbit NK_2 receptors than at rat or hamster NK_2 receptors, while the cyclic peptide antagonist, L 659,877 (Williams *et al.*, 1988) or the cyclic pseudopeptide MEN 10,573 (Quartara *et al.*, 1992a) present the converse pattern of affinities (Maggi *et al.*, 1990b; Van Giersbergen *et al.*, 1991).

These species-related variations in antagonist potencies probably reflect the existence of species-related changes in the primary sequence of the NK_1 and NK_2 receptor protein: point mutation studies have established that species-related changes in aminoacid sequence at two discrete positions of the NK_1 receptor are responsible for the species-dependent variations in the affinities of CP 96,345 and RP 67,580 (Fong *et al.*, 1992b). The molecular bases for species-related variations in antagonists potencies at the NK_2 receptor have not been similarly established.

In addition, pharmacological evidence supports the idea that receptor subtypes (intraspecies heterogeneity) may exist for tachykinin NK_1 and NK_2 receptors. For example, septide or [Glp^6,Pro^9]SP(6-11), a synthetic ligand originally developed as a selective NK_1 receptor agonist, displays a contractile activity in the guinea-pig ileum which cannot easily be explained by an interaction with the 'classical' NK_1 receptor (Petitet *et al.*, 1992). Petitet *et al.* (1992) proposed that a novel, 'septide-sensitive', tachykinin receptor exists in the guinea-pig ileum. A 'septide-sensitive' receptor was also detected in the rat urinary bladder, which is recognized by NK_1 receptor antagonists with higher affinity than that observed toward a 'classical' NK_1 receptor agonist like [Sar^9]SP sulphone (Meini *et al.*, 1994). Likewise, (±)-CP 96,345 was found to be significantly more potent in antagonizing septide than [Sar^9]SP sulphone in the circular muscle of the guinea-pig ileum (Maggi *et al.*, 1993c). Thus, the 'septide-sensitive' receptor may be an NK_1 receptor subtype.

An intraspecies heterogeneity of the NK_2 receptor has also been evidenced by pharmacological studies (Xu *et al.*, 1991; Brunelleschi *et al.*, 1992; Nimmo *et al.*, 1992); it is at present uncertain whether or not these examples fit with the criteria used to define species-related differences in the NK_2 receptor (Maggi *et al.*, 1993a for review).

The aim of this study was to characterize the tachykinin NK_1 and NK_2 receptors mediating contraction in the circular muscle of two distinct intestinal segments (ileum and colon) from the same species (guinea-pig) by use of synthetic receptor selective agonists to stimulate the receptors and a panel of antagonists of both peptide and non peptide nature to block them. Some of these results were presented at the Third meeting of the European Neuropeptide Club, Cambridge, April 5–7, 1993.

Methods

Male albino guinea-pigs weighing 200–250 g were stunned and bled. A 10–15 cm length of ileum and 2–3 cm segment of proximal colon were excised and placed in warmed (37°C) and oxygenated (96% O_2 and 4% CO_2, pH 7.4) Krebs solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, KCl 4.7, $CaCl_2$ 2.5 and glucose 11.

Guinea-pig ileum

The longitudinal muscle and attached myenteric plexus were removed from the ileum as described by Paton & Zar (1968) and discarded. All experiments were performed on longitudinal muscle-myenteric plexus-free ileal rings (2–3 mm wide) in the presence of 10 μM indomethacin. The ileal rings were suspended in 5 ml baths for isolated organs by means of two stainless steel hooks and connected to an isotonic transducer (load 5 mN) to record the mechanical activity of the circular muscle. The preparations were allowed to equilibrate for 90 min with renewal of the bathing solution every 15 min. The rings were then exposed to 10 μM carbachol at 15 min intervals until two reproducible responses were observed. This usually occurred at 150–180 min from setup. The response to 1 μM [βAla^8]NKA(4-10) was also determined in some experiments before addition of various tachykinin receptor agonists. The addition of tachykinin receptor agonists produced an increase in both phasic activity and a tonic contraction of the circular muscle of the ileum (Figure 1). To enable a more accurate quantitative evaluation of the effect of agonists, the signal recorded from the isotonic transducer was delivered to a Basile 7083 integrator and the contractile activity of the rings was integrated every 10 s. The level of integration was set at about 10% of the spontaneous mechanical activity of the rings. The overall effect produced by the

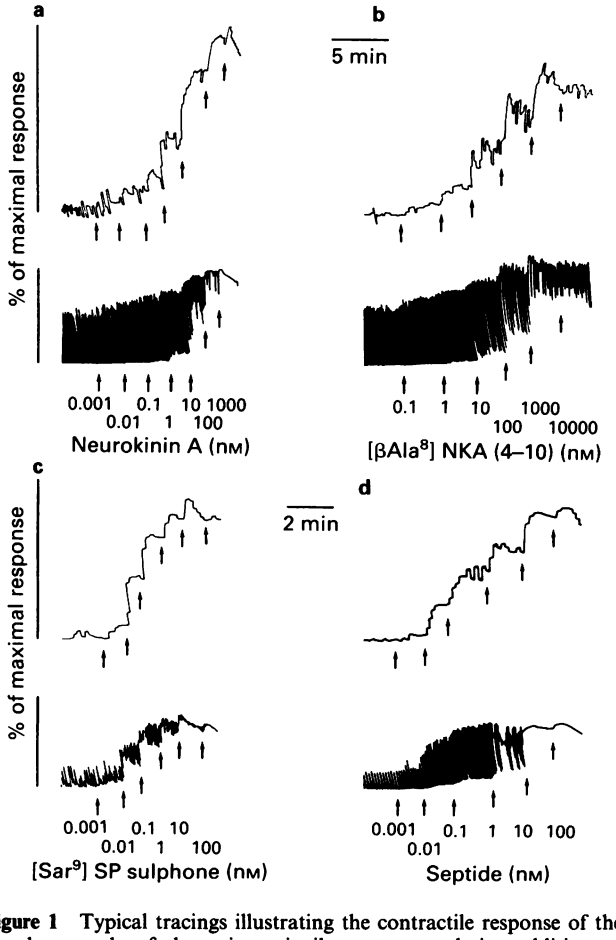

Figure 1 Typical tracings illustrating the contractile response of the circular muscle of the guinea-pig ileum to cumulative addition of neurokinin A (a), [βAla^8]NKA(4-10) (b), [Sar^9]SP sulphone (c) and septide (d). For each panel the lower tracing shows the mechanical activity recorded from isotonic transducers and the upper tracing the integrated mechanical activity.

various concentrations of agonists was calculated from the integrated mechanical activity.

Guinea-pig colon

Strips of mucosa-free circular muscle from the proximal colon were prepared as described in a previous study (Giuliani *et al.*, 1993). All experiments were performed in the presence of 10 μM indomethacin. The strips were suspended in 5 ml organ baths and connected to isotonic transducers (load 10 mN). After setup, the strips were allowed to equilibrate for 90 min with renewal of the bathing solution every 15 min. The strips were then exposed to 80 mM KCl at 15 min intervals until two reproducible responses were observed. This usually occurred at 120 min from setup. The response to 1 μM [βAla^8]NKA(4-10) was also determined in some experiments before addition of various tachykinin receptor agonists.

Experimental protocol

In both preparations, concentration-response curves to the agonists were constructed in a cumulative manner. The concentration of the agonist was increased by a factor of 10 for each successive dose of the cumulative curve, the next dose being added when the effect of the preceding one had reached its maximum. At the end of the concentration-response curve, the agonist was removed by repeated washing. Preliminary experiments showed that a second concentration-response curve to the various agonists produced at 20 min interval from the first one was fully reproducible in both ileum and colon. For each antagonist, contact time before application of the agonist was 15 min. In some experiments, performed with SR 48,968 in the ileum, contact time was 45 min (see results). In some experiments performed with the natural agonists, SP and NKA, a control curve was established first, then a mixture of peptidase inhibitors (bestatin, captopril and thiorphan 1 μM each) was added and a new curve obtained 15 min later.

Experiments with nifedipine

In a previous electrophysiological study (Zagorodnyuk *et al.*, 1993b) we showed that electrical and contractile responses to [Sar^9]SP sulphone and [βAla^8]NKA(4-10) in the circular muscle of guinea-pig proximal colon differ in a number of characteristics, including sensitivity to nifedipine. Owing to the possibility that septide stimulates a novel type of tachykinin receptor (Petitet *et al.*, 1992), we assessed the effect of nifedipine (1 μM) on the concentration-response curve to [Sar^9]SP sulphone, septide and [βAla^8]NKA(4-10). Responses are expressed as % of the maximal response produced by the agonist in the control curve. Contact time of nifedipine (45 min) was determined in preliminary experiments showing full blockade of the contractile response to KCl (80 mM).

Data evaluation and statistical analysis

The increase in integrated mechanical activity (ileum) or tone (colon) produced by the various agonists was expressed as % of the response to the internal standard, [βAla^8]NKA(4-10), for experiments aiming to compare the maximal effects (E_{max}) produced by different agonists. EC_{50} and 95% CL were calculated by the least square method. Dose-ratios were calculated and the Schild plots constructed for each agonist/antagonist pair tested. When the results of this analysis were consistent with competitive antagonism (slopes of Schild plot not significantly different from unity) pK_B values were calculated by the constrained Schild plot method; pK_B values are presented in Tables 2 and 3 with the corresponding 95% CL. In the ileum, SR 48,968 caused nonparallel rightward shifts of the concentration-response curves and decreased the E_{max} to [βAla^8]NKA(4-10). The method described by Kenakin (1987a) for noncompetitive and/or pseudoirreversible antagonist was used to evaluate the equilibrium dissociation constant (K_B) of SR 48,968. In practice, a double-reciprocal plot of equieffective concentrations of agonist (A) in the absence (1/A) and in the presence (1/A') of SR 48,968 (B) was constructed, and K_B derived from the equation: $K_B = [B]/\text{slope} - 1$ (Kenakin, 1987a). In order to obtain more accurate estimates of K_B we selected the experiments in which E_{max} to the agonist was depressed to 50% or less than 50% of control by SR 48,968. For the same reason, equieffective concentrations of [βAla^8]NKA(4-10) were selected from the upper region of the depressed dose-response curve, as suggested by Kenakin (1987a).

The statistical significance of differences in pK_B values obtained for a given antagonist toward different agonists was evaluated by comparing the Schild plot regression lines by means of analysis of covariance to detect differences in the elevation (position) and slopes of regression lines, as described by Kenakin (1987b).

Drugs

Drugs used were: bestatin, substance P, [Sar^9]SP sulphone and septide ([pGlu^6,Pro^9]SP(6-11)) (Peninsula), GR 82,334 ([D-Pro^9,(spiro-γ-lactam)Leu^{10},Trp^{11}]physalaemin(1-11)) (Hagan *et al.*, 1991), (Neosystem), amastatin, indomethacin and captopril (Sigma), thiorphan (Bachem), carbachol (Merck).

RP 67,580 {(3α**R**,7α**R**)-7, 7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)ethyl] perhydroisoindol-4-one} (Garret *et al.*, 1991) was a kind gift of Dr C. Garret, Rhone Poulenc, Vitry, France.

SR 48,968 ((S)-N-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl] benzamide) (Emonds-Alt *et al.*, 1992) or Dr X. Emonds-Alt, Sanofi Recherche, Montpellier, France. GR 94,800 or PhCO-Ala-Ala-D-Trp-Phe-D-Pro-Pro-NleNH$_2$ (McElroy *et al.*, 1992) was a kind gift from Dr R.M. Hagan, GGR, Ware England.

(±)-CP 96,345 or ((2S,3S) -*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo [2.2.2] octan-3-amine] (Snider *et al.*, 1991), neurokinin A, MEN 10,376 ([Tyr^5,D-Trp^{6,8,9},Lys^{10}]NKA(4-10), MEN 10,573 (cyclo(LeuΨ[CH$_2$NMe] Leu-Gln-Trp-Phe-Gly)), MDL 28,564 ((Leu$^9\Psi$(CH$_2$NH)Leu^{10}] NKA(4-10)), FK 888 ((2-(N-Me)indolil)-CO-Hyp-Nal-NMe-Bzl) and [βAla^8]NKA(4-10) were synthesized in the Chemistry Department of Menarini Pharmaceuticals.

Results

Circular muscle of the ileum

Effect of agonists In the presence of 10 μM indomethacin, longitudinal muscle-myenteric plexus-free ileal rings developed regular spontaneous phasic contractions (4–10 min^{-1}), their amplitude ranging between 20–60% of the maximal response to 10 μM carbachol. SP and NKA produced a concentration-dependent contraction (Figure 1 and 2, Table 1), SP being about 10 times more potent than NKA. The action of SP and NKA was not modified by bestatin, captopril and thiorphan (1 μM each). In fact, the EC_{50}s of SP were 100 pM (63–174 pM are 95% CL) and 99 pM (48–263 pM) in the absence and presence of peptidase inhibitors, respectively ($n = 6$ in each group). The corresponding values for NKA were 1.16 nM (0.85–1.38 nM) and 0.83 nM (0.70–1.11 nM), respectively ($n = 6$). When SP or NKA (both 1 μM) were added to the bath as a single concentration, E_{max} was not different from that produced by [βAla^8]NKA(4-10), inducing a total closure of the ileal lumen. The E_{max} to NKA or [βAla^8]NKA(4-10) during the cumulative concentration-response curve equalled that observed in response to single administration of 1 μM concentration of each agonist. The E_{max} to SP during the cumulative concentration-response curve was slightly less than that produced by [βAla^8]NKA(4-

Table 1 EC_{50}s (95% confidence limits in brackets) and E_{max} (expressed as % of the maximal response to [βAla^8]NKA(4-10), 1 μM) of natural tachykinins and receptor-selective synthetic agonists-induced contraction in the circular muscle of the guinea-pig ileum and colon

Agonist	*Ileum*		*Colon*	
	EC_{50}	E_{max}	EC_{50}	E_{max}
Substance P	100 pM (63–174)	69 ± 4*	5 nM (4–6)	73 ± 4*
Neurokinin A	1.2 nM (1.1–1.4)	90 ± 5	19 nM (10–55)	96 ± 4
[Sar^9]SP sulphone	83 pM (54–139)	60 ± 4*	8 nM (4–15)	81 ± 4*
Septide	36 pM (20–86)	68 ± 6*	0.7 nM (0.3–1.2)	88 ± 3
[βAla^8]NKA(4-10)	10 nM (6–24)	100	12 nM (6–28)	100
MDL 28,564	474 nM (400–650)	86 ± 4	55 nM (35–72)	97 ± 2

Each value is from 6–12 experiments. *Significantly different ($P<0.05$) from E_{max} of [βAla^8]NKA(4-10)

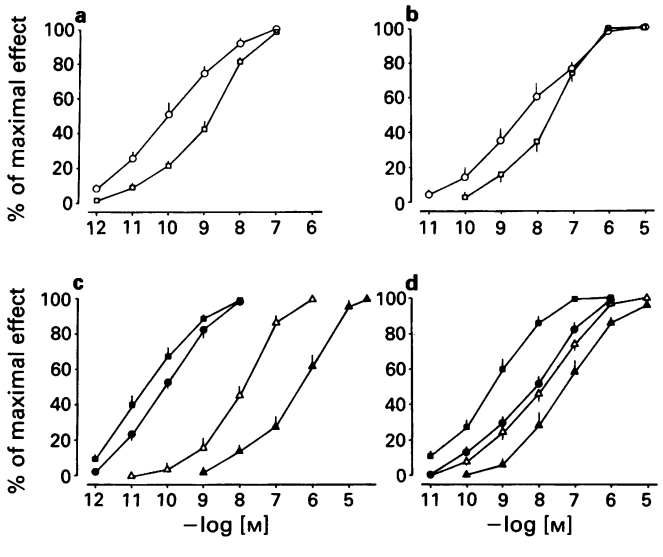

Figure 2 Concentration-dependent contraction of the circular muscle of the guinea-pig ileum (left a, c) and colon (b, d) to natural tachykinins, SP (○) and NKA (□) and to synthetic tachykinin receptor agonists septide (■), [Sar^9]SP sulphone (●), [βAla^8]NKA(4-10) (△) and MDL 28,564 (▲). The contractile response was expressed as % of the maximal response produced by each agonist. Each value is mean ± s.e.mean of 6–12 experiments.

10) or NKA (Table 1). The synthetic agonists [Sar^9]SP sulphone, septide and [βAla^8]NKA(4-10) all produced concentration-dependent contractions of ileal rings. Septide and [Sar^9]SP sulphone were distinctly (about 2 orders of magnitude) more potent than [βAla^8]NKA(4-10) (Figure 1 and 2, Table 1). The E_{max} produced by septide or [βAla^9]SP sulphone during the cumulative concentration-response curve was significantly less than that produced by [βAla^8]NKA(4-10) and was similar to the E_{max} produced by SP (Table 1). The pseudopeptide derivative of NKA (4-10), MDL 28,564 was less potent than NKA and [βAla^8]NKA(4-10) (400 and 47 times, respectively), but its E_{max} was not significantly different from that produced by NKA or [βAla^8]NKA(4-10) (Table 1, Figure 2). The response to tachykinin receptor agonists developed quite rapidly in the circular muscle of the ileum (Figure 1). For SP and [Sar^9]SP sulphone the maximal response produced by each dose developed within 30–45 s from agonist application; for [βAla^8]NKA(4-10), MDL 28,564 and NKA within 60–120 s, the time course of the response to septide was intermediate and the effect of each dose required 45–90 s to develop maximal effects. In the presence of antagonists (see below), a slower time course of the response to the agonists was observed.

Effect of NK_1 receptor antagonists

(±)-CP 96,345, FK 888, GR 82,334 or RP 67,580 displayed no significant agonist activity, and did not affect the response to [βAla^8]NKA(4-10) ($n=4$ for each antagonist, Table 2). (±)-CP 96,345 (1–300 nmM, Figure 3), GR 82,334 (30 nM–10 μM) and FK 888 (30 nM–30 μM) produced concentration-dependent rightward shifts of the concentration-response curve to both [Sar^9]SP sulphone and septide with slopes of Schild plots not significantly different from unity (Figures 4 and 5, Table 2). The affinity of (±)-CP 96,345, GR 82,334 and FK 888 toward septide-induced response was greater (Table 2) than that toward [Sar^9]SP sulphone. At 1 μM, RP 67,580 was inactive toward septide and [Sar^9]SP sulphone (Table 2). At 3 μM RP 67,580 produced a significant depression of the maximal response to the two agonists (40–50% reduction, $n=4$ for each agonist).

Effect of NK_2 receptor antagonists

SR 48,968, GR 94,800, MEN 10,376 or MEN 10,573 displayed no significant agonist activity. MEN 10,376 (0.3–10 μM), MEN 10,573 (0.3–10 μM) and GR 94,800 (3–30 nM, Figure 6) produced concentration-dependent rightward shifts of the curve to [βAla^8]NKA(4-10) with slopes of Schild plots not significantly different from unity (Figure 7, Table 2). The affinity of MEN 10,376 for NK_2 receptors in the circular muscle of the ileum was about 1 log unit lower than that measured in other guinea-pig smooth muscle preparations bearing NK_2 receptors (see Discussion). To assess whether this may involve breakdown of MEN 10,376 by peptidases and generation of shorter fragments with lower affinity for NK_2 receptors (Quartara *et al.*, 1992b), the effect of MEN 10,376 toward [βAla^8]NKA(4-10) was investigated in the presence of captopril, bestatin, thiorphan (1 μM each, 15 min beforehand) and amastatin (10 μM, 45 min beforehand): the pK_B determined in these experiments (6.59 ± 0.14, $n=4$) was not significantly different from that measured in the absence of peptidase inhibitors. GR 94,800 was ineffective (up to 1 μM) against septide or [Sar^9]SP sulphone ($n=4$ for each agonist), while MEN 10,376 and MEN 10,573 competitively antagonized the response to septide; MEN 10,573 also antagonized competitively the response to [Sar^9]SP sulphone

Table 2 Effect of various tachykinin receptor antagonists on contractions produced by [Sar^9]SP sulphone, septide or [βAla^8] NKA(4-10) in the circular muscle of the guinea-pig ileum

Antagonist	*[Sar^9]SP sulphone*	*Septide*	*[βAla^8]NKA(4-10)*
(±)-CP 96,345			
slope	−1.03 (0.59–1.46)	−0.87 (0.63–1.10)	inactive up to 1 μM
pK_B	8.17 (7.95–8.38)	9.24 (9.07–9.41)*	
GR 82,334			
slope	−1.23 (0.91–1.54)	−1.15 (0.76–1.54)	inactive up to 10 μM
pK_B	7.17 (6.97–7.37)	7.52 (7.30–7.74)*	
FK 888			
slope	−0.95 (0.75–1.15)	−1.21 (0.82–1.51)	inactive up to 3 μM
pK_B	7.53 (7.39–7.67)	8.30 (8.08–8.52)*	
RP 67,580	inactive up to 1 μM	inactive up to 1 μM	inactive up to 1 μM
MEN 10,376			
slope	inactive up to 10 μM	−0.93 (0.66–1.19)	−0.90 (0.72–1.09)
pK_B		6.40 (6.29–6.51)	6.44 (6.33–6.55)
MEN 10,573			
slope	−0.97(0.55–1.38)	−1.24(0.91–1.57)	−1.14 (0.75–1.53)
pK_B	5.95 (5.75–6.15)	6.48(6.31–6.64)*	7.18 (7.01–7.35)**
GR 94,800			
slope	inactive up to 1 μM	inactive up to 1 μM	−0.91 (0.63–1.20)
pK_B			8.85 (8.72–8.98)

For each agonist/antagonist combination slopes of Schild plot and pK_B values (with 95% CL) are shown. Schild plots were constructed with at least 3 different concentrations of the test antagonist:each concentration was tested in at least 3 experiments on preparations from different animals. For slopes of Schild plot not significantly different from unity, pK_B values were calculated using the constrained plot method. Statistical significance of differences in pK_B was evaluated by analysis of covariance to detect differences in the position of Schild regression lines and differences in slope. *Position of Schild regression line significantly different ($P<0.05$) from that measured against [Sar^9]SP sulphone. Slopes of Schild plots not significantly different from each other. **Position of Schild regression line significantly different ($P<0.05$) from that measured against septide. Slopes of Schild plots not significantly different from each other.

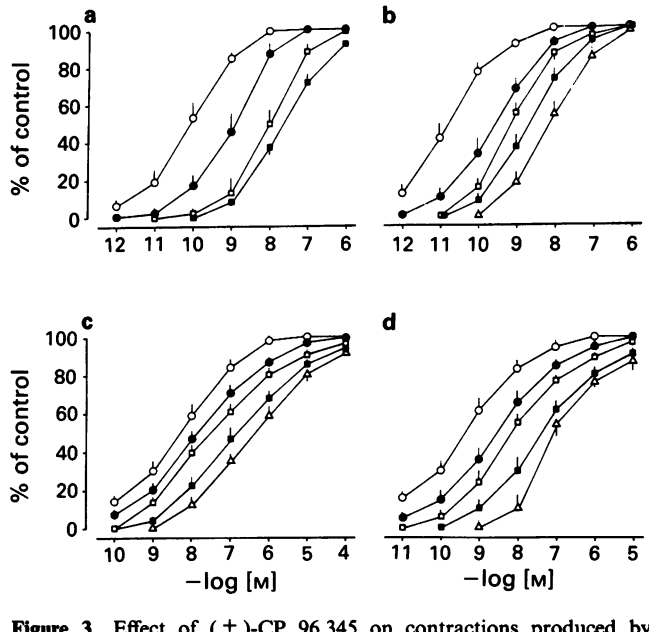

Figure 3 Effect of (±)-CP 96,345 on contractions produced by [Sar^9]SP sulphone in the circular muscle of the guinea-pig ileum (a) and colon (c) and toward septide-induced contraction in the circular muscle of the guinea-pig ileum (b) and colon (d). In each panel (○) represents the control curve. Concentrations of (±)-CP 96,345 tested were: 30 (●) 300 (□) and 500 nM (■) toward [Sar^9]SP sulphone in the ileum; 10 (●), 30 (□), 100 (■) and 300 nM (△) toward [Sar^9]SP sulphone in the colon; 10 (●), 30 (□), 100 (■) and 300 nM (△) toward septide in ileum; 10 (●), 30 (□), 100 (■) and 300 nM (△) toward septide in the colon. Each value is mean ± s.e.mean of at least 3 determinations.

(Figures 4 and 5), while MEN 10,376 (10 μM, $n=4$) was ineffective (Table 2). As can be seen from Table 2, MEN 10,376 was equipotent against [βAla^8]NKA(4-10) and septide (pK_B about 6.4 toward both agonists). Thus MEN 10,376 is at least 30 times more potent toward septide than [Sar^9]SP sulphone (Table 2). MEN 10,573 was slightly but significantly more potent towards [βAla^8]NKA(4-10) than towards septide and significantly more potent towards septide than towards [Sar^9]SP sulphone (Table 2).

SR 48,968 (10 nM–3 μM, $n=28$, Figure 6), displayed a more complex pattern of antagonism: it produced both non-parallel rightward shifts of the curve to [βAla^8]NKA(4-10) and depression of E_{max}, both effects being concentration-dependent (Figure 6). Schild plot analysis revealed the non-competitive nature of antagonism with a slope of −0.72 (0.48–0.90). To assess whether the depression of E_{max} to [βAla^8]NKA(4-10) by SR 48,968 could be reversed, ileal rings were exposed to 3 μM [βAla^8]NKA(4-10) at 30 min intervals until reproducible responses were obtained (Figure 8). SR 48,968 (3 μM for 30 min) reduced the response to the agonist to 42 ± 6% of controls ($n=4$, $P<0.05$): as shown in Figure 8, a slow, time-dependent, recovery of inhibition by SR 48,968 was observed up to 87 ± 8% of control at 150 min. Having determined that the depression of E_{max} to [βAla^8] NKA(4-10) by SR 48,968 is not a consequence of irreversible interaction with the receptors, the pK_B of SR 48,968 was estimated by the double reciprocal plot method described by Kenakin (1987a). For this analysis the experiments obtained with 1 and 3 μM SR 48,968 were used (Figures 6 and 9): the corresponding pK_B value was 7.83 (7.15–8.51, $n=7$). Owing to the lower potency of SR 48,968 in antagonizing responses to [βAla^8]NKA(4-10) in the ileum vs. colon (see below), the question was raised as to whether a 15 min contact time was sufficient for this antagonist to reach equilibrium with NK_2 receptors in the guinea-pig ileum. To check this point, the contact time of the antagonist was extended to 45 min. For these experiments a concentration of 1 μM SR 48,968 was selected which, after 15 min contact time, produced both a rightward shift of the curve to the agonist (dose-ratio 30 ± 6, $n=9$) and a depression of E_{max} (76 ± 4% of control response). The corresponding values measured after 45 min contact time (dose ratio 32 ± 11, $E_{max}=80\pm5$% of control, $n=4$) were not significantly different from those obtained at 15 min, i.e. no evidence was found for time-dependency of antagonist action. SR 48,968, up to 0.1 μM, was ineffective

toward [Sar^9]SP sulphone or septide (Table 2). At 1 μM the E_{max} to these two agonists was reduced by 25 and 32%, respectively ($n = 5$ in each case). This effect was not due to nonspecific depression of contractility because the concentration-response curve to carbachol (10 nM–10 μM, EC_{50} 164 nM, 100–352 nM) was unaffected by 15 min contact time with 1 μM SR 48,968 (EC_{50} 199 nM, 86–411 nM, $n = 4$).

Circular muscle of the colon

Effect of agonists In the presence of 10 μM indomethacin, muscle strips of guinea-pig proximal colon developed a low amplitude (<20% of maximal response to KCl) irregular phasic activity. SP and NKA produced concentration-dependent contractions of the strips (Figure 2), SP being about four times more potent than NKA (Table 1). The action of SP and NKA was not significantly modified in the presence of peptidase inhibitors. The EC_{50}s of SP were 5 nM (4–6 nM) and 6 nM (4–9 nM) in the absence and presence of peptidase inhibitors, respectively ($n = 6$ in each group). The corresponding values for NKA were 19 nM (10–55 nM) and 15 nM (9–27 nM), respectively ($n = 6$). When 1 μM SP or NKA was added to the bath as a single concentration, the E_{max} was not different from that produced by 1 μM [βAla^8]NKA(4-10). The E_{max} produced by NKA or [βAla^8]NKA(4-10) during the cumulative concentration-response curve equalled that observed in response to a single administration of 1 μM concentration of each agonist. The E_{max} produced by SP during the cumulative concentration-response curve was slightly less than that produced by [βAla^8]NKA(4-10) or NKA (Table 1). The synthetic agonists [Sar^9]SP sulphone, septide and [βAla^8]NKA(4-10) all produced concentration-dependent contractions of the strips (Figure 2). The E_{max} to [Sar^9]SP sulphone, but not that to septide, was slightly less than that produced by [βAla^8]NKA(4-10) (Table 1). Septide was the most potent

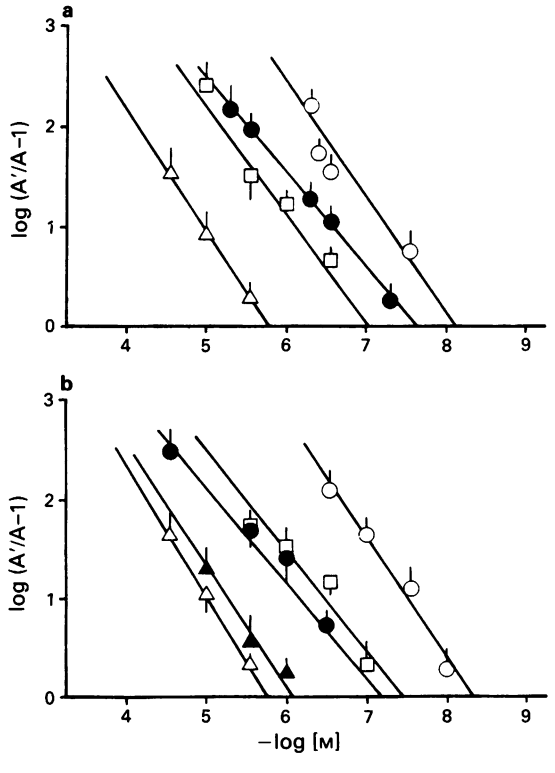

Figure 4 Schild plots for antagonism of contraction produced by [Sar^9]SP sulphone in the circular muscle of the ileum (a) and colon (b) by (±)-CP 96,345 (○), FK 888 (●), GR 82,334 (□), MEN 10.573 (△) and RP 67,580 (▲). Each value is mean ± s.e.mean of at least three determinations. Slopes of Schild plots are given in Tables 1 and 2.

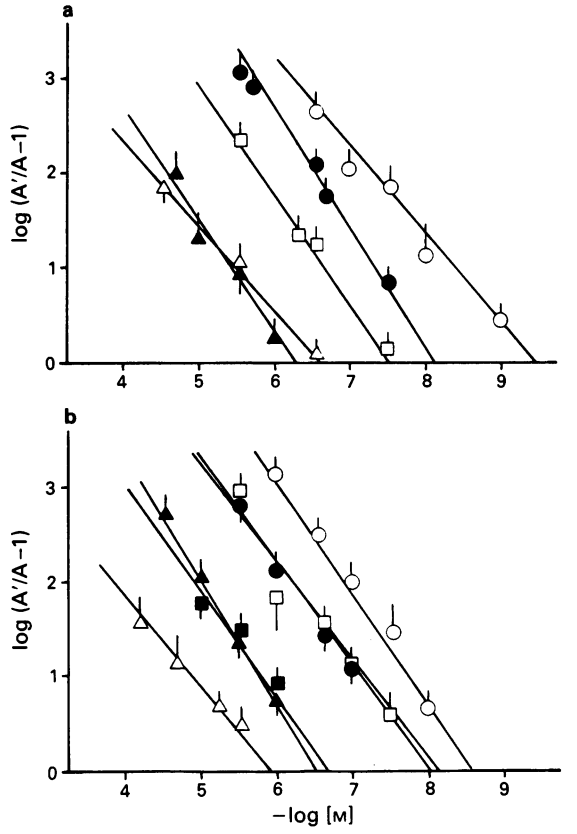

Figure 5 Schild plots for antagonism of contraction produced by septide in the circular muscle of the ileum (a) and colon (b) by (±)-CP 96,345 (○), FK 888 (●), GR 82,334 (□), RP 67,580 (■), MEN 10,376 (△) and MEN 10,573 (▲). Each value is mean ± s.e. mean of at least three determinations. Slopes of Schild plots are given in Tables 1 and 2.

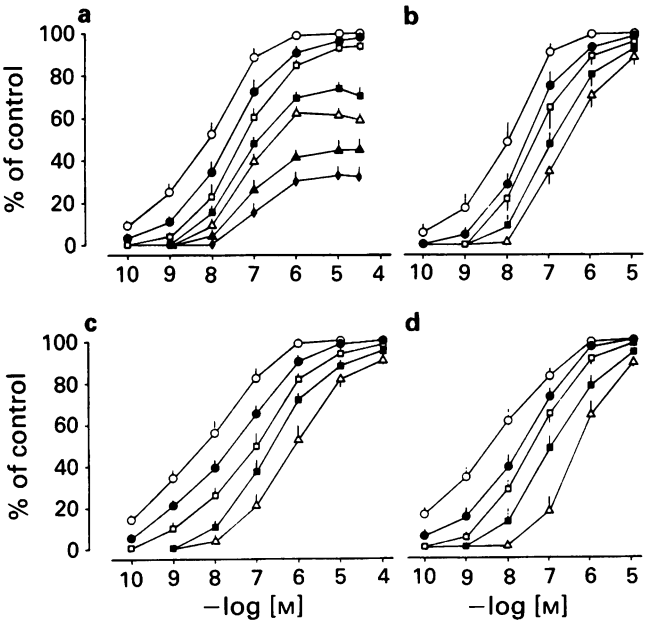

Figure 6 (a, c) Effect of SR 48,968 on the concentration-response curve to [βAla^8]NKA(4-10) in the circular muscle of the guinea-pig ileum (a) and colon (c). (b, d) Effect of GR 94,800 on the concentration-response curve to [βAla^8]NKA(4-10) in the circular muscle of the guinea-pig ileum (b) and colon (d). In each panel (○) represents the control curve obtained in the absence of the antagonist. Concentrations of antagonists tested were: 10 (●), 30 (□), 100 (■), 300 (△), 1000 (▲) and 3000 (◆) for SR 48,968 in the ileum; 3 (●), 10 (□), 30 (■), 100 nM (△) for SR 48,968 in the colon; 3 (●), 5 (□), 10 (■), and 30 nM (△) for GR 94,800 in the ileum; 1 (●), 3 (□), 10 (■) and 30 nM (△) for GR 94,800 in the colon. Each value is mean ± s.e.mean of at least 3 determinations.

agonist tested, being about seven times more potent than SP; [Sar^9]SP sulphone and [βAla^8]NKA(4-10) were equipotent to SP and NKA, respectively (Table 1). The pseudopeptide derivative of NKA (4-10), MDL 28,564 was about two times less potent than NKA and about four times less potent than [βAla^8]NKA(4-10) (Figure 2, Table 1). Its E_{max} was not significantly different from that produced by NKA or [βAla^8]NKA(4-10) (Table 1). As observed in the ileum, the time course of the contractile response produced by SP or [Sar^9[SP sulphone (45–120 s for maximum effect of each concentration) was faster than that of NKA, [βAla^8]NKA(4-10) or MDL 28,564 (90–300 s), while the time course of the response to septide was intermediate (90–180 s). In the presence of antagonists (see below), a slower time course of the response to the agonists was observed.

Effect of NK_1 receptor antagonists (±)-CP 96,345, FK 888, GR 82,334 or RP 67,580 displayed no agonist activity, nor did they affect the concentration-response curve to [βAla^8]NKA (4-10) (Table 3, $n = 4$ for each antagonist). (±)-CP 96,345 (10 nM–1 μM, Figure 3), FK 888 (0.1–30 μM), GR 82,334 (0.1–3 μM) or RP 67,580 (1–10 μM) produced concentration-dependent rightward shifts of the curve to septide and [Sar^9]SP sulphone with slopes of Schild plot significantly different from unity (Table 3, Figures 4 and 5). As observed in the ileum, the affinity of NK_1 receptor antagonists was significantly higher toward septide than toward [Sar^9]SP sulphone, the only exception being CP 96,345, for which the difference was not statistically significant (Table 3).

Effect of NK_2 receptor antagonists SR 48,968, GR 94,800, MEN 10,376 or MEN 10,573 displayed no agonist activity. SR 48,968 (3–100 nM), MEN 10,376 (0.3–10 μM), MEN 10,573 (0.3–10 μM) and GR 94,800 (1–100 nM) produced concentration-dependent rightward shifts of the curve to [βAla^8]NKA(4-10): slopes of Schild plots indicated competitive antagonism (Table 3, Figures 6 and 7). SR 48,968 (up to 3 μM) and GR 94,800 (up to 1 μM) were ineffective against [Sar^9]SP sulphone or septide (Table 3). MEN 10,376 and MEN 10,573 competitively antagonized the response to septide. MEN 10,573, but not MEN 10,376 (10 μM, $n = 4$) also antagonized the response to [Sar^9]SP sulphone (Table 3). MEN 10,376 was about ten times more potent against [βAla^8]

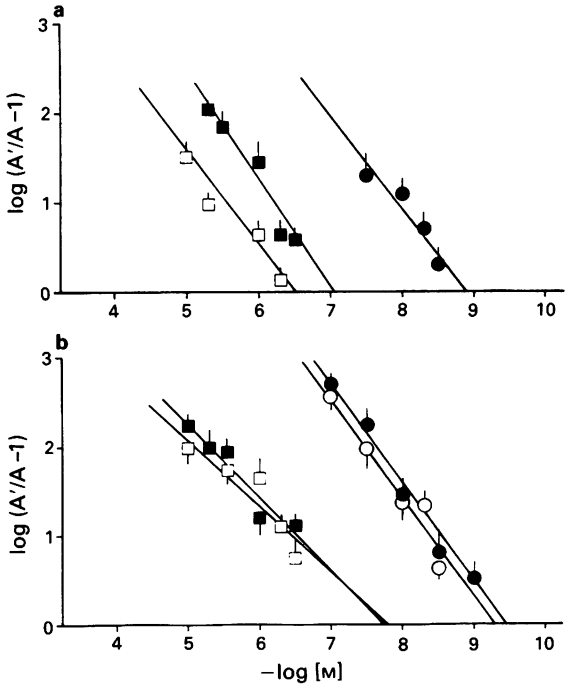

Figure 7 Schild plots for antagonism of contraction produced by [βAla^8]NKA(4-10) in the circular muscle of the ileum (a) and colon (b) by SR 48,968 (○), GR 94,800 (●) MEN 10,376 (□) and MEN 10,573 (■). Each value is mean ± s.e.mean of at least three determinations. Slopes of Schild plots are given in Tables 2 and 3,

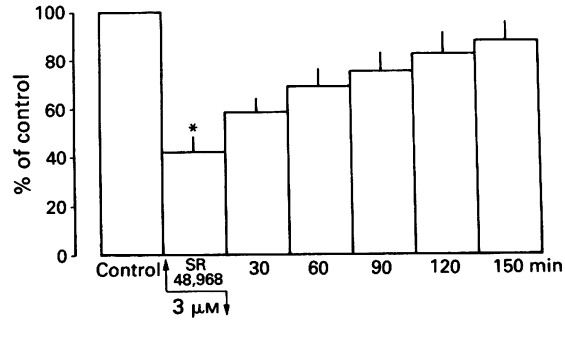

Figure 8 Reversal, by washing, of the depressant effect of SR 48,968 on the maximal response to [βAla^8]NKA(4-10) in the circular muscle of the ileum. Each value is mean ± s.e.mean of 4 determinations. *Significantly different from control, $P < 0.05$.

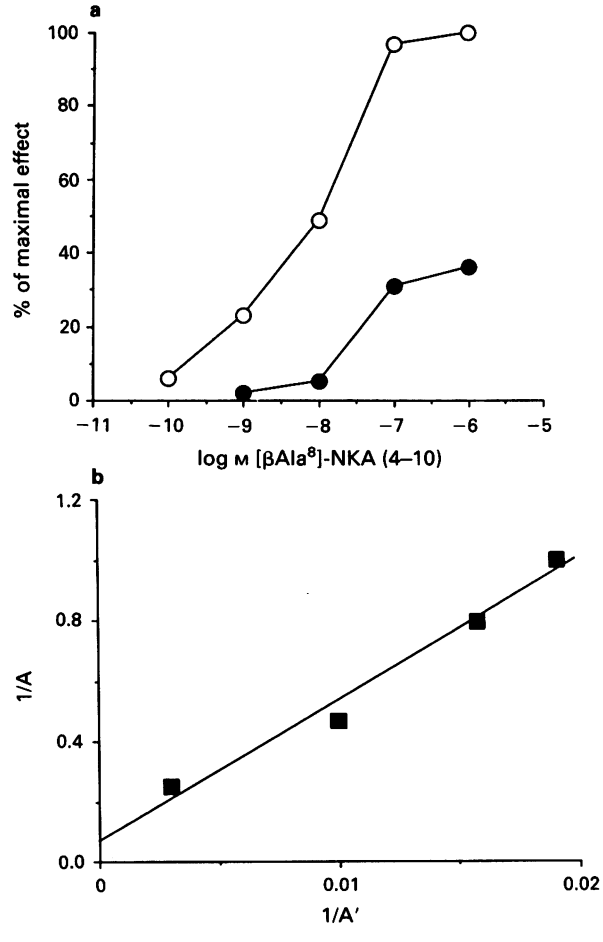

Figure 9 (a) Example of concentration-response curve to [βAla^8]NKA(4-10) in the absence (○) and presence (●) of SR 48,968 (1 μM) in the guinea-pig ileum circular muscle. (b) Double-reciprocal plot of equiactive concentrations of [βAla^8]NKA(4-10) from dose-response curve shown in (a) in the absence (1/A) and in the presence (1/A') of SR 48,968 (1 μM) in the guinea-pig ileum circular muscle. Pairs of equieffective concentrations of [βAla^8[NKA(4-10) have been chosen producing 35, 31, 25 and 23% of maximal effect. Slope of regression line is 46.5. pK_B value for SR 48,968 in the experiment reported, determined as − log ([SR 48,968] slope − 1), is 7.67.

Table 3 Effect of various tachykinin receptor antagonists on contractions produced by [Sar^9]SP sulphone, septide or [βAla^8] NKA(4-10) in the circular muscle of the guinea-pig colon

Antagonist	*[Sar^9]SP sulphone*	*Septide*	*[βAla^8]NKA(4-10)*
(±)-CP 96,345			
slope	−1.16 (0.78–1.53)	−0.95 (0.69–1.22)	inactive up to 1 μM
pK_B	8.41 (8.20–8.62)	8.89 (8.64–9.13)	
GR 82,334			
slope	−0.96 (0.58–1.34)	−1.04 (0.80–1.29)	inactive up to 10 μM
pK_B	7.49 (7.29–7.69)	8.12 (7.94–8.32)*	
FK 888			
slope	−0.98 (0.64–1.32)	−1.08 (0.90–1.26)	inactive up to 1 μM
pK_B	7.13 (6.95–7.31)	8.13 (8.03–8.22)*	
RP 67,580			
slope	−1.25 (0.95–1.52)	−1.18 (0.67–1.69)	inactive up to 1 μM
pK_B	6.25 (6.10–6.40)	6.82 (6.60–7.04)*	
SR 48,968			
slope	inactive up to 3 μM	inactive up to 3 μM	−1.15 (0.65–1.64)
pK_B			9.41 (9.24–9.58)
MEN 10,376			
slope	inactive up to 10 μM	−1.04 (0.77–1.31)	−0.86 (0.57–1.14)
pK_B		5.85 (5.70–6.00)	7.27 (7.10–7.44)**
MEN 10,573			
slope	−1.31 (0.85–1.76)	−1.12 (0.79–1.45)	−0.80 (0.38–1.22)
pK_B	5.98 (5.84–6.11)	6.72 (6.55–6.89)*	7.28 (7.11–7.45)**
GR 94,800			
slope	inactive up to 1 μM	inactive up to 1 μM	−1.06 (0.88–1.24)
pK_B			9.49 (9.28–9.70)

For each agonist/antagonist combination slopes of Schild plot and pK_B values (with 95% CL) are shown. Schild plots were constructed with at least 3 different concentrations of the test antagonist:each concentration was tested in at least 3 experiments on preparations from different animals. For slopes of Schild plot not significantly different from unity, pK_B values were calculated using the constrained plot method. Statistical significance of differences of pK_B was evaluated by analysis of covariance to detect differences in the position of Schild regression lines and differences in slope. *Position of Schild regression line significantly different ($P<0.05$) from that measured against [Sar^9]SP sulphone. Slopes of Schild plots not significantly different from each other. **Position of Schild regression line significantly different ($P<0.05$) from that measured against septide. Slopes of Schild plots not significantly different from each other.

NKA(4-10) than toward septide (Table 3). MEN 10,573 was slightly but significantly more potent towards [βAla^8]NKA(4-10) than towards septide, and significantly more potent towards septide than towards [Sar^9]SP sulphone (Table 3).

Effect of nifedipine on the response to [Sar^9]SP sulphone, septide and [βAla^8]NKA(4-10) in the ileum and colon

Nifedipine (1 μM) abolished spontaneous activity of the strips in both the ileum and colon. In the ileum, the response to septide, [Sar^9]SP sulphone or [βAla^8]NKA(4-10) (up to 1 μM, for each agonist) was totally abolished by nifedipine ($n=4$ for each agonist). In the colon, the concentration-response curves to [Sar^9]SP sulphone and septide were markedly depressed and shifted to the right by nifedipine (Figure 10). In the presence of nifedipine, the E_{max} to [Sar^9]SP sulphone and septide (30 μM in each case) averaged 21 ± 4 and 27 ± 3% of controls, respectively ($n=7$) (Figure 10). On the contrary, the response to [βAla^8]NKA(4-10) was only depressed but not shifted to the right by nifedipine (Figure 10, $n=5$): in the presence of nifedipine, the E_{max} to [βAla^8] NKA(4-10) averaged 80 ± 4% of control (Figure 10). Qualitatively, the contractile response produced by the three tachykinin receptor agonists became slower in the presence of nifedipine, each concentration requiring at least 3–5 min to produce its maximal effect.

Discussion

The aim of this study was to perform a systematic analysis of the affinities of various peptide and nonpeptide tachykinin antagonists at NK_1 and NK_2 receptors in the guinea-pig ileum and colon. Since pharmacological evidence has been presented to indicate intraspecies heterogeneity of NK_1 and NK_2 receptors (see Introduction), a major aim of the study was to unravel possible differences in receptor antagonist potencies in preparations from the same species. Tachykinin NK_3 receptors are also present in the circular muscle of the ileum and their stimulation produces an indirect contractile response (Maggi *et al.*, 1990a). No information is available about the possible presence of NK_3 receptors in the guinea-pig colon, although in preliminary experiments we found senktide effective in producing concentration-dependent (threshold concentration 1 nM) contractions of this preparation (Maggi, unpublished observations). Since high concentrations of SP and NKA are capable of stimulating NK_3 receptors with similar efficiency to neurokinin B, the value of natural tachykinins for characterizing NK_1 and NK_2 receptors in the intestine, and detect possible intraspecies receptor heterogeneity is limited. For this reason, the receptor-selective agonist, [Sar^9]SP sulphone, septide and [βAla^8]NKA(4-10) were used for studying the effect of antagonist. The use of these agonists relies on the assumption, supported by previous literature data, of negligible, if any, affinity for NK_3 receptors (Lee *et al.*, 1986; Wormser *et al.*, 1986; Dion *et al.*, 1987; Laufer *et al.*, 1988; Rovero *et al.*, 1989).

NK_1 receptors in the circular muscle of the ileum and colon

The results of this study indicate that: (i) various NK_1 receptor-selective antagonists are significantly more potent against septide than [Sar^9]SP sulphone; (ii) MEN 10,376, previously characterized as a selective NK_2 receptor antagonist, displays a sizeable affinity toward the septide-induced

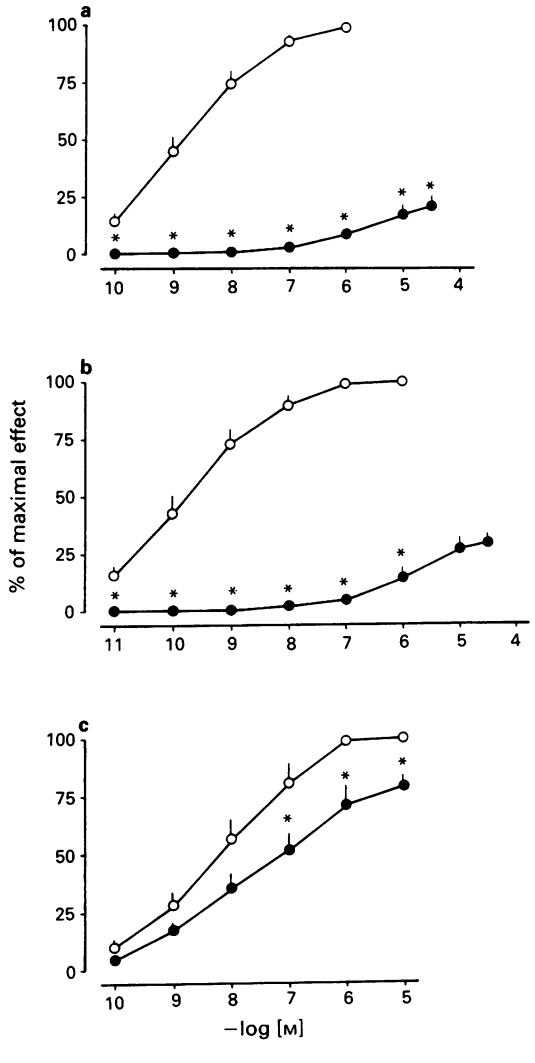

Figure 10 Effect of nifedipine (1 μM, ●) on the concentration-response curve to [Sar^9]SP sulphone (a), septide (b) and [βAla^8]NKA(4-10) (c) in the circular muscle of the guinea-pig colon. Each value is mean ± s.e.mean of 5–7 determinations. *Significantly different from control, $P<0.05$.

contraction; (iii) in both preparations (±)-CP 96,345 was markedly more potent than RP 67,580, in keeping with the known species-related pattern of NK_1 receptor antagonist affinity.

Petitet *et al.* (1992) raised the issue of the possible existence of a novel, 'septide-sensitive', tachykinin receptor to account for the marked discrepancy between the high potency of septide in stimulating NK_1 receptors in the longitudinal muscle of the guinea-pig ileum and its relative low potency in displacing SP from NK_1 receptors. The observation that the responses to septide in the guinea-pig ileum and rat urinary bladder are blocked by NK_1 receptor antagonists with higher potency than responses produced by SP or 'classical' NK_1 receptor agonists (i.e. agonists for which a high binding affinity parallels the high biological activity) (Petitet *et al.*, 1992; Meini *et al.*, 1994; Maggi *et al.*, 1993a,b,c; and present findings), raises the possibility that the 'septide-sensitive' receptor is an NK_1 receptor subtype.

The present findings demonstrate that various peptide and nonpeptide NK_1 receptor antagonists (with the exception of CP 96,345 in the colon) are significantly more potent in antagonizing the response to septide than that to [Sar^9]SP sulphone. The observed differences in pK_B values range between 0.5–1 log unit, and notably on no occasion did we find an NK_1 receptor antagonist to be more potent toward [Sar^9]SP sulphone than toward septide. Although this latter finding may be incidental, it calls for a word of caution against the existence of a 'septide-sensitive' receptor as a distinct entity from the classical NK_1 receptor.

Further discrimination between the responses to septide and [Sar^9]SP sulphone was seen with MEN 10,376, a linear NKA(4-10) derivative possessing high affinity for NK_2 receptors expressed in the human, bovine, guinea-pig and rabbit species (Maggi *et al.*, 1993a). We reported previously that the potency of MEN 10,376 in antagonizing septide-induced contraction in the guinea-pig isolated bronchus is somewhat intermediate between that displayed toward the NK_2 receptor-selective agonist, [βAla^8]NKA(4-10), and the NK_1-receptor-selective agonist, SP methylester (Maggi *et al.*, 1991b). In the circular muscle of the guinea-pig ileum, the affinity of MEN 10,376 for NK_2 receptors is lower than that determined in other guinea-pig smooth muscle preparations (for e.g. in the circular muscle of the colon); in the ileum, MEN 10,376 was equipotent toward [βAla^8]NKA(4-10) (pK_B 6.44) and septide (pK_B 6.40). On the other hand, MEN 10,376 is at least 30 times less potent ($pK_B<5$) toward [Sar^9]SP sulphone. Therefore, in absolute values, MEN 10,376 is the antagonist which better discriminates between [Sar^9]SP sulphone- and septide-induced contraction in the circular muscle of the guinea-pig ileum, although its affinity for the septide-stimulated receptor is about three orders of magnitude lower than that of (±)-CP 96,345.

Assuming that a 'septide-sensitive' NK_1 receptor exists, the rank order of potency of natural tachykinins at this receptor is an obvious question of physiological relevance. Our previous observations (Meini *et al.*, 1994) suggest that neurokinin B could be a better ligand than SP at the 'septide-sensitive' receptor in the rat urinary bladder. Owing to the presence of NK_3 receptors in the gut and the lack of a suitable NK_3 receptor antagonist, functional experiments with neurokinin B at this level would not be informative.

The present findings are compatible with the existence of pharmacologically distinct NK_1 receptor subtypes in guinea-pig. On the other hand, other possibilities could be considered to account for these results: the possibility that physicochemical properties of septide determine its unusual pharmacological properties is unconvincing, because other NK_1 receptor agonists, including the undecapeptides [Apa^{9,10}]SP and [Pro^{9,10}]SP show a 'septide-like' profile of action in the guinea-pig ileum (Petitet *et al.*, 1992). Since the recognition epitopes for certain NK_1 receptor antagonists by the NK_1 receptor protein appear to be distinct from the agonist binding site(s) (Gether *et al.*, 1993), the possibility of an allosteric modulation between agonist and anagonist binding sites should be taken into serious consideration for the NK_1 receptor. It may be that the recognition epitopes of the NK_1 receptor protein for SP and classical NK_1 receptor agonist do not overlap with the epitopes recognizing the 'septide-like' agonists, whereby septide and other agonists with a similar profile are more easily displaced by antagonists. This interpretation may explain why several NK_1 antagonists were in general more potent in blocking septide than 'classical' NK_1 receptor agonists while no example of the converse pattern has been reported yet. On the other hand, the available data from mutation experiments indicate that septide and SP recognize similar epitopes of the NK_1 receptor protein (Fong *et al.*, 1992a). However, a systematic study of the effects of mutations of different regions of the NK_1 receptor protein on the binding of septide vs. SP is not available. It is to be noted, finally, that in cell systems expressing the cloned, full length, NK_1 receptor protein, septide is distinctly less potent than SP in both binding and functional assays (Fong *et al.*, 1992a see also Hermans *et al.*, 1993), in sharp contrast with the high potency of this hexapeptide in various bioassays (e.g. Laufer *et al.*, 1988; Hall & Morton, 1991; and present data).

The possibility that a 'septide-sensitive' NK_1 receptor sub-

type exists as a distinct receptor entity remains a matter for investigation, which cannot be negated or supported from the presently available data on the molecular biology of the NK_1 receptor. Although only one gene encoding the NK_1 receptor has been isolated thus far (Gerard *et al.*, 1993), the possibility exists that different forms of the receptor protein are generated from the same gene (Fong *et al.*, 1992b; Kage *et al.*, 1993).

NK_2 receptors in the circular muscle of the ileum and colon

The present findings substantiate the conclusion that NK_2 receptors mediate contraction in the circular muscle of the guinea-pig ileum and colon (Maggi *et al.*, 1990a; Giuliani *et al.*, 1993). In fact, various NK_1 antagonists such as (±)-CP 96,345, FK 888 and GR 82,334 failed to affect the response to [βAla^8]NKA(4–10) while GR 94,800 and SR 48,968 were effective at concentrations that do not significantly shift the curve to septide or [Sar^9]SP sulphone.

In both preparations, the pseudopeptide derivative of NKA(4–10), MDL 28,564 behaves as a full agonist relative to NKA or [βAla^8]NKA(4–10). This behaviour has been observed previously in various guinea-pig smooth muscles (Buck *et al.*, 1990; Maggi *et al.*, 1991b; 1992). The agonist activity of MDL 28,564 has been one of the criteria to identify putative species-dependent variants of the NK_2 receptor (Maggi *et al.*, 1993a for review) and the two preparations investigated here appear homogeneous in this respect. Two out of the four NK_2 receptor antagonists tested, the linear heptapeptide GR 94,800 and the cyclic pseudopeptide MEN 10,573, displayed similar affinities for NK_2 receptors in the circular muscle of the ileum and colon. By contrast, the non peptide antagonist, SR 48,968 and the linear heptapeptide, MEN 10,376 were more potent at NK_2 receptors in the circular muscle of the colon than ileum. The estimate of the affinity of SR 48,968 for NK_2 receptors in the ileum was complicated by the depression of E_{max} to the agonist: up to 0.1 μM SR 48,968 is selective for NK_2 receptors. At 1.0 μM a depression of E_{max} to NK_1 receptor agonists but not to carbachol was observed indicating that at this concentration SR 48,968 is effective at NK_1 receptors as well. E_{max} depression by SR 48,968 is not due to irreversible changes in NK_2 receptor function, being reversed, even if slowly, by repeated washings: this depressant effect, detected previously in the rabbit pulmonary artery (Maggi *et al.*, 1993b), may arise from pseudoirreversible antagonism. The estimate of affinity for pseudoirreversible antagonism according to Kenakin (1987) yielded a pK_B value (7.83) which is remarkably lower than the affinity of SR 48,968 measured in the guinea-pig colon (pK_B 9.41) where its profile of action was fully compatible with competitive antagonism. Although the exact mechanism responsible for the very different profile of action of SR 48,968 in the guinea-pig ileum and colon remains to be determined, these observations, along with the higher potency of MEN 10,376 at NK_2 receptor in the guinea-pig colon vs the ileum, raise the possibility of an intraspecies heterogeneity of the NK_2 receptor.

Effect of nifedipine

In a previous study we showed that the responses to equieffective concentrations of [Sar^9]SP sulphone and [βAla^8]NKA(4-10) in the guinea-pig colon exhibit a marked difference in their sensitivity to nifedipine (Zagorodnyuk *et al.*, 1993b): the response to the NK_1 receptor agonist was greatly diminished (about 80% inhibition) while that to the NK_2 receptor agonist was largely unaffected (<20% inhibition) by nifedipine. The present findings demonstrate that: (i) the contraction to NK_1 or NK_2 receptor agonists is strictly dependent upon influx of extracellular calcium through nifedipine-sensitive calcium channels in the circular muscle of the ileum; (ii) the contraction to NK_2 receptor stimulation shows a marked regional difference in the degree of usage of nifedipine-sensitive calcium channels in the guinea-pig intestine. In both preparations, a similar depression of the contractile response to septide and [Sar^9]SP sulphone by nifedipine occurs. Thus, if septide were really acting at a tachykinin receptor different from that activated by 'classical' NK_1 receptor agonists, like [Sar^9]SP sulphone, it would follow that the two receptors have a similar degree of usage of nifedipine-sensitive calcium channels for inducing contraction in the circular muscle of the guinea-pig intestine. In view of the above discussed differences in the action of SR 48,968 and MEN 10,376 at NK_2 receptors in the ileum and colon, the remarkable difference in the effectiveness of nifedipine for inhibiting the response to [βAla^8]NKA(4-10) suggest that the putative NK_2 receptor subtypes couple with different effector systems to produce smooth muscle contraction in the guinea-pig intestine. This working hypothesis needs further evaluation.

Conclusions

In conclusion, the present findings demonstrate, through the use of a series of agonists and antagonists, that both NK_1 and NK_2 receptors mediate contraction in the circular muscle of the guinea-pig ileum and colon. Our data are compatible with the idea of the existence of a 'septide-sensitive' receptor which could be an NK_1 receptor subtype, although other explanations cannot be excluded at the present stage of knowledge. Furthermore, the existence of NK_2 receptor subtypes in guinea-pig intestinal smooth muscles is suggested. While pharmacological data suggest heterogeneity, the existence of NK_1 and NK_2 receptor subtypes should find structural support from the molecular biology approach before being regarded as conclusive.

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