British Journal of Pharmacology (1998) 124, 1375–1384
http://www.stockton-press.co.uk/bjp

Roles of neuronal NK_1 and NK_3 receptors in synaptic transmission during motility reflexes in the guinea-pig ileum

[1,3]P.J. Johnson, [1]J.C. Bornstein & [2]E. Burcher

[1]Department of Physiology, University of Melbourne, Parkville, Victoria 3052; and [2]School of Physiology and Pharmacology, University of New South Wales, Sydney, NSW 2052, Australia

1 The role of NK_1 and NK_3 receptors in synaptic transmission between myenteric neurons during motility reflexes in the guinea-pig ileum was investigated by recording intracellularly the reflex responses of the circular muscle to distension or compression of the mucosal villi. Experiments were performed in a three-chambered organ bath that enabled drugs to be selectively applied to different sites along the reflex pathways.

2 When applied in the recording chamber, an NK_1 receptor antagonist, SR140333 (100 nM), reduced by 40–50% the amplitudes of inhibitory junction potentials (i.j.ps) evoked in the circular muscle by activation of descending reflex pathways. This effect was abolished when synaptic transmission in the stimulus region was blocked with physiological saline containing 0.1 mM Ca^{2+} plus 10 mM Mg^{2+}, leaving only the component of the descending reflex pathway conducted via long anally directed collaterals of intrinsic sensory neurons.

3 SR140333 (100 nM) had no effect on descending reflex i.j.ps when applied to the stimulus region. Ascending reflexes were also unaffected by SR140333 in the stimulus region or between the stimulus and recording sites.

4 Septide (10 nM), an NK_1 receptor agonist, enhanced descending reflexes by 30–60% when in the recording chamber. [Sar^9,$Met(O_2)^{11}$]substance P had no effect at 10 nM, but potentiated distension-evoked reflexes at 100 nM.

5 A selective NK_3 receptor antagonist, SR142801 (100 nM), when applied to the stimulus region, reduced the amplitude of descending reflex responses to compression by 40%, but had no effect on responses to distension. SR142801 (100 nM) had no effect when applied to other regions of the descending reflex pathways.

6 SR142801 (100 nM) only inhibited ascending reflexes when applied at the recording site. However, after nicotinic transmission in the stimulus region was blocked, SR142801 (100 nM) at this site reduced responses to compression.

7 Contractions of the circular muscle of isolated rings of ileum evoked by low concentrations of septide, but not [Sar^9,$Met(O_2)^{11}$]substance P, were potentiated by tetrodotoxin (300 nM).

8 Contractile responses evoked by an NK_3 receptor agonist, senktide, were non-competitively inhibited by SR142801. After excitatory neuromuscular transmission was blocked, senktide produced inhibitory responses that were also antagonised by SR142801, but to a lesser extent and in an apparently competitive manner.

9 These results indicate that tachykinins acting via NK_1 receptors partly mediate transmission to inhibitory motor neurons. NK_3 receptors play a role in transmission from intrinsic sensory neurons and from ascending interneurons to excitatory motor neurons during motility reflexes.

Keywords: Enteric nervous system; motility; enteric reflexes; tachykinins; NK_1 receptors; NK_3 receptors; neurokinin; substance P; septide; ileum

Introduction

Intestinal motility is regulated by ascending excitatory and descending inhibitory reflexes that modulate the activity of intestinal circular muscle (Bayliss & Starling, 1899; Costa & Furness, 1976). These reflexes are conducted via nerve pathways within the gut wall that comprise intrinsic sensory neurons which respond to stimuli such as distension or mechanism distortion of the mucosa, interneurons which conduct the reflexes along the gut, and motor neurons to the circular muscle (Bornstein, 1994; Furness *et al*, 1994).

A number of substances have been implicated in transmission during motility reflexes, including acetylcholine (ACh), tachykinins, 5-hydroxytryptamine, nitric oxide and vasoactive intestinal peptide (Furness *et al*., 1994). In the guinea-pig small intestine, tachykinins are found in several functional classes of myenteric neurons and are implicated in both neuro-neuronal and neuromuscular transmission (see Holzer & Holzer-Petsche, 1997). Mammalian tachykinins, including substance P and neurokinin A, contract the longitudinal and circular muscle layers (Maggi *et al*., 1990), and mimic slow synaptic responses when applied to myenteric neurons (Katayama & North, 1978; Johnson *et al*., 1981; Galligan *et al*., 1987).

Tachykinins exert their effects via three classes of receptor, known as NK_1, NK_2 and NK_3 receptors, all of which are present in the small intestine (see Maggi, 1995 for review). NK_3 receptors appear to be located exclusively on neurons, as selective agonists depolarise these cells, release ACh and tachykinins from the myenteric plexus, and evoke contractions of circular muscle which are abolished by toxins which prevent

[3] Author for correspondence.

nerve conduction (Laufer *et al*., 1985; Guard & Watson, 1987; Hanani *et al*., 1988; Yau *et al*., 1992; Maggi *et al*., 1990; 1994b). Furthermore, densitisation of NK_3 receptors inhibits motility reflexes (Johnson *et al*., 1996). Selective antagonists for these receptors have now been developed, and preliminary studies indicate that NK_3 receptors play roles in synaptic transmission during motility reflexes (Emonds-Alt *et al*., 1995; Costa *et al*., 1996b).

In contrast, selective NK_1 and NK_2 receptor agonists evoke contractions which are largely insensitive to neurotoxins, and it has been presumed that these receptors are located predominantly on the muscle layers (Maggi *et al*., 1990). However, selective NK_1 receptor agonists depolarise myenteric neurons, evoke release of ACh and inhibit peristalsis (Hanani *et al*., 1988; Guard *et al*., 1991; Holzer *et al*., 1995). Indeed, antisera raised against the NK_1 receptor have revealed that these receptors are found on inhibitory motor neurons and interstitial cells of Cajal (Portbury *et al*., 1996). As yet the role of neuronal NK_1 receptors in reflex transmission is unknown.

The present study used established divided organ bath methods and selective NK_1 and NK_3 agonists and antagonists to identify the endogenous roles of these receptors in transmission between functionally-defined enteric neurons during motility reflexes in the guinea-pig ileum.

Methods

Guinea-pigs of either sex (200–300 g) were stunned by a blow to the head and killed by severing the carotid arteries and spinal cord. Segments of ileum were removed from the region between 10 and 20 cm from the ileocaecal junction and flushed with physiological saline containing (in mM): NaCl, 118; $NaHCO_3$ 25; KCl, 4.8; $CaCl_2$ 2.5; $MgSO_4$ 1.2; NaH_2PO_4, 1.0 and glucose 11, bubbled with 95% O_2, 5% CO_2.

Motility reflex experiments

Segments of guinea-pig ileum were opened and pinned flat, mucosa uppermost, in a divided organ bath as described in detail elsewhere (Yuan *et al*., 1994; Johnson *et al*., 1996). The organ bath allowed the preparation to be separately perfused in three adjacent chambers. In the first two chambers (far and near), reflex pathways were activated either by distending the serosa via balloons embedded in the base of the bath or by compressing the mucosal villi with a sponge block. In the third chamber (recording), the reflex responses of the circular muscle were measured using conventional intracellular recording methods. The distances from the stimulation sites in the near and far chambers to the recording site were approximately 12 and 27 mm respectively. Stimulus conditions and parameters were identical to those of Johnson *et al*. (1996). Based on current knowledge of the projections of myenteric neurons, this arrangement allowed transmission at different functional synapses along intrinsic reflex pathways to be selectively investigated. Stimuli applied to the far chamber activate reflex pathways in which the majority of synapses occurring within the near chamber would be between interneurons; accordingly transmission at these synapses was examined by application of drugs to the near chamber. When reflexes were evoked from the near chamber, drugs applied here would access synapses predominantly between the local terminals of intrinsic sensory neurons and interneurons. Drugs applied to the recording chamber would affect transmission to the final motor neurons.

While the stimuli used might be expected to also activate extrinsic sensory neurons, reflex responses evoked by such stimuli in the guinea-pig ileum are unaffected by extrinsic denervation (Furness *et al*., 1995) and are thus predominantly due to activation of intrinsic nerve pathways.

Analysis of results

Reflex responses in the circular muscle in the recording chamber consisted of compound excitatory junction potentials (e.j.ps) oral to the stimulus and inhibitory junction potentials (i.j.ps) anal to the stimulus. These responses were analysed to determine their peak amplitudes, latencies, times to peak and half-peak durations. In many preparation, slow wave oscillations of the resting membrane potential were observed with a frequency of approximately 0.3 Hz and these were subtracted from junction potential responses in the analysis.

The compound i.j.ps were almost certainly due to the simultaneous release of transmitter from many inhibitory motor neurons (Bornstein *et al*., 1986). To obtain an estimate of the amount of transmitter released, and hence the number of active motor nerve terminals, the amplitudes of recorded i.j.ps were corrected for nonlinear summation using the equation derived by McLachlan & Martin (1981):

$$A' = A(1 - A/(\text{r.m.p.} - E_K))^{-1},$$

where A′ is the corrected amplitude, A is the recorded i.j.p. amplitude, r.m.p. is the resting membrane potential; and E_K is the reversal potential for i.j.ps (−90 mV; see Bornstein *et al*., 1986).

E.j.p. amplitudes were not corrected, as these were usually smaller, and the reversal potential for these events was considerably further from resting membrane potential.

All data are expressed as mean ± s.e.mean. Statistical comparisons were made using Student's paired *t* test, or analysis of variance as appropriate. Differences were considered significant if $P < 0.05$. In the text, *n* refers to the number of animals used.

Contractile organ bath studies

Experiments were also performed to test the effect of an NK_3 receptor antagonist, SR142801, on excitatory and inhibitory contractile responses of the circular muscle to an NK_3 receptor agonist, and to compare the effects of NK_1 receptor agonists.

Rings of ileum 3–5 mm wide were attached to stainless steel hooks, placed in organ baths (volume 6 ml) and bathed with physiological saline at 37°C, bubbled with 95% O_2, 5% CO_2 and containing indomethacin (10 μM) to induce rhythmic contractile activity (Maggi *et al*., 1994b). The rings were suspended connected to isotonic force transducers with loads of 0.5 g. Contractile responses were recorded using the BIOPAC system. After 1.5–2 h, such preparations developed rhythmic circular muscle contractions at a frequency of approximately 0.3 Hz.

Non-cumulative concentration-effect curves for senktide were established by applying increasing concentrations of senktide for 2 min at 30 min intervals. As senktide activates NK_3 receptors on both excitatory and inhibitory nerve pathways to the circular muscle (Maggi *et al*., 1993), these effects were examined separately. Purely contractile responses to senktide were studied by performing experiments in the presence of apamin (300 nM) and nitro-L-Arginine (100 μM) to block inhibitory neuromuscular transmission (Humphreys *et al*., 1991; Lyster *et al*., 1992). Results were expressed as a percentage of the mean contractile response to carbachol

(10 μM). Inhibitory responses to senktide were examined by conducting similar experiments in the presence of hyoscine (100 nM), SR140333 (100 nM) and the NK_2 receptor antagonist SR48968 (100 nM) to block excitatory neuromuscular transmission. Inhibition of spontaneous contractions was calculated by integrating the response and measuring the percentage inhibition over 90 s after application of senktide. In both experiments, the effect of SR142801 was tested by comparing responses in the absence and presence of SR142801, applied to the tissue 20 min prior to the agonist.

Finally, the contractile responses to NK_1 agonists septide and [Sar^9,$Met(O_2)^{11}$]substance P were examined in the presence and absence of tetrodotoxin (TTX; 300 nM).

Drugs

The drugs used in this study were: nicardipine, hexamethonium bromide, hyoscine hydrobromide; indomethacin, naloxone (Sigma, U.S.A.); tetrodotoxin (Alomone Labs, Israel); carbachol (BDH Chemicals Inc. U.S.A.); SR140333, SR142801, SR48968 (gifts from Dr Emonds-Alt, Sanofi Recherche, Montpellier, France); [$pGlu^6$,Pro^9]-substance P 6-11 (septide), [Sar^9,$Met(O_2)^{11}$]-substance P, [succinyl-Asp^6, $Me-Phe^8$]-substance P 6-11 (senktide), nitro-L-arginine (NOLA; RBI, U.S.A.) and CP99994 (gift from Dr S. McLean, Pfizer Central Research, Groton, Conn. U.S.A.). Apamin was synthesised by Dr. Roger Murphy of the Department of Pharmacology, University of Melbourne. Peptides were dissolved in 0.01 N acetic acid to give stock solutions of 1 mM and stored in frozen aliquots until required; indomethacin, SR140333, SR142801 and SR48968 were dissolved in ethanol. Nicardipine was dissolved in warm distilled water and stored in frozen aliquots. Other drugs were dissolved in distilled water. Dilutions were freshly prepared every day.

Results

Effects of NK_1 receptor antagonists on descending reflexes

When stimuli were applied oral to the recording site, distension and compression in the near chamber consistently evoked biphasic compound i.j.ps (see Figure 1). In contrast, responses to far chamber stimuli were usually monophasic.

Application of the selective NK_1 receptor antagonist SR140333 (100 nM; Emonds-Alt *et al*., 1993) to the recording chamber reduced the amplitudes (by 40–60%) of both peaks of the i.j.ps evoked by distension or mucosal compression in the near chamber (see Table 1; Figure 1a). The amplitudes of i.j.ps evoked by mucosal compression in the far chamber were reduced by 55%, but i.j.ps evoked by distension in this chamber were unaffected. In all cases, responses returned to control levels after washout of the drug.

Another NK_1 receptor antagonist, CP99994 (1 μM; McLean *et al*., 1993), was less effective when applied in the recording

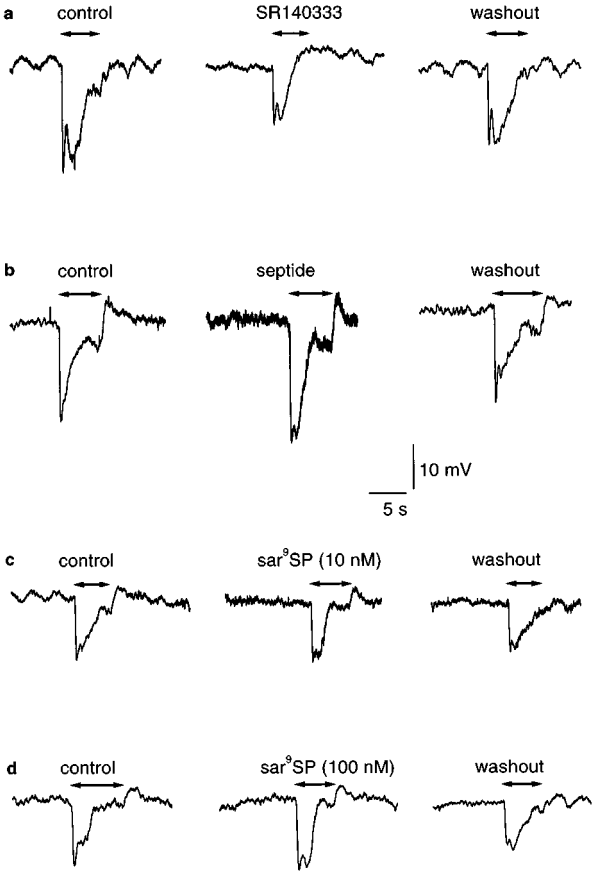

Figure 1 Effect of NK_1 receptor antagonists and agonists in the recording chamber on descending i.j.ps evoked by distension in the near chamber (bars indicate distension period). (a) SR140333 (100 nM) reduced i.j.p. amplitude. (b) Septide (10 nM) enhanced reflex responses. (c) [Sar^9,$Met(O_2)^{11}$]-substance P had no effect at 10 nM, but at 100 nM (d), i.j.ps were potentiated.

Table 1 Effect of NK_1 receptor antagonists applied in the recording chamber or in the near chamber on descending reflexes evoked by distension or compression in the near or far stimulation chamber

Treatment (drug & location)	*Near chamber distension*		*Near chamber compression*		*Far chamber distension*	*Far chamber compression*
	First peak	*Second peak*	*First peak*	*Second peak*	*Absolute peak*	*Absolute peak*
SR140333 (100 nM) in recording chamber	51±12* (6)	50±13* (6)	63±7* (6)	42±6* (6)	84±24 (6)	44±7* (6)
CP99994 (1 μM) in recording chamber	98±14 (8)	77±8* (8)	97±6 (7)	61±14* (6)	101±19 (7)	95±9 (7)
SR140333 (100 nM) in near stimulation chamber	121±48 (4)	144±29 (4)	83±8 (4)	80±8 (4)	83±5* (4)	82±15 (4)
CP99994 (1 μM) in near stimulation chamber	82±8 (4)	118±25 (4)	109±22 (4)	107±30 (4)	87±15 (4)	101±16 (4)

Numbers indicate amplitudes of i.j.ps in the presence of antagonist expressed as a percentage of control i.j.p. amplitudes (mean±s.e. mean. (n)) *significantly different from control ($P<0.05$).

chamber. It reduced the amplitudes of the second peaks of the i.j.ps evoked by distension or compression in the near chamber by 25–40%, but had no effect on the first peaks. No effect on longer pathways was observed.

To identify the site of action of SR140333, physiological saline containing 0.1 mM Ca^{2+} and 10 mM Mg^{2+} was added to the near chamber to block synaptic transmission within this chamber (Dingledine & Goldstein, 1976) and reveal only the component of the i.j.p. response mediated by long anally projecting intrinsic sensory neurons (Brookes *et al*., 1995; Johnson *et al*., 1996). This treatment completely abolished the second peak of i.j.ps evoked by near chamber stimuli (see Figure 2). The amplitudes of the first peak of responses to distension and compression were reduced by 60% and 85% respectively ($P<0.01$; $n=7$) and the mean duration at half absolute peak amplitude of responses to distension was reduced by 40% ($P<0.05$; $n=7$). The mean latency of the i.j.ps evoked by distension increased significantly from 210 ± 6 ms to 235 ± 8 ms ($P<0.05$). Subsequent application of SR140333 (100 nM) to the recording chamber did not further reduce the amplitudes of responses to either stimulus.

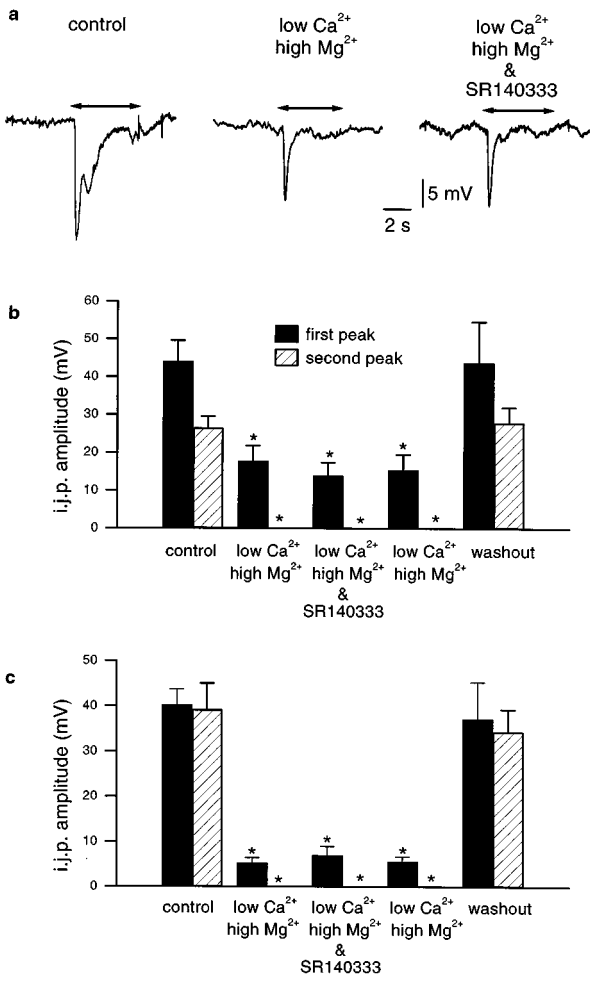

Figure 2 Effect of SR140333 (100 nM) in the recording chamber on descending reflexes after synaptic blockade in the near stimulation chamber with physiological saline containing 0.1 mM Ca^{2+} and 10 mM Mg^{2+}. (a) Electrophysiological records of i.j.ps evoked by distension in the near stimulation chamber. (b) Amplitude of first and second peaks of i.j.ps evoked by near chamber distension (mean± s.e.mean). (c) Amplitude of first and second peaks of responses to near chamber compression. *Significantly different from control ($P<0.001$; $n=7$).

When added to the near chamber, neither NK_1 antagonist affected distension or compression reflex responses evoked from the near chamber (Table 1). However, SR140333 in this chamber reduced the mean amplitude of i.j.ps evoked by distension in the far chamber by 15%.

Effect of NK_1 receptor agonists on descending reflexes

An NK_1 receptor agonist, septide (Wormser *et al*., 1986), when added to the recording chamber perfusate at 10 nM (an intermediate concentration which would be expected to activate but not desensitise NK_1 receptors; see Figure 3), substantially enhanced descending reflex responses, but did not alter the resting membrane potential of circular muscle cells (Figure 1b). The amplitudes of the first peaks of the i.j.ps evoked by distension or compression in the near chamber were enhanced by 30–40% and second peak amplitudes were potentiated by approximately 60–70% (see Table 2). I.j.ps evoked by far chamber stimuli were also greatly enhanced. These increases were all blocked by application of SR140333 (100 nM) 15 min before the agonist in the recording chamber.

In contrast, the same concentration (10 nM) of another selective NK_1 receptor agonist, [Sar^9,$Met(O_2)^{11}$]substance P (Drapeau *et al*., 1987), had no effect on the reflex responses evoked from either near or far chambers when added to the

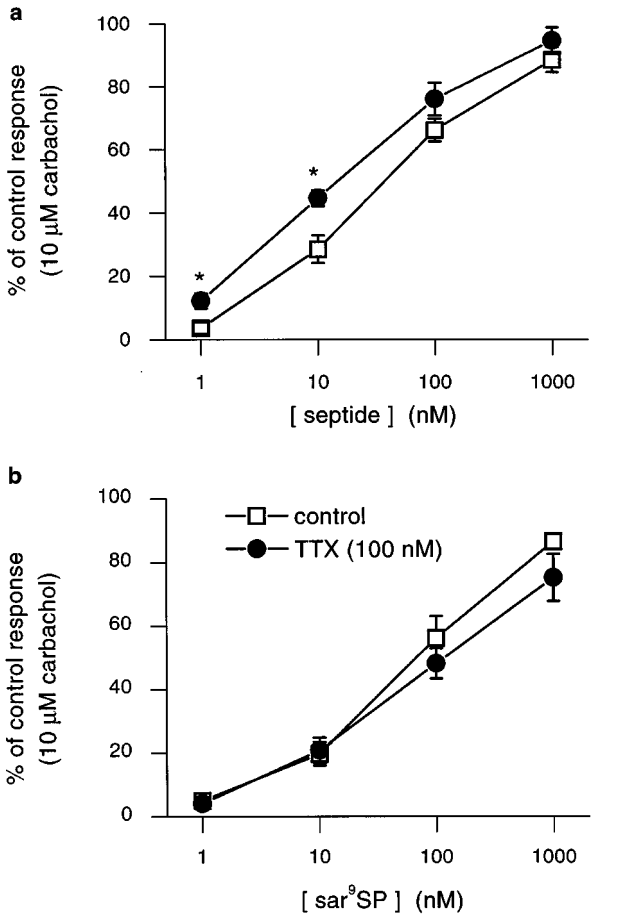

Figure 3 Concentration-effect curves for (a) septide ($n=10$) and (b) [Sar^9,$Met(O_2)^{11}$]substance P ($n=8$) in the absence and presence of TTX (300 nM). TTX potentiated contractile responses of the circular muscle to septide but not [Sar^9,$Met(O_2)^{11}$]substance P. *Significantly different from control ($P<0.01$).

recording chamber (Figure 1c; Table 2). However, a 10 fold higher concentration of [Sar^9,Met(O$_2$)^{11}]substance P (100 nM) significantly potentiated the mean amplitude of the second peak of i.j.ps evoked by near chamber distension by 60% (Table 2; Figure 1d). Amplitudes of responses to near chamber compression or far chamber stimuli remained unaffected. At this concentration, [Sar^9,Met(O$_2$)^{11}]substance P also depolarised circular muscle cells by 5–10 mV ($P<0.05$; $n=8-9$).

No effect of NK$_1$ receptor blockade on ascending excitatory reflexes

SR140333 (100 nM), when applied to the near chamber in ascending reflex experiments, had no effect on the amplitudes of e.j.ps evoked by distension or compression either within this chamber or the far chamber ($n=5$; data not shown). The effect of NK$_1$ receptor antagonists in the recording chamber in the ascending pathway was not tested, as effects on neuro-neuronal transmission could not be discriminated from effects on circular muscle (Maggi *et al*., 1994a).

Contractile organ bath experiments–effect of NK$_1$ receptor agonists

Septide and [Sar^9,Met(O$_2$)^{11}]substance P (1–1000 nM) evoked similar concentration-dependent enhancements of the rhythmic contractions of the circular muscle in the presence of indomethacin. Their EC$_{50}$ values were 24 nM and 60 nM respectively (Figure 3). Responses to low concentrations of septide (1–10 nM), but not [Sar^9,Met(O$_2$)^{11}]substance P, were significantly potentiated by TTX (300 nM; $P<0.01$; $n=8$; see also Burcher & Stamatakos, 1994).

Effect of an NK$_3$ receptor antagonist on descending reflexes

Initially, the effects of the NK$_3$ receptor antagonist SR142801 (100 nM) in the near stimulation chamber on descending reflexes were tested over a period of 75 min of exposure with repeated trials at 15 min intervals. The peak amplitudes of the i.j.ps evoked by mucosal compression in the near chamber were depressed by SR142801 by 40% when compared to vehicle (Table 3). This effect was maximal after 30 min, although previous studies indicated that SR142801 requires a considerably longer incubation time (Patacchini *et al*., 1995). In subsequent experiments, a 30 min exposure time was used and vehicle controls were eliminated.

SR142801 had no effect on reflexes evoked by distension in the near chamber or those evoked by either stimulus in the far chamber. When added to the recording chamber, the antagonist did not alter the amplitude or time course of descending reflexes evoked from either stimulation chamber ($n=6$; data not shown).

Effect of NK$_3$ receptor blockade on ascending reflexes

When applied to the near chamber in experiments on the ascending pathway, SR142801 (100 nM) did not alter the amplitudes of e.j.ps evoked by distension or compression in either stimulation chamber (Table 4). However, when applied to the recording chamber, the mean amplitudes of e.j.ps evoked by compression of the mucosa in the near and far chambers were reduced by 30%. Responses to distension in the near chamber were also decreased by 30%.

Hexamethonium, a nicotinic receptor antagonist (200 μM), reduced the amplitude of e.j.ps evoked by distension and

Table 2 Effect of NK$_1$ receptor agonists applied in the recording chamber on descending reflexes evoked by distension or compression in the near or far chamber

Treatment (in recording chamber)	*Near chamber distension*		*Near chamber compression*		*Far chamber distension*	*Far chamber compression*
	First peak	*Second peak*	*First peak*	*Second peak*	*Absolute peak*	*Absolute peak*
septide (10 nM)	142±30* (8)	172±24* (7)	133±23* (6)	163±33* (6)	166±18* (7)	167±33* (6)
septide (10 nM) & SR140333 (100 nM)	88±7 (5)	91±8 (5)	105±17 (4)	101±21 (4)	114±22 (4)	89±8 (4)
[Sar^9, Met(O$_2$)^{11}]-SP (10 nM)	88±9 (7)	98±12 (7)	94±13 (7)	98±22 (7)	82±13 (7)	103±13 (7)
[Sar^9, Met(O$_2$)^{11}]-SP (100 nM)	100±7 (10)	158±18* (9)	99±14 (9)	135±37 (8)	113±23 (7)	112±26 (9)

Numbers indicate amplitudes of i.j.ps in the presence of agonist expressed as a percentage of control i.j.p. amplitudes (mean±s.e. mean (n)) *Significantly different from control ($P<0.05$).

Table 3 Effect of an NK$_3$ receptor antagonist (SR142801) in the near chamber and comparison with effect of senktide desensitisation on descending reflexes

Treatment	*Near chamber distension*	*Near chamber compression*	*Far chamber distension*	*Far chamber compression*
Vehicle (0.1% ethanol)	113±11 (6)	110±8 (6)	110±10 (6)	92±11 (6)
SR142801 (100 nM)	101±13 (7)	64±6* (7)	107±22 (6)	77±9 (6)
Senktide (1 μM)	65±7** (6)	60±7** (6)	25±7** (6)	36±8** (6)
Senktide (1 μM) & SR142801 (100 nM)	86±5 (6)	46±6** (6)	36±7** (6)	30±12** (6)
Sektide (1 μM) & naloxone (1 μM)	43±3** (5)	42±3** (5)	19±4** (5)	32±6** (5)

Numbers indicate amplitudes of i.j.ps in the presence of drug treatment expressed as a percentage of control i.j.p. amplitudes (mean±s.e.mean (n)) *significantly different from vehicle ($P<0.01$); **significantly different from control ($P<0.05$).

Table 4 Effect of an NK_3 receptor antagonist applied in the near or recording chamber on ascending reflexes evoked by distension or compression in the near or far chamber.

Treatment	*Near chamber distension*	*Near chamber compression*	*Far chamber distension*	*Far chamber compression*
Control	14±2 (5)	16±1 (5)	11±3 (5)	13±3 (5)
SR142801 (100 nM) in near chamber	14±2 (5)	13±2 (5)	10±3 (5)	11±3 (5)
Control	13±1 (6)	13±1 (7)	11±2 (7)	14±1 (7)
SR142801 (100 nM) in recording chamber	9±1* (6)	9±1* (7)	9±2 (7)	10±1* (7)

Numbers indicate absolute e.j.p. amplitudes (mV; mean±s.e.mean (n)) *significantly different from control ($P<0.05$).

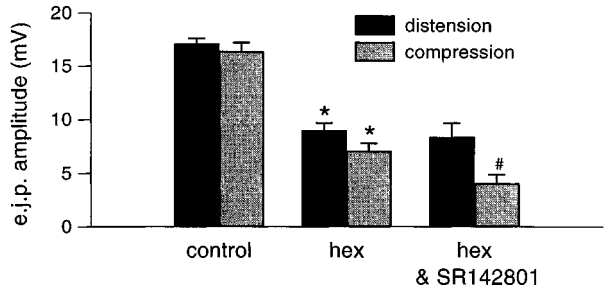

Figure 4 Effect of hexamethonium and SR142801 on ascending reflexes. Amplitudes of e.j.ps evoked by distension and mucosal compression in the absence and presence of hexamethonium (hex; 200 μM) and SR142801 (100 nM; $n=6$; mean±s.e.mean). *Significantly different from control ($P<0.005$); #significantly different from hexamethonium ($P<0.05$).

compression in the near chamber by 50–60% when applied to this chamber (Figure 4). Subsequent application of SR142801 (100 nM) reduced the amplitude of the hexamethonium resistant responses to compression by a further 40% ($P<0.05$; $n=7$). Responses to distension were not further altered by SR142801.

Comparison of effects of SR142801 and senktide desensitisation

In a previous study using an identical divided bath system, it was found that a desensitising concentration of the selective NK_3 receptor agonist senktide (1 μM) in the near chamber substantially inhibited descending reflexes evoked by stimulation in either the near or far chamber (Johnson *et al.*, 1996; see Table 3). In the present study, pretreatment with SR142801 (100 nM for 30 min) blocked the effect of senktide desensitisation on responses to near chamber distension, but did not inhibit the effect of senktide in the near chamber on reflexes evoked from the far chamber (Table 3).

It has been reported that senktide can inhibit contractile reflexes by evoking release of endogenous opioids (Izzo *et al.*, 1995). However, in the present study the inhibitory effects of senktide on descending reflexes persisted in the presence of the opioid receptor antagonist naloxone (1 μM).

Contractile organ bath experiments–effect of NK_3 receptor agonists and antagonists

In light of the differences between the effects of SR142801 and senktide desensitisation, the interactions between these drugs were re-evaluated using isolated organ bath methods.

Senktide (0.3–300 nM) evoked a concentration-dependent enhancement of rhythmic circular muscle contractions observed in indomethacin and this was significantly inhibited by

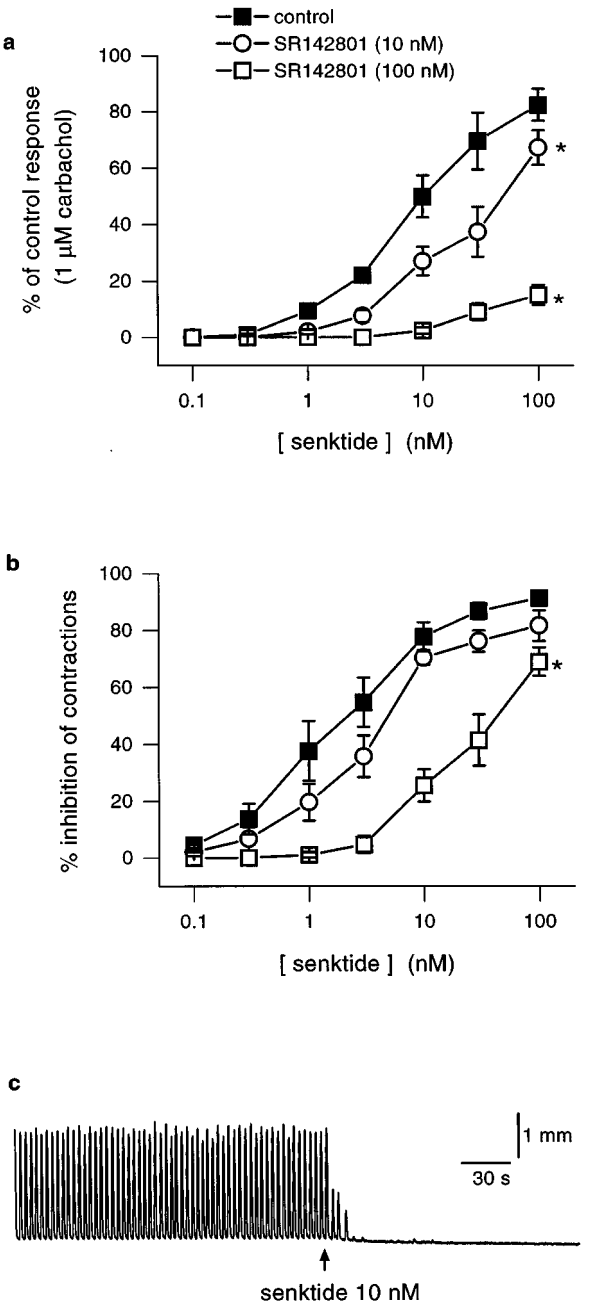

Figure 5 Effect of SR142801 on contractile responses of isolated rings of ileum to senktide. Concentration-effect curves for senktide alone and in the presence of SR142801 for (a) excitatory circular muscle contractions in the presence of NOLA (100 mM) and apamin (100 nM; $n=6$) and (b) inhibition of contractions in the presence of indomethacin (10 μM), hyoscine (100 nM), SR 140333 (100 nM) and SR48968 (100 nM). Results are expressed as mean±s.e.mean. *Significantly different from control ($P<0.05$). (c) Representative record illustrating the inhibitory effect of senktide (10 nM; applied at the arrow) on rhythmic circular muscle contractions.

SR142801 (10 – 100 nM). The antagonist shifted the concentration-effect curve for senktide in an apparently non-competitive and insurmountable manner (Figure 5a).

When excitatory neuromuscular transmission was blocked with the muscarinic antagonist hyoscine (100 nM) and the selective NK_1 and NK_2 receptor antagonists SR140333 and SR48968 (100 nM; Emonds-Alt *et al*, 1992), the contractile response to senktide was converted to an inhibition of rhythmic contractions (Figure 5c). The effect of SR142801 on this inhibitory action of senktide appeared to be competitive and surmountable (Figure 5b). Indeed, the antagonistic effect of SR142801 (10 nM) on inhibitory responses to senktide was significantly less than its effect on excitatory senktide responses ($P<0.05$).

Discussion

The present study extends and refines the conclusions of previous studies that tachykinin receptors play significant roles in transmission between neurons during motility reflexes in the guinea-pig small intestine. The results indicate that NK_3 receptors partly mediate transmission from intrinsic sensory neurons to ascending and descending interneurons and between ascending interneurons and excitatory motor neurons. They also provide direct evidence that neural NK_1 receptors are also activated during motility reflexes and contribute to transmission to inhibitory motor neurons (Figure 6).

Properties of the descending inhibitory reflex pathway

Descending reflex pathways can be subdivided on the basis of sensory modality, projections of the intrinsic sensory neurons and sensitivity to NK_1 and NK_3 receptor agonists and antagonists.

Activation of descending reflexes by stimuli in an adjacent chamber evoked biphasic i.j.ps in the recording chamber. The second phase was abolished by blockade of synaptic transmission in the stimulation chamber, indicating that it was due to synaptic activation of interneurons in this chamber by intrinsic sensory neurons with local or circumferential projections. The early phase of the response to distension was relatively insensitive to synaptic blockade, and may have been largely mediated by intrinsic sensory neurons that projected from the stimulus chamber into the recording chamber (Brookes *et al*., 1995; Johnson *et al*., 1996). Responses to mechanical compression of the mucosa were much more sensitive to blockade of transmission in the stimulus chamber than responses evoked by distension. If the descending intrinsic sensory neurons acted on motor neurons via interneurons, such interneurons would make further contacts more anally (Costa *et al*., 1996a), thereby maintaining the two components of the responses over longer conduction distances. However, descending reflex responses evoked by stimuli applied in the far chamber were consistently monophasic. Thus, the first phase of the i.j.ps evoked by near chamber stimuli is probably due to monosynaptic transmission from descending intrinsic sensory neurons to inhibitory motor neurons.

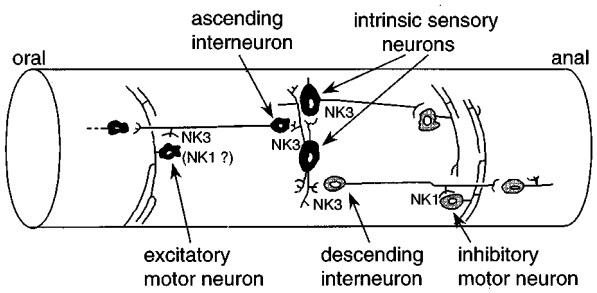

Figure 6 Diagram of the motility reflex nerve circuitry in the guinea-pig ileum (Bornstein 1994; Furness *et al*., 1994) showing the synapses at which NK_1 and NK_3 receptors play a role in transmission between neurons. Functional classes of neuron that contain tachykinins are shown in black; those which do not are shown in grey.

Blockade of the polysynaptic pathway significantly increased the latency of i.j.p. responses. Electrophysiological estimates of the conduction velocities of intrinsic sensory neurons (about 30 cm s^{-1}) and descending interneurons (about 50 cm s^{-1}; Stebbing & Bornstein, 1996) suggest that conduction along the polysynaptic pathway (i.e. second peak of i.j.p.) should be faster than in the monosynaptic pathway. Thus, although the polysynaptic component of the reflex takes longer to reach its peak, this component may have a shorter latency and partly underly the first peak of the i.j.p. This is consistent with the reduction in the first peak seen when synaptic transmission is blocked in the stimulation chamber.

Role of NK_1 and NK_3 receptors in descending reflexes

NK_1 antagonists inhibited descending reflexes when applied in the recording chamber. Both SR140333 and CP99994 depressed the second phase of i.j.ps evoked by distension or compression in the near chamber. SR140333 also reduced reflexes evoked from the far stimulation chamber and inhibited the first phase of responses to near chamber stimuli, possibly due to removal of the initial part of the second phase. After the polysynaptic component of the reflex was removed by blockade of synaptic transmission in the near chamber, SR140333 in the recording chamber had no further effect, suggesting that NK_1 receptors do not contribute to transmission from descending intrinsic sensory neurons to inhibitory motor neurons. This indicates that activation of NK_1 receptors is necessary for the normal excitation of inhibitory motor neurons by descending interneurons (however, see Natural ligands).

These results are consistent with other evidence that NK_1 receptors are present on inhibitory motor neurons. NK_1 receptors have been localised to neurons identified as inhibitory motor neurons by their immunoreactivity for nitric oxide synthase (Portbury *et al*., 1996). The present observation that descending reflexes are enhanced by NK_1 agonists is also consistent with this hypothesis. However, septide was much more effective than [Sar^9,$Met(O_2)^{11}$]substance P in enhancing descending reflexes. In addition, TTX enhanced the contractile effect of septide on the circular muscle, but not that of [Sar^9,$Met(O_2)^{11}$]substance P, a result that had previously been observed for longitudinal muscle (Burcher & Stamatakos; 1994) and suggested as evidence for the presence of a 'septide-preferring' receptor on inhibitory motor neurons. However, recent data indicates that both agonists activate the same population of receptors, suggesting that the higher potency of septide seen in these experiments could be explained by the recently proposed 'dual conformer' NK_1 receptor model (Maggi & Schwartz, 1997).

The selective NK_3 receptor antagonist, SR142801 did not affect descending reflexes evoked by distension, but depressed responses to mucosal compression when applied in the stimulation chamber. Only the second peak of the i.j.ps was affected, suggesting that the antagonist inhibited transmission from compression-sensitive intrinsic sensory neurons to

descending interneurons. These results differ markedly from those of our previous study in which exposure to a selective NK_3 receptor agonist, senktide, was used to presumably desensitise NK_3 receptors (Johnson *et al*., 1996). In those experiments, transmission from distension-sensitive intrinsic sensory neurons and between descending interneurons was also markedly reduced. Such difference could not be due to a senktide-evoked release of inhibitory opioids, as naloxone did not modify the effect of senktide desensitisation in the present study.

It is worth noting that while SR142801 prevented the depression of distension reflexes normally produced by senktide desensitisation in the stimulus region, it did not inhibit the effect of senktide desensitisation on transmission between descending interneurons. This suggests that NK_3 receptors on myenteric neurons in the guinea-pig intestine are heterogeneous and differentially sensitive to SR142801. This is consistent with the results of the present contractile experiments in which the nature of the antagonism by SR142801 clearly differed when the actions of senktide on excitatory and inhibitory pathways were examined separately. Similar evidence has been provided by Guiliani & Maggi (1995), who found that, in the isolated guinea-pig colon, senktide produced a slowly developing (>15 min) and sustained inhibition of cholinergic twitch contractions evoked by electrical field stimulation. This action of senktide was only poorly antagonised by SR142801, but senktide-induced relaxations of the same preparation were inhibited more potently. Interestingly, an NK_3-like receptor with a distinctly different pharmacological profile to classical NK_3 receptors has recently been identified in a human c-DNA library (Krause *et al*., 1996).

Ascending reflexes

The picture for the ascending reflex pathway is considerably simpler than for the descending pathway. Morphological and physiological evidence indicates that intrinsic sensory neurons have no significant oral projection (Bornstein *et al*., 1991; Johnson *et al*., 1996), thus drugs applied to the stimulus chamber would have access to all active synapses from intrinsic sensory neurons to their targets. NK_1 receptor blockade did not alter ascending reflexes when applied to either the region of output of intrinsic sensory neurons or to the intermediate chamber where interneuron to interneuron synapses would be affected, suggesting that NK_1 receptors do not mediate transmission along ascending reflex pathways. The NK_3 receptor antagonist alone also had no effect when applied to the site of stimulus, however, after nicotinic transmission in this region was abolished by hexamethonium, the residual response to mucosal compression was reduced by SR142801. This supports the earlier conclusion that NK_3 receptors are involved in transmission at synapses between intrinsic sensory neurons and ascending interneurons (Johnson *et al*., 1996).

NK_3 receptor blockade in the recording chamber also reduced the amplitude of ascending reflexes, presumably by inhibiting transmission from ascending interneurons to excitatory motor neurons. This is consistent with the observation that activation of NK_3 receptors releases ACh from the myenteric plexus (Yau *et al*., 1992). Similar results have been reported in abstract form by Costa *et al*. (1996b) who found that SR142801 inhibited a high threshold component of ascending reflexes after both nicotinic and muscarinic receptors were blocked (see also Tonini & Costa, 1990).

Natural ligands

Immunohistochemical studies have shown that tachykinins are present in all myenteric ascending interneurons and most intrinsic sensory neurons in both myenteric and submucous ganglia (Costa *et al*., 1996a). This correlates with the physiological localisation of the functional sites of NK_3 receptors deduced in the present study. However, because tachykinins are not found in descending interneurons, the inhibition of descending reflexes by NK_1 receptor blockade in the recording chamber may not be due to a blockade of transmission from these neurons. An alternative source of tachykinins in this region of the pathway may be the terminals of local intrinsic sensory neurons. Kunze *et al*. (1997) have shown that the excitability of myenteric neurons close to intact mucosa is enhanced, apparently as a result of spontaneous activity in intrinsic sensory neurons with processes in the mucosa. This enhancement is largely confined to neurons of the descending reflex pathway and appears to be due to ongoing slow synaptic potentials, which would be consistent with an action of a tachykinin on either NK_1 or NK_3 receptors. Another explanation is that the natural ligand acting at NK_1 receptors on inhibitory motor neurons is not a tachykinin but an unidentified neurotransmitter.

There are a number of possible explanations for the discrepancies between the effects of the drugs used in this study on reflexes evoked by distension and mucosal compression. Distension and compression stimulate separate populations of intrinsic sensory neurons (Smith *et al*., 1991; 1992; Johnson *et al*., 1996), and it is possible that reflex responses are conducted by different neural pathways utilising distinct transmitters, although the final motor neurons are common (Smith *et al*., 1992). Alternatively, the discrepancies may relate to the relative strengths of the stimuli. The relative contribution of tachykinins to excitatory neuromuscular transmission increases with stimulus strength (Costa *et al*, 1985; Bartho *et al*., 1992). The compression stimuli used in this study were nearer to maximal than the distension stimuli (Furness *et al*., 1995), so that NK_3 receptors may play a greater role in the response to mucosal compression.

Conclusion

NK_1 receptors play a significant role in determining the effectiveness of transmission to inhibitory circular muscle motor neurons during motility reflexes of the guinea-pig ileum. In contrast, NK_3 receptors appears to be involved in transmission from intrinsic sensory neurons in both ascending and descending reflex pathways, and also from ascending interneurons to excitatory motor neurons (Figure 6).

This work was supported by a grant from the National Health & Medical Research Council of Australia. We wish to thank Professor J.B. Furness for helpful comments on the manuscript, and Dr. Emonds-Alt from Sanofi Recherche for the generous gifts of SR140333, SR142801 and SR48968.

References

BARTHO, L., SANTICIOLI, P., PATACCHINI, R. & MAGGI, C.A. (1992). Tachykininergic transmission to the circular muscle of the guinea-pig ileum: Evidence for the involvement of NK2 receptors. *Br. J. Pharmacol.,* **105,** 805–810.

BAYLISS, W.M. & STARLING EH. (1899). The movements and innervation of the small intestine. *J. Physiol.,* **24,** 99–143.

BORNSTEIN, J.C. (1994). Local neural control of intestinal motility: nerve circuits deduced for the guinea-pig small intestine. *Clin. Exp. Pharmacol. Physiol.,* **21,** 441–452.

BORNSTEIN, J.C., COSTA, M., FURNESS, J.B. & LANG, R.J. (1986). Electrophysiological analysis of projections of enteric inhibitory motor neurones in the guinea-pig small intestine. *J. Physiol.,* **370,** 61–74.

BORNSTEIN, J.C., HENDRIKS, R., FURNESS, J.B. & TRUSSELL, D.C. (1991). Ramifications of the axons of AH-neurons injected with the intracellular marker biocytin in the myenteric plexus of the guinea pig small intestine. *J. Comp. Neurol.,* **314,** 437–451.

BROOKES, S.J.H., SONG, Z.-M., RAMSAY, G.A. & COSTA, M. (1995). Long aboral projections of Dogiel type II, AH neurons within the myenteric plexus of the guinea-pig small intestine. *J. Neuroscience,* **15,** 4013–4022.

BURCHER, E. & STAMATAKOS, C. (1994). Septide but not substance P stimulates inhibitory neurons in guinea-pig ileum. *Eur. J. Pharmacol.,* **258,** R9–R10.

COSTA, M. & FURNESS, J.B. (1976). The peristaltic reflex: an analysis of nerve pathways and their pharmacology. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **294,** 47–60.

COSTA, M., BROOKES, S.J.H., STEELE, P.A., GIBBINS, I., BURCHER, E. & KANDIAH, C.J. (1996a). Neurochemical classification of myenteric neurons in the guinea-pig ileum. *Neuroscience,* **75,** 949–967.

COSTA, M., FURNESS, J.B., PULLIN, C.O. & BORNSTEIN, J.C. (1985). Substance P enteric neurons mediate non-cholinergic transmission to the circular muscle of the guinea-pig intestine. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **328,** 446–453.

COSTA, M., IZZO, A., BROOKES, S.J.H. & HUMPHREYS, C. (1996b). The action of a new NK3 receptor antagonist on enteric reflexes and peristalsis. *Proc. Aust. Neuroscience Soc.,* **7,** 66.

DINGLEDINE, R. & GOLDSTEIN, A. (1976). Single neuron studies of opiate action in the guinea-pig myenteric plexus. *Life Sci.,* **17,** 57–62.

DRAPEAU, G., D'ORLEANS-JUSTE, P., DION, S., RHALEB, N.E., ROUISSI, N.E. & REGOLI, D. (1987). Selective agonists for substance P and neurokinin receptors. *Neuropeptides,* **10,** 43–54.

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., LE FUR G. & BRELIERE, J.-C. (1995). SR142801, the first potent non-peptide antagonist of the tachykinin NK_3 receptor. *Life Sci.,* **56,** PL27–32.

EMONDS-ALT, X., DOUTREMEPUICH, J.-D., HEAULME, M., NELIAT, G., SANTUCCI, V., STEINBERG, R., VILAIN, P., BICHON, D., DUCOUX, J.-P., PROIETTO, V., VAN BROECK, D., SOUBRIE, P., LE FUR, G. & BRELIERE, J.-C. (1993). In vitro and in vivo biological activities of SR140333, a novel potent non-peptide tachykinin NK_1 receptor antagonist. *Eur. J. Pharmacol.,* **250,** 403–413.

EMONDS-ALT, X., VILAIN, P., GOULAOUIC, 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,** PL101–106.

FURNESS, J.B., BORNSTEIN, J.C., POMPOLO, S., YOUNG, H.M., KUNZE, W.A.A. & KELLY, H. (1994). The circuitry of the enteric nervous system. *Neurogastroenterol. Mot.,* **6,** 241–253.

FURNESS, J.B., JOHNSON, P., POMPOLO, S. & BORNSTEIN, J.C. (1995). Evidence that enteric motility reflexes can be initiated through entirely intrinsic mechanisms in the small intestine. *Neurogastroenterol. Mot.,* **7,** 89–96.

GALLIGAN, J.J., TOKIMASA, T. & NORTH, R.A. (1987). Effects of three mammalian tachykinins on single enteric neurons. *Neuroscience Letters.,* **82,** 167–171.

GUARD, S., MCKNIGHT, A.T., WATLING, K. & WATSON, S.P. (1991). Evidence for two types of tachykinin receptors on cholinergic neurons of the guinea-pig ileum myenteric plexus. *Annals N.Y. Acad. Sci.,* **632,** 400–403.

GUARD, S. & WATSON, S.P. (1987). Evidence for neurokinin-3 receptor-mediated tachykinin release in the guinea-pig ileum. *Eur. J. Pharmacol.,* **144,** 409–412.

GIULIANI, S. & MAGGI, C.A. (1995). Effect of SR 142801 on nitric oxide-dependent and independent responses to tachykinin NK_3 receptor agonists in isolated guinea-pig colon. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **352,** 512–519.

HANANI, M., CHOREV, M., GILON, C. & SELINGER, Z. (1988). The actions of receptor-selective substance P analogues on myenteric neurons: an electrophysiological investigation. *Eur. J. Pharmacol.,* **153,** 247–253.

HOLZER, P. & HOLZER-PETSCHE, U. (1997). Tachykinins in the gut. Part I. Expression, release and motor function. *Pharmacol-Ther.,* **73,** 173–217.

HOLZER, P., SCHULET, W. & MAGGI, C.A. (1995). Substance P stimulates and inhibits intestinal peristalsis via distinct receptors. *J. Pharm. Exp. Ther.,* **274,** 322–328.

HUMPHREYS, C.M.S., COSTA, M. & BROOKES, S.J.H. (1991). Nitric oxide mediates the apamin-insensitive component of transmission from enteric inhibitory motor neurons to the circular muscle of the guinea-pig small intestine and colon. *Proc. Aust. Physiol. Pharmacol. Soc.,* **22,** 144P.

IZZO, A., COSTA, M., HUMPHREYS, C. & BROOKES, S.J.H. (1995). The effect of an NK3 agonist on the enteric ascending excitatory reflex in the guinea-pig small intestine. *Proc. Aust. Physiol. Pharmacol. Soc.,* **26,** 199P.

JOHNSON, S.M., KATAYAMA, Y., MORITA, K. & NORTH, R.A. (1981). Mediators of slow synaptic potentials in the myenteric plexus of the guinea-pig ileum. *J. Physiol.,* **320,** 175–186.

JOHNSON, P.J., BORNSTEIN, J.C., YUAN, S.Y. & FURNESS, J.B. (1996). Analysis of contributions of acetylcholine and tachykinins to neuro-neuronal transmission in motility reflexes in the guinea-pig ileum. *Br. J. Pharmacol.,* **118,** 973–983.

KATAYAMA, Y. & NORTH, R.A. (1978). Does substance P mediate slow synaptic excitation within the myenteric plexus? *Nature,* **274,** 387–388.

KRAUSE, J.E., STVETEIG, P.T., MENTZER, J.N., SCHMIDT, S.K., TUCKER, J.B., BRODBECK, R.M., BU, J.Y. & KARPITSHIY, V.V. (1996). Functional expression of a novel human neurokinin-3 receptor homolog that binds [3H]senktide and [125I-MePhe7]-neurokinin B, and is responsive to tachykinin peptide agonists. *Proc. Nat. Acad. Sci.,* **94,** 310–315.

KUNZE, W.A.A., BERTRAND, P.P., FURNESS, J.B. & BORNSTEIN, J.C. (1997). Influence of the mucosa on the excitability of myenteric neurons. *Neuroscience,* **76,** 619–634.

LAUFER, R., WORMSER, U., FRIEDMAN, Z.Y., GILON, C., CHOREV, M. & SELINGER, Z. (1985). Neurokinin B is a preferred agonist for neuronal substance P receptor and its action is antagonized by enkephalin. *Proc. Nat. Acad. Sci. U.S.A.,* **82,** 7444–7448.

LYSTER, D.J., BYWATER, R.A.R., TAYLOR, G.S. & WATSON, M.J. (1992). Effects of a nitric oxide synthase inhibitor on non-cholinergic junction potentials in the circular muscle of the guinea-pig ileum. *J. Auton. Nerv. Sys.,* **41,** 187–196.

MAGGI, C.A. (1995). The mammalian tachykinin receptors. *Gen. Pharmacol.,* **26,** 911–944.

MAGGI, C.A., PATACCHINI, R., BARTHO, L., HOLZER, P. & SANCTICIOLI, P. (1994a). Tachykinin NK_1 and NK_2 receptor antagonists and atropine-resistant ascending excitatory reflex to the circular muscle of the guinea-pig ileum. *Br. J. Pharmacol.,* **112,** 161–168.

MAGGI, C.A., PATACCHINI, R., GIACHETTI, A. & MELI, A. (1990). Tachykinin receptors in the circular muscle of the guinea-pig ileum. *Br. J. Pharmacol.,* **101,** 996–1000.

MAGGI, C.A., PATACCHINI, R., MEINI, S. & GIULIANI, S. (1993). Nitric oxide is the mediator of tachykinin NK_3 receptor-induced relaxation in the circular muscle of the guinea-pig ileum. *Eur. J. Pharmacol.,* **240,** 45–50.

MAGGI, C.A., PATACCHINI, R., MEINI, S. & GUILIANI, S. (1994b). Effect of longitudinal muscle-myenteric plexus removal and indomethacin on the response to tachykinin NK-2 and NK-3 receptor agonists in the circular muscle of the guinea-pig ileum. *J. Auton. Pharmacol.,* **14,** 49–60.

MAGGI, C.A. & SCHWARTZ, T.W. (1997). The dual nature of the tachykinin NK_1 receptor. *TIPS,* **18,** 351–355.

MACLACHLAN, E.M. & MARTIN, A.R. (1981). Non-linear summation of end-plate potentials in the frog and mouse. *J. Physiol.,* **311,** 307–324.

MACLEAN, S., SNIDER, R.M., DESAI, M.C., ROSEN, T., BRYCE, D.K., LONGO, K.P., SCHMIDT, A.W. & HEYM, J. (1993). CP 99,994, a non-peptide antagonist of the tachykinin NK1 receptor. *Regul. Pept.,* **46,** 329–331.

PATACCHINI, R., BARTHO, L., HOLZER, P. & MAGGI, C.A. (1995). Activity of SR 142801 at peripheral tachykinin receptors. *Eur. J. Pharmacol.,* **278,** 17–25.

PORTBURY, A.L., FURNESS, J.B., YOUNG, H.M., SOUTHWELL, B.R. & VIGNA, S.R. (1996). Localisation of NK_1 receptor immunoreactivity to neurons and interstitial cells of the guinea-pig gastrointestinal tract. *J. Comp. Neurol.,* **367,** 342–351.

SMITH, T.K., BORNSTEIN, J.C. & FURNESS, J.B. (1991). Interaction between reflexes evoked by distension and mucosal stimulation: electrophysiological studies of guinea-pig ileum. *J. Auton. Nerv. Syst.,* **34,** 69–76.

SMITH, T.K., BORNSTEIN, J.C. & FURNESS, J.B. (1992). Convergence of reflex pathways excited by distension and mechanical stimulation of the mucosa onto the same myenteric neurons of the guinea pig small intestine. *J. Neurosci.,* **12,** 1502–1510.

STEBBING, M.J. & BORNSTEIN, J.C. (1996). Electrophysiological mapping of fast excitatory synaptic inputs to morphologically and chemically characterised myenteric neurons of guinea-pig small intestine. *Neuroscience,* **73,** 1017–1028.

TONINI, M. & COSTA, M. (1990). A pharmacological analysis of the neuronal circuitry involved in distension-evoked enteric excitatory reflex. *Neuroscience,* **38,** 787–795.

WORMSER, U., LAUFER, R., HART, Y., CHOREV, M., GILON, C. & SELINGER, Z. (1986). Highly selective agonists for substance P receptor subtypes. *EMBO J.,* **5,** 2805–2808.

YAY, W.M., MANDEL, K.G., DORSETT, J.A. & YOUTHER, M.L. (1992). Neurokinin$_3$ receptor regulation of acetylcholine release from myenteric plexus. *Am. J. Physiol.,* **263,** G659–G664.

YUAN, S.Y., BORNSTEIN, J.C. & FURNESS, J.B. (1994). Investigation of the role of 5-HT_3 and 5-HT_4 receptors in ascending and descending reflexes to the circular muscle of guinea-pig small intestine. *Br. J. Pharmacol.,* **112,** 1095–1100.

(Received December 8 1997
revised April 3 1998
accepted May 1 1998)