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Antisecretory and Relaxatory Effects of Tachykinin Antagonists in the Guinea-pig Intestinal Tract

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Abstract

Existing models used to study the mechanism of action and antagonism of tachykinergic effects on intestinal contraction and secretion suffer from technical problems and have not been fully characterized using specific tachykinin antagonists. Contraction of ileal segments by substance P, colonic circular muscle by β-alanine-neurokinin A, and longitudinal muscle by senktide were used as models for neurokinin-induced contraction in the guinea-pig. Guinea-pig colonic epithelial tissue was stimulated by substance P and senktide to assess NK_1- and NK_3-mediated secretion. Using these models the potency of therapeutically useful compounds was determined.

NK_1 and NK_2 activation directly contracted smooth muscle, while NK_1-mediated secretion was nerve-mediated. NK_3 stimulation of contraction and secretion was neurally mediated, involving cholinergic nerves and 5-HT release. NK_1-mediated contraction and secretion were antagonized by SR140333 ($pD'_2 = 9{\cdot}29$ and $pK_b = 8{\cdot}53$); NK_2-mediated contraction was antagonised by SR48968 ($pD'_2 = 8{\cdot}35$) and NK_3-mediated contraction and secretion were antagonized by SB223412 ($pK_b = 8{\cdot}97$ and 8·79). The mixed antagonist MDL103392 blocked NK_1- and NK_2-mediated contraction with pK_b values of 7·92 and 6·71 respectively and NK_1-mediated secretion with a pK_b value of 6·57.

This data characterizes existing tachykinin antagonists, and should orientate the development of improved compounds as therapies for intestinal disease.

The tachykinins belong to a family of peptides including the products of two genes, the preprotachykinin (PPT) I gene which produces substance P and neurokinin A (Nawa et al 1983) and the PPT II gene which produces neurokinin B (Kotani et al 1986). These tachykinins preferentially bind to NK_1, NK_2 and NK_3 receptors respectively. The guinea-pig has been used extensively to characterize tachykinin-receptor antagonists in man as there are considerable inter-species differences in the pharmacological properties of both NK_1 and NK_2 receptors. There is a particularly sharp division reported between man and guinea-pigs, and rats (Barr & Watson 1993; Maggi et al 1993). A wide range of specific and potent receptor agonists and antagonists exist for these receptor subtypes. Sarcosine-substance P, β-alanine-neurokinin A and senktide (Drapeau et al 1987; Laufer et al 1988) are the most frequently used receptor agonists (NK_1, NK_2 and NK_3 respectively), while SR140333 (NK_1; Emonds-Alt et al 1993); SR48968 (NK_2; Maggi et al 1993; Patacchini et al 1994); SB223412 (NK_3; Sarau et al 1997); and MDL103392 (NK1/NK2/NK3; Kudlacz et al 1996) have all been widely employed as receptor antagonists.

The tachykinins stimulate various physiological functions, possibly the most well-characterized being smooth muscle contraction. NK_2 receptors play a major contractile role throughout the intestinal tract of man and guinea pigs (Giuliani et al 1991; Maggi et al 1992) mediating neural control of muscle tone, peristalsis and response to sensory stimulation (Bartho et al 1994; Johnson et al 1996).

In addition to playing a role in motility, the tachykinins are potent secretagogues. The response to the tachykinins has been studied in the small and large intestinal mucosa. Substance P has been reported to stimulate NK_1 receptors on cholinergic nerves in the guinea-pig ileum (Keast et al 1985; Perdue et al 1987; Reddix & Cooke 1992), and

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NK_1 and NK_3 receptors on nerves and mast cells in the colon (Kuwahara & Cooke 1990; Cooke et al 1997).

Tachykinin-receptor antagonists in clinical development block pain pathways (Julia et al 1994) and hypermotility in animal models of irritable bowel syndrome (Ikeda et al 1995), and inflammation in an animal model of inflammatory bowel disease (Wallace et al 1998). To develop these receptor antagonists, in-vitro models have been developed using guinea-pig intestinal smooth muscle. Understanding of these models is incomplete, with the interaction between the tachykinins and other major intestinal neurotransmitters such as acetylcholine and 5-hydroxytryptamine being particularly unclear. Existing models, especially those used to measure NK_2 and NK_3 activity, have a number of additional disadvantages. NK_2-activation of circular muscle has high-level phasic activity which requires signal integration (Maggi et al 1994). NK_3-activation of intact ileal smooth muscle requires the continuous presence of NK_1 antagonists (Croci et al 1995; Nguyen-Le et al 1996).

The aim of this study was to improve the methods available to assess tachykinergic antagonists within intestinal smooth muscle. Specifically, the NK_2 model was modified by reducing Ca^{2+} levels in the bathing solution, while a modified longitudinal muscle/myenteric plexus (LMMP) preparation was developed to assess NK_3 activity without the need for nerve stimulation. Compounds are in development to reduce tachykinin-mediated secretion and therefore a model to test the anti-secretory potential of tachykinin antagonists was also developed. For each of these studies the sensitivity to agonists and their antagonism by tachykinergic and non-tachykinergic antagonists was tested.

Materials and Methods

Drugs

The Krebs solution used was of the following composition (mM): NaCl 118, KCl 4·7, $MgSO_4.7H_2O$ 1·64, KH_2PO_4 1·18, glucose 11·5, $NaHCO_3$ 24·88, $CaCl_2.2H_2O$ 2·52. The concentration of $CaCl_2$ was lowered to 1·3 mM for the NK_2 functional assay. Agonists used were purchased from Sigma unless specified and were as follows: substance P acetate; [Sar^9 Met $(O_2)^{11}$]-substance P; [βAla^8]-neurokinin A (4–10) TFA salt (RBI); senktide (succinyl-[Asp^6, N-Me-Phe^8]-SP fragment 6–11); acetylcholine chloride; prostaglandin E_2; 5-hydroxytryptamine creatinine sulphate and histamine dihydrochloride. Antagonists were synthesized by the chemistry department of Synthélabo Recherche unless otherwise indicated and included: the cyclooxygenase inhibitor, indomethacin (sigma); the $5HT_3/5HT_4$ antagonist, tropisetron; the $5HT_3$ antagonist, ondansetron; the $5HT_4$ antagonist, SB204070 ((1-butyl-4-piperidinyl)-methyl 8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride); atropine sulphate (RBI); phosphoramidon (N-(α-rhamnopyranosyloxy- hydroxyphosphoinyl)-Leu-Trp sodium salt sigma); the neural toxin, tetrodotoxin (TTX; sigma); the NK_1 antagonist, SR140333 ((S) 1-[-[3-(3,4-dichlorophenyl)-1-[(3-isopropoxyphenyl) acetyl] piperidin-3-yl] ethyl] -4-phenyl-1-azoniabicyclo[2.2.2]octane); the NK_2 antagonist, SR48968 ((*S*)-*N*-methyl-*N*-[4-(4-acetylamino - 4 - phenylpiperidin - 1-yl] - 2 - (3,4-dichlorophenyl)butyl]benzamide); the NK_3 antagonist, SB223412 ((*RS*)-*N*(α-ethyl-benzyl)-3-hydroxyl-2-phenylquinoline-4-carboxamide); and the mixed tachykinergic antagonist, MDL 103392 ((*R*, *S*)-1-[3-[3,4-dichlorophenyl]-1-(3,4,5-trimethoxybenzoyl)-pyrrolidin-3-yl] ethyl-4-phenylpiperidine-4-carboxamide). Peptides were stored as stock solutions in 0·1 M acetic acid at −20°C and diluted in distilled water. MDL 103392, SR140333, SR48968, and SB223412 were dissolved and diluted in dimethylsulphoxide (100%). All other compounds were dissolved and diluted in distilled water. Solutions were diluted 100-fold by the bathing medium.

Animals

Male Hartley guinea-pigs, 450–800 g (Charles River, France), were used throughout this study and were given free access to food and water. Animals were killed by desanguination after a stunning blow to the head. Tissue was immediately excised and used for the study of smooth muscle or epithelial function.

Smooth muscle preparation

Three different contractile systems were employed: the intact terminal ileum (NK_1); circular muscle strips from the proximal colon (NK_2) and LMMP from the distal ileum (NK_3). For each preparation, segments of intestine were flushed free of luminal contents and mounted in 20-mL organ baths, with one end attached to an isometric tension transducer (Hugo Sachs, Germany), and the other anchored to the bath floor. Tissue was bathed in a Krebs solution at 37°C, continuously gassed with carbogen, and allowed to stabilize for 60 min.

The method used to prepare the intact ileum (NK_1) has previously been described (Holzer & Lembeck 1980; Maggi et al 1994). Briefly, non-

cholinergic muscle contraction was evoked in the presence of atropine (3 μM) in 2–3 cm segments of distal ileum (10 cm from the caecum) at 15-min intervals by 5 hydroxytryptamine (10 μM) until consistent responses were achieved. A cumulative response curve to substance P was then constructed (0·1–100 nM). After wash out, the preparation was allowed to restabilize for 15 min before addition of the test antagonist. A further 30-min equilibration period was allowed before the construction of a second response curve. Preliminary results showed that studies performed in this way yielded reproducible response curves.

The colonic circular muscle preparation (NK_2) was modified from methods previously described (Maggi et al 1994). The mucosa was removed from a section of proximal colon and two adjacent strips were cut in the circular muscle axis. Tissue was bathed in modified Krebs solution containing a reduced Ca^{2+} concentration (1·3 mM) (to reduce phasic contractions) and indomethacin (10 μM), and contracted at 15-min intervals by KCl (80 mM) until consistent responses were achieved. One muscle strip was then incubated for 30 min in the presence of the antagonist with its pair acting as a vehicle control, after which, cumulative response curves to β-alanine-neurokinin A were constructed (0·1–300 nM).

The LMMP preparation (NK_3) has been previously described (Croci et al 1995). The longitudinal muscle and myenteric plexus was dissected away from its underlying circular muscle and mucosa and a strip mounted in the longitudinal muscle axis. Tissue was bathed in Krebs solution and contracted at 15-min intervals by histamine (10 μM) until consistent responses were achieved. A cumulative response curve to senktide was then constructed (0·1–100 nM). After wash out, the preparation was allowed to restabilize for 20 min before addition of the test antagonist. A further 30-min equilibration period was allowed before the construction of a second response curve. Preliminary studies showed that studies performed in this way yielded reproducible response curves.

Epithelial tissue preparation

The colonic epithelial preparation has been previously described (Kuwahara & Cooke 1990). Briefly, the distal 5–10 cm of colon was removed, rinsed in cold Krebs buffer and placed over a glass rod (6 mm diameter). The outer muscle layer was scored along its anti-mesenteric surface using a dull scalpel blade and peeled away from the mucosa using a piece of gauze previously dampened with Krebs buffer. The preparation was opened along its anti-mesenteric surface, and the resultant epithelial preparation mounted as a flat sheet between two halves of an Ussing chamber (exposed area 1·24 cm^2). To prevent desensitization, responses were determined in paired tissue, with one preparation always acting as a control. Two consecutive series of senktide challenges separated by an equilibration period of 30 min yielded similar responses and so the first curve was treated as a control while the second was used to test the activity of a given antagonist. In both protocols the antagonist was incubated with the tissue for at least 30 min. Both the mucosal and serosal surfaces were circulated with 4 mL Krebs buffer using a gas-lift (95%O_2/5%CO_2; pre-humidified by bubbling through distilled water), and maintained at $37 \pm 1°C$. Short-circuit current (SCC) generated by the epithelium was continuously monitored using an EVC4000 voltage clamp (WPI, USA) connected to one voltage- and one current-passing electrode inserted into each half chamber via a pre-amplifier. The voltage generated by the epithelium was continuously short-circuited using the current-passing electrodes. After a 30-min equilibration in the presence of mucosal and serosal antagonist or vehicle, various agonists were added in a cumulative way to the serosal solution. In studies performed using substance P, the endopeptidase inhibitor phosphoramidon (10 μM) was added to both halves of the Ussing chamber to prevent peptide breakdown. This treatment was not necessary for any of the other studies. Thirty minutes after the final washout, tissues were challenged with either acetylcholine or in the case of atropine pre-treatment, prostaglandin E_2 to determine tissue viability. Tissues responding to these agonists with an increase in SCC of less than 50 and 25 μA, respectively were excluded.

Data analysis

Peak agonist responses in grams of tension or μA were collected by an acquisition package and normalized to control E_{max} values, plotted and the pD_2 calculated. Antagonist effect on E_{max} values was assessed statistically using a one-sample *t*-test, with comparisons made with a hypothetical mean of 100%. If no reduction was observed, the pD_2 was compared in the presence and absence of the antagonist. If these values were statistically different, as determined using Student's *t*-test, the dose ratio (DR), and subsequently the pK_b values were calculated. For non-competitive antagonism pD'_2 values were calculated according to Van Rossum (1963) by performing linear regression analysis of log [antagonist] plotted against log ((E_{max}(control)/E_{max}(antagonist)).

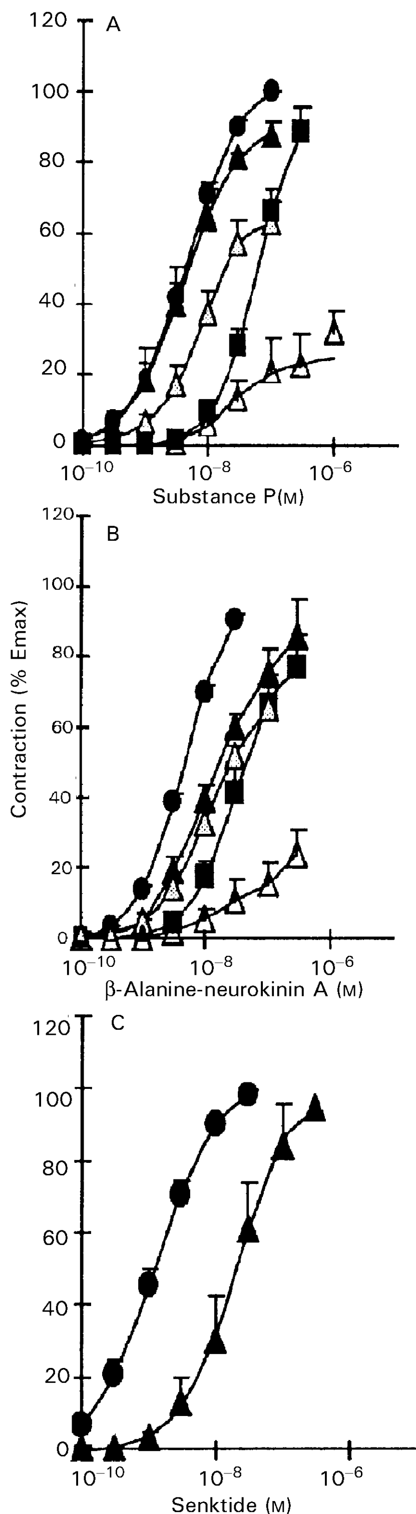

Figure 1. Contractile response of guinea-pig intestinal smooth muscle after 30-min incubation with tachykinin antagonists. A. Response of intact guinea-pig ileum to substance P. Control (●), 0·1 μM MDL103392 (■), 0·1 nM (▲), 0·3 nM (△), 1 nM (△) SR140333. B. Response of guinea pig colonic circular muscle to β-alanine neurokinin A. Control (●), 1 μM MDL103392 (■), 0·1 nM (▲), 0·3 nM (△), 1 nM (△) SR48968, C. Response of guinea pig ileum longitudinal muscle/myenteric plexus preparation to senktide. Control (●), 100 nM SB223412 (▲). Values are mean ± s.e.m (Antagonist data, n = 4; control-data is pooled).

Results

Contractile activity

Substance P, β-alanine-neurokinin A and senktide potently and concentration-dependently contracted the NK_1, NK_2 and NK_3 smooth muscle preparations, respectively (Figure 1). It was found that reducing Ca^{2+} concentration in the bathing medium from 2·5 mM to 1·3 mM gave circular muscle responses with less superimposable phasic activity. All further studies using the colonic circular muscle preparation were therefore performed in reduced Ca^{2+}.

NK_1 antagonism. The sensitivity of the intact ileum to substance P was reduced by MDL103392 (Table 1; Figure 1a). SR140333 also antagonized the response to substance P; however, this effect was non-competitive, reducing the E_{max} in a concentration-dependent way (Table 1; Figure 1a). In contrast the response was not altered by the NK_2 antagonist, SR48968 (n = 4) or the NK_3 antagonist, SB223412 (n = 6; Table 1), suggesting that the response of guinea-pig ileum to substance P is specifically mediated by NK_1 receptors. The response to substance P was not reduced by tetrodotoxin (1 μM; E_{max} = 97 ± 6% of control; n = 4) or the 5-HT antagonist, tropisetron (10 μM; E_{max} = 129 ± 4; n = 4).

NK_2 antagonism. The response of the colonic circular muscle to β-alanine-neurokinin A was antagonized by SR48968 and MDL103392 (Table 1; Figure 1b). The response of this preparation to β-alanine-neurokinin A was specifically mediated by NK_2 receptors as it was insensitive to NK_1 (n = 4) or NK_3 (n = 4) antagonists at the concentrations tested (Table 1). Likewise the response to NK_2 stimulation was not reduced by tetrodotoxin (E_{max} = 131 ± 4% of control; 1 μM; n = 4), atropine (E_{max} = 118 ± 9% of control; 1 μM; n = 4) or tropisetron (E_{max} = 100 ± 10% of control; 10 μM; n = 5).

NK_3 antagonism. The response of the ileal LMMP preparation to senktide was antagonized by SB223412 (Table 1; Figure 1c). Although SR140333 and SR48968 antagonized the response to senktide (n = 4), antagonism was weak and non-competitive. The response to senktide was specifically mediated by NK_3 receptors. In contrast, the response to NK_3 stimulation was inhibited by tropisetron (E_{max} = 64 ± 6% of control; 10 μM; n = 4; $P < 0{\cdot}05$) and atropine (E_{max} = 24 ± 3% of control; 1 μM; n = 4; $P < 0{\cdot}05$) and completely abolished by tetrodotoxin (1 μM; n = 4; $P < 0{\cdot}05$). To investigate

Table 1. pKb and pD'_2 values for antagonism of tension or short-circuit current (SCC) responses to substance P, β-alanine-neurokinin A and senktide.

Antagonist		Substance P		β-alanine neurokinin A	Senktide	
		Tension	SCC	Tension	Tension	SCC
SR140333 (0·1–1000 nM)	NK_1	9·29[a]	8·53	< 6	5·57[a]	6·95
SR48968 (1 nM–10 μM)	NK_2	< 7		8·35[a]	5·87[a]	
SB223412 (10 nM–100 nM)	NK_3	< 7	< 7	< 7	8·97	8·79[†]
MDL103392 (0·1–10 μM)	NK_1, NK_2, NK_3	7·92	6·57	6·71		

Tension was measured in intact ileum, colonic circular muscle and ileal longitudinal muscle/myenteric plexus respectively. SCC was measured across colonic epithelium. [a]pD'_2 values in cases where the E_{max} was reduced. [†]Apparent pK_b value in cases where the E_{max} was reduced.

whether blockade by tropisetron was due to $5HT_3$ or $5HT_4$ antagonism, the effect of the $5HT_3$-specific antagonist, ondansetron (10 μM) and the $5HT_4$-specific antagonist SB204070 (0·01 μM) on the response to senktide was determined. Ondansetron had no significant effect on the E_{max} (93 ± 8% of control values; n = 4; $P > 0·05$); however, SB204070 shifted the senktide response curve to the right ($pK_b = 7·73$) while leaving the E_{max} unchanged (88 ± 16% of control values; n = 4).

Secretory activity

Substance P and senktide potently and concentration-dependently increased colonic SCC (Figure 2) generating maximum values of 295 ± 37 $\mu A\,cm^{-2}$ (n = 12) and 186 ± 43 $\mu A\,cm^{-2}$ (n = 5). In contrast β-alanine-neurokinin A was a very weak agonist, and further experiments were therefore restricted to NK_1- and NK_3-mediated effects.

NK_1 antagonism. The response to substance P was shifted to the right by SR140333 and MDL103392 (Table 1; Figure 2a). In contrast the response was not altered by high concentrations of the NK_3 antagonist, SB223412 (n = 4; Table 1). The response to substance P appeared to result from neural stimulation as it was abolished by tetrodotoxin (E_{max} = 12 ± 8% of control; 10 μM; n = 4; $P < 0·05$) but did not involve $5HT_3/5HT_4$ receptors as tropisetron had no effect on the E_{max} (E_{max} = 110 ± 28% of control values; 10 μM; n = 4).

NK_3 antagonism. The sensitivity of the response to senktide was reduced by SB223412 (Table 1; Figure 2b) and much more weakly by SR140333 (n = 4; Table 1). SB223412 acted in a surmountable way reducing the E_{max} to 57 ± 9% of control values ($P < 0·05$; n = 4). As seen in the LMMP, the secretory response to NK_3 stimulation was inhibited in a non-competitive way by tropisetron (E_{max} = 41 ± 10% of control; 10 μM; n = 4; $P < 0·05$) and atropine (E_{max} = 49 ± 4% of control; 10 μM; n = 3; $P < 0·05$) and abolished by tetrodotoxin (E_{max} = 5 ± 3% of control; 10 μM; n = 3; $P < 0·05$). To investigate whether blockade by tropisetron was due to $5HT_3$ or $5HT_4$ antagonism, the effect of the $5HT_3$-specific antagonist, ondansetron (10 μM) and the $5HT_4$-specific antagonist SB204070 (10 μM) on the response to senktide was determined. The E_{max} was reduced by each of these antagonists (E_{max} = 66 ± 11% and 70 ± 8% of control values respectively; $P < 0·05$; n = 4).

Discussion

The tachykinins are potent and multi-functional neurotransmitters in the intestinal tract, and the antisecretory and relaxatory activity of their antagonists are of potential use in the treatment of diseases such as irritable bowel syndrome and inflammatory bowel disease. This study improved the methods used to assess activity and subsequently determine the potency and specificity of a range of antagonists. Finally questions regarding mechanisms involved in tachykinin-mediated secretion and contraction have been addressed.

In the past, intact guinea-pig ileum, colonic circular muscle and electrically stimulated LMMP or intact ileal preparations have been used to test NK_1, NK_2 and NK_3 antagonists (Holzer & Lembeck 1980; Maggi et al 1994; Croci et al 1995; Nguyen-Le et al 1996). NK_1-mediated contraction of the guinea-pig ileum is perhaps the most well-characterized intestinal model of tachykinergic activity. It has been shown that SR140333 blocked the response of the guinea-pig ileum to substance P with a similar potency and non-competitive mechanism to data previously published (Emonds-

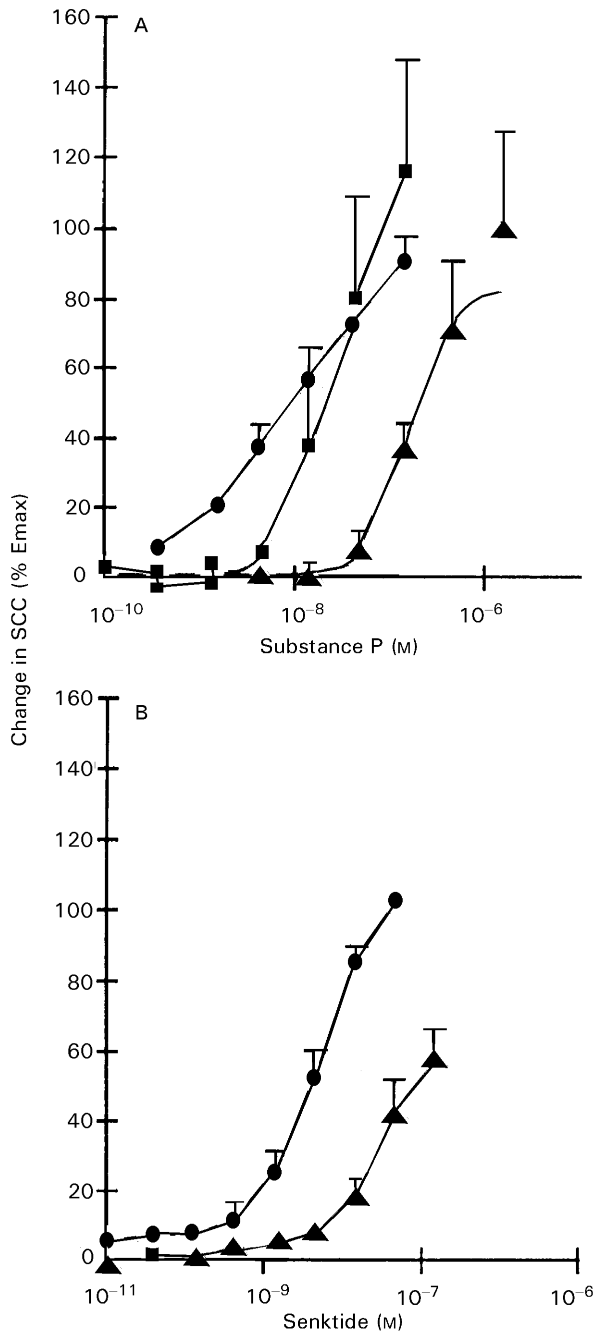

Figure 2. Secretory response of guinea pig distal colon after 30-min incubation with tachykinin antagonists. A. Response to substance P. Control (●), MDL103392 (■), SR140333 (▲). B. Response to senktide. Control (●), SB223412 (▲). Values are mean ± s.e.m. (pooled data, n = 4, 5 or 8 animals).

Alt et al 1993). We characterized this model further by excluding the involvement of NK_2 or NK_3 receptors through the absence of effect of SR48968 and SB223412. Like SR140333, MDL103392 has been in preclinical development. This study shows for the first time that this mixed antagonist blocks NK_1-mediated intestinal smooth muscle contraction with a value similar to that reported in human cell lines (Kudlacz et al 1996). Mechanistically, tachykinergic neurotransmission in this model was found to differ from other tissues (Ramirez et al 1994) as it did not interact with serotonergic systems. In fact, the guinea pig ileum appears mechanistically very simple, as tetrodotoxin was without effect suggesting a direct smooth muscle effect.

Non-intestinal tissue such as the rabbit pulmonary artery has almost exclusively been used to assess NK_2 antagonism. In a few cases, colonic circular muscle has been used even though it has inherent problems related to the high levels of phasic contraction induced by β-alanine-neurokinin A (Maggi et al 1994). We were able to overcome this problem by reducing the Ca^{2+} concentration in the bathing solution. Under these conditions the preparation responded to β-alanine-neurokinin A with a sensitivity similar to that in the presence of normal Ca^{2+} concentrations. This model was homologous for NK_2 receptors as it was blocked by SR48968 but was insensitive to the NK_1 antagonist SR140333 and the NK_3 antagonist SB223412. In addition to blocking NK_1 activity, using this model we show for the first time that MDL103392 also blocks NK_2-mediated activity in the intestinal tract. Like NK_1-mediated ileal contraction, NK_2 activation of colonic circular muscle was insensitive to atropine, tropisetron and tetrodotoxin, suggesting a direct smooth muscle effect. Guinea-pig colonic circular muscle is a particularly good model for the human gastrointestinal musculature since both tissues are predominantly activated by NK_2 receptors rather than NK_1 or NK_3 subtypes (Giuliani et al 1991; Maggi et al 1992; Huber et al 1993). Modification of existing models described in this study has improved our ability to identify and optimize compounds such as SR48968, that may have relaxatory activity in the intestinal tract of man.

Intestinal NK_3 assays have previously involved electrical stimulation of LMMP which leads to complicated analysis, or intact tissue that requires the continuous presence of NK_1 antagonists (Croci et al 1995). We found that the LMMP preparation was contracted in a dose-dependent way by senktide. This response was very insensitive to NK_1 or NK_2 antagonists, but blocked by SB223412 with a similar potency to that previously reported in the rabbit iris (Sarau et al 1997). This shows for the first time that SB223412 is potentially active within the intestinal tract. As described in previous reports (Croci et al 1995), we showed that the contractile response to NK_3 activation is largely mediated by cholinergic nerves. The response to senktide is also blocked by tropisetron suggesting that serotonin mediates the response to senktide via $5HT_3$ and/or

$5HT_4$ receptors. Ondansetron had little effect on the response to senktide arguing against $5HT_3$ involvement, while SB204070 was active suggesting that $5HT_4$ receptors play an important role. Although SB204070 did not bind to the NK_3 receptor (data not shown) it behaved in a competitive way, and further confirmation of $5HT_4$ involvement is therefore required. 5-HT has previously been shown to stimulate tachykinergic nerves in the guinea-pig ileum and colon (Ramirez et al 1994; Kojima & Shimo 1995; Yamano & Miyata 1996), tachykinin release of 5-HT in the periphery has been reported only once previously (Fewtrell et al 1982). In addition to characterizing the unstimulated LMMP preparation for use as an assay of NK_3 activity, this study has revealed important mechanistic considerations for NK_3 control of the intestinal musculature.

Consequently, when NK_3 antagonist activity is assessed in the guinea-pig ileum, care must be taken to ensure that effects are not due to non-specific antagonism of serotonergic receptors, cholinergic receptors or neural blockade.

Since the tachykinins are secretogues, our final aim was to characterize a model capable of assessing antisecretory activity of tachykinin antagonists. The guinea-pig colon responded to substance P and senktide with an increase in SCC, and much more poorly to β-alanine-neurokinin A. This confirms that this organ is a good model for assaying NK_1- and NK_3-receptor activity in agreement with previous reports (Cooke et al 1997). We proceeded to assess the activity of the NK_1 antagonist SR140333 against substance P. SR140333 antagonized the response to substance P with a pK_b value in reasonable agreement with its potency calculated using smooth muscle. To our knowledge, this is the first time that the anti-secretory activity of SR140333 has been reported. MDL103392 also has antisecretory activity and this is likely to be due to its NK_1 antagonism as substance P-induced secretion appears to be entirely NK_1-mediated.

Senktide evoked a secretory response that for the first time was shown to be antagonized by SB223412. NK_3-receptor activation also releases acetylcholine and 5-HT. Release of these transmitters stimulates secretion via muscarinic, $5HT_3$ and $5HT_4$ receptors as the response to senktide is reduced by atropine, ondansetron and SB204070. In this respect, stimulation of colonic contraction and secretion by senktide shares many mechanistic similarities.

In this study we have described improved smooth muscle assays to test NK_2 and NK_3 antagonists designed for use in the gut. A model has been developed to determine the antisecretory activity of tachykinin antagonists, and we have elucidated the mechanism of action of the tachykinins on intestinal smooth muscle and epithelium, and determined the relaxatory and antisecretory potency of therapeutically relevant tachykinin antagonists. Since the tachykinin receptors expressed by man and guinea-pig tissue are similar, the characterization of these methods should allow improved intestinally active tachykinin antagonists to be identified.

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