

Evidence for functional NK₁-tachykinin receptors on motor neurones supplying the circular muscle of guinea-pig small and large intestine

X-C. BIAN,* P. P. BERTRAND,* J. B. FURNESS† & J. C. BORNSTEIN*

*Departments of Physiology and †Anatomy & Cell Biology, University of Melbourne, Parkville, Australia

Abstract *The guinea-pig intestine was investigated to determine which neurones are excited via NK₁ receptors. The specific NK₁ receptor agonists [Sar⁹, Met(O₂)¹¹]-SP and septide contracted the circular muscle of all regions via a tetrodotoxin (TTX)-insensitive mechanism. In the proximal colon, they also evoked a TTX-sensitive relaxation; in the distal colon, the contractions were larger when nerve impulses were blocked with TTX, indicating that the agonists excited inhibitory motor neurones. In the duodenum and ileum, TTX reduced agonist-evoked contractions indicating that excitatory motor neurones were activated. In the presence of indomethacin, TTX enhanced contractions of ileal circular muscle evoked by these agonists suggesting that NK₁ receptors were on inhibitory motor neurones. Blockade of nitric oxide synthase (NOS) enhanced NK₁ receptor agonist evoked contractions of duodenal circular muscle, indicating that the agonists excite inhibitory motor neurones in duodenum. Neurones immunoreactive for NK₁ receptors were studied in the duodenum and distal colon. As reported previously for the ileum,¹ some neurones were immunoreactive for NOS and had Dogiel type I morphology; features characteristic of inhibitory motor neurones. In conclusion, there are functional NK₁ receptors on excitatory and inhibitory motor neurones in the guinea-pig small intestine and on inhibitory motor neurones in the colon.*

Keywords *enteric nervous system, immunohistochemistry, NK₁ receptors, pharmacology, tachykinin.*

Address for correspondence

Dr X-C. Bian, Department of Physiology,
University of Melbourne,
Parkville, VIC 3010, Australia.
Tel.: + 61 3 8344 4466; fax: + 61 3 8344 5818;
e-mail: x.bian@physiology.unimelb.edu.au
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INTRODUCTION

Tachykinins, including substance P, NKA (and possibly NPK and NPγ) are cotransmitters with acetylcholine in excitatory motor neurones supplying intestinal smooth muscle, and are likely to be transmitters at synapses between neurones in the enteric nervous system (for recent reviews see^{2–4}). These peptides are found in many enteric neurones including intrinsic primary afferent neurones, in both the myenteric and submucosal plexuses, excitatory motor neurones supplying the circular and longitudinal muscle layers, and interneurones.⁵ They are also in the terminals of extrinsic primary afferent neurones.⁵ All three known subtypes of tachykinin receptors, NK₁, NK₂ and NK₃, are found in the mammalian gastrointestinal tract, although their individual locations differ markedly from each other. For example, immunohistochemical studies indicate that NK₁ receptors are located on the cell bodies of enteric neurones and on interstitial cells of Cajal in the guinea-pig small intestine,⁶ while NK₂ receptors are prominent on the smooth muscle and are seen in some nerve terminals of the same preparation.⁷ By contrast, a large body of pharmacological evidence indicates that NK₁ receptors are also on the muscle and that NK₃ receptors are located exclusively on enteric neurones and are absent from the smooth muscle layers.^{8–10}

Activation of NK₁ receptors by exogenously applied agonists produces contractions of the circular muscle in almost all regions of the guinea-pig intestine.³ In the ileum, this effect is partially mediated via intrinsic neurones and partly via a direct action on the smooth muscle or the interstitial cells of Cajal that are electrically coupled to the muscle.¹¹ However, immunohistochemical studies of the location of NK₁ receptors in the myenteric plexus of the guinea-pig ileum indicate that they are predominantly on the inhibitory motor neurones supplying the circular muscle, with

only a small proportion on the excitatory motor neurones.^{1,6} Thus, the pharmacological data on the effects of tachykinins on neural NK₁ receptors contrasts with immunohistochemical data on the locations of these receptors. Furthermore, although tachykinins are predominantly found in neurones of the ascending excitatory reflex pathway, a study of their role in ascending reflexes suggests that NK₁ receptors play no role in transmission between neurones in this pathway.¹² Rather, this study identified a significant, but subtle, role in transmission between neurones during descending inhibitory reflexes.¹² Thus, the relative contributions of the NK₁ receptors on different neuronal subtypes to contractions of the guinea-pig ileum remain unclear and even fewer data are available about the specific locations of NK₁ receptors elsewhere in the gastrointestinal tract.

The goal of the present study was to determine the specific neuronal subtypes that are excited via NK₁ receptors within the guinea-pig ileum, duodenum, proximal colon and distal colon. The major part of the study involved a pharmacological analysis of the responses of the different intestinal regions to specific NK₁-receptor agonists. The results of this study were then correlated with an immunohistochemical study of the locations of NK₁-receptor-like immunoreactivity within the duodenum and distal colon and with the results of Lomax *et al.*¹ on the ileum.

MATERIALS AND METHODS

Guinea-pigs of both genders in the weight range 200–300 g were killed by a blow to the head followed by severing of the carotid arteries. Experiments were approved by the University of Melbourne Animal Experimentation Ethics Committee.

Segments (5–6 cm in length) of duodenum, ileum, proximal colon or distal colon were taken immediately after the guinea-pig was killed and placed in physiological saline (composition in mmol L⁻¹: NaCl 118; KCl 4.8; NaH₂PO₄ 1.0; NaCO₃ 25; MgSO₄ 1.2; D-glucose 11.12 and CaCl₂ 2.5) bubbled with 95% O₂, 5% CO₂ at room temperature. In some experiments, indomethacin (1 μM) was added to the saline. In each case, the tissue was pinned down in a small dissecting dish without any stretching, any mesenteric attachments were removed and intact rings of intestine (3 mm wide) were then cut from the segment and opened to create circumferentially orientated strips. The strips were then suspended between stationary hooks and isotonic force transducers in organ baths containing physiological saline

at 37 °C. The mechanical activity of the strips was recorded via an A/D converter (Biopac Systems Inc., CA, USA) onto the hard drive of a personal computer.

After the muscle strips were set up, they were equilibrated for 60–120 min during which the physiological saline was exchanged every 10–15 min. Carbachol (10 μM), applied to the strips for 2 min and then washed out, was used to contract the tissue at intervals of 10–30 min. Consistent contractions to carbachol were usually obtained within a further 60 min. Responses to other agonists were expressed as a percentage of the mean carbachol response at the end of this equilibration period (see below). To ensure that the preparation was stable over the time course of the experiment, carbachol was added between construction of concentration–effect curves and at the end of the experiment (see below). When tissues were pretreated with phosphoramidon (1 μM) or tetrodotoxin (TTX, 300 nM), these drugs were added 10–15 min before responses to agonists were tested and were then present throughout the experiment.

Two different protocols were used in these experiments. In the first, strips were set up in paired organ baths with one serving as the control for the other. In this case, noncumulative concentration–effect curves for NK₁ receptor agonists (pGlu⁶, Pro⁹]-SP(6–11) (Septide) and [Sar⁹, Met(O₂)¹¹]-SP) were constructed in each organ bath, with drugs expected to modify these curves being added to one bath, but not the other. In each experiment, four organ baths were used so that the two agonists were studied in parallel on tissues from the same animal. In the second case, paired organ baths were also used, but two noncumulative concentration–effect curves were constructed for each strip, with a drug expected to modify the curves added to one bath between construction of the first and second curve and the other bath serving as a time control. In practice, these two protocols led to identical results and the data are pooled in the analysis below.

Drugs used

The drugs used were: apamin (Alomone Labs, Ltd. Jerusalem, Israel), carbachol (Merck & Co., USA), hexamethonium, indomethacin, phosphoramidon (Sigma Chemical Co., MO, USA), N^G-nitro-L-arginine (NOLARG; Research Biochemical International, Natick, MA, USA), [Sar⁹, Met(O₂)¹¹]-SP, [pGlu⁶, Pro⁹]-SP(6–11) (septide; Auspep Pty. Ltd, VIC, Australia) and tetrodotoxin (TTX; Sapphire Bioscience Pty. Ltd, NSW, Australia).

Analysis of results

All data analyses were based on 5–6 replicates for each experiment. Responses to agonists were assessed by measuring the maximum change in length of the preparation during a 2-min exposure to the drug. Responses to agonists were expressed as percentages of the mean contraction caused by carbachol during the equilibration period to allow for variations in circular muscle contractility between preparations. Statistical analysis was performed by paired *t*-test or by analysis of variance when applicable. *P* < 0.05 was considered statistically significant.

Immunohistochemistry

Segments of duodenum and distal colon were removed and placed in phosphate-buffered saline (PBS: 0.9% NaCl in 0.01 M sodium phosphate buffer, pH = 7.0), containing the L-type Ca²⁺ channel blocker nifedipine (10⁻⁵ M) to relax the muscle. The segments were cut open along the mesenteric border and the contents were flushed away with PBS. The tissue was pinned taut on balsa board with the mucosal surface facing down and placed in Zamboni's fixative (2% formaldehyde and 0.2% picric acid in 0.1 M sodium phosphate buffer, pH = 7.0) for 24 h at 4 °C. After fixation, the tissue was cleared with 3 × 10 min washes in dimethylsulphoxide (DMSO), followed by 3 × 10 min washes in PBS. Wholmount preparations of myenteric plexus with attached longitudinal muscle or of submucosal plexus were prepared from both the duodenum and distal colon for each of six animals.

Wholmount preparations of myenteric and submucosal plexuses were preincubated in 10% normal horse serum in PBS for 30 min at room temperature. After blotting off excess serum, the tissues were incubated in two primary antisera raised against the NK₁ receptor and against nitric oxide synthase (NOS). The primary antiserum to NK₁ receptor was raised in a rabbit against the C-terminal 15 amino acids of the rat NK₁ receptor (antiserum code, #94168; Grady *et al.* 1996) and was diluted to 1:200 in hypertonic PBS (NaCl 0.5 M). The antiserum against NOS was raised in a sheep and diluted to 1:1000 (Emson; H205). Tissue was incubated for 24 h at room temperature, and then excess serum was washed off with 3 × 10 min changes in PBS and incubated in 1:100 donkey antirabbit IgG coupled to biotin (Jackson; #30801) in PBS for 2 h at room temperature. After this, the tissue was washed again in PBS and incubated in 1:50 streptavidin coupled to Texas Red (Amersham; Batch 31) and 1:12.5 donkey antisheep FITC (Jackson; #27979) for 1 h at room

temperature. The tissue was washed in PBS for 3 × 10 and then mounted in buffered glycerol (0.5 M sodium carbonate buffer in glycerol, pH = 8.6).

After immunohistochemical staining, all preparations were examined under a Zeiss Axioplan microscope with a 63x oil immersion objective (using filter set 10 for FITC, filter set 14 for Texas Red). The fluorescence images were collected by an Image Point cooled CCD camera (Photo-metrics Ltd, AZ, USA) and V for Windows imaging software (Digital Optics Ltd, Auckland, New Zealand). The images were further processed by PhotoPaint and CorelDraw (Corel Corp., Dublin, Ireland).

RESULTS

Pharmacology

Both [Sar⁹, Met(O₂)¹¹]-SP and septide (1 nM–10 μM) caused concentration-dependent contractions of circular muscle strips of duodenum, ileum and distal colon (Fig. 1). The EC₅₀ for [Sar⁹, Met(O₂)¹¹]-SP was 67 nM in duodenum, 450 nM in ileum and 170 nM in distal colon and for septide was 160 nM in duodenum, 200 nM in ileum and 60 nM in distal colon. The maximum responses were very similar to the amplitudes of the responses to carbachol (10 μM) in the same preparations. The contractions evoked by the NK₁ receptor agonists were not significantly altered by pretreatment with phosphoramidon, a peptidase inhibitor, in any of these preparations (*P* > 0.05, two-way ANOVA).

The effects of [Sar⁹, Met(O₂)¹¹]-SP and septide on circular strips of proximal colon were biphasic. In six experiments with [Sar⁹, Met(O₂)¹¹]-SP and four out of six experiments with septide, the preparations initially contracted on exposure to the agonist and then showed a late relaxation. In order to distinguish actions of the agonists on neurones and muscle, the effects of TTX (300 nM) on the evoked responses were examined. It was assumed that, because TTX blocks action potential initiation and propagation in enteric neurones, contractions seen in the presence of this toxin would reflect only direct actions on the smooth muscle. TTX abolished the relaxation in each case, but had no effect on the initial contraction (Fig. 2). When seen, the relaxations were evoked by concentrations of agonist as low as 10 nM and appeared to be maximal once evoked. This made calculation of the EC₅₀ for either the myogenic contraction or the neurogenic relaxation unreliable.

Sites of action of [Sar⁹, Met(O₂)¹¹]-SP and septide in duodenum, ileum and distal colon Three

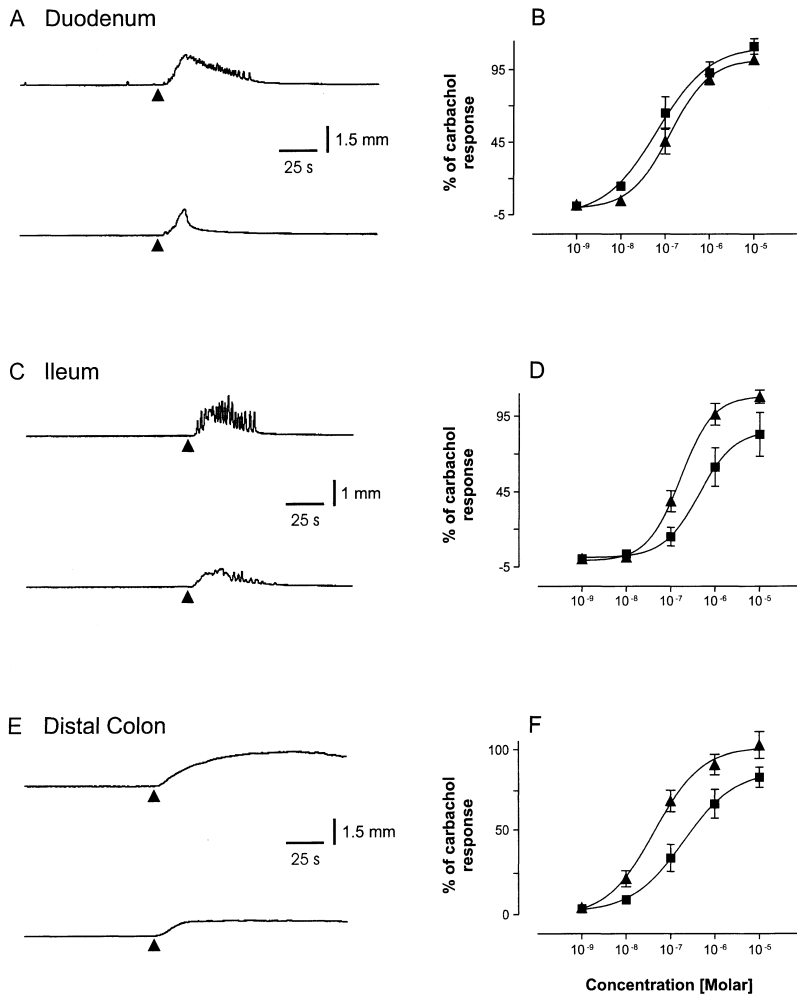


Figure 1 Original tracings (A, C, E), and concentration-effect curves (B, D, F) for the contractions of circular muscle strips evoked by septide (upper traces) and [Sar⁹, Met(O₂)¹¹]-SP; (A, B) duodenum (C, D) ileum (E, F) distal colon. In A, C and E, ▲ indicates the time of application of the agonist. In B, D and F, ■ denotes the responses to septide and ▲ denotes the responses to [Sar⁹, Met(O₂)¹¹]-SP; all responses are expressed as percentages of the response to carbachol (10 μM) and each point shows the mean ± SEM of 5–6 experiments.

concentrations of each agonist were chosen that bracketed the EC₅₀s of the original concentration–effect curves (Fig. 1). These three concentrations were then used to construct noncumulative concentration–effect relations in the presence or absence of TTX for duodenum, ileum and distal colon.

In both duodenum and ileum, TTX significantly depressed the contractions evoked by either agonist (*P* < 0.001; two-way ANOVA) (Fig. 3A,B). For example, in

the duodenum, the response to [Sar⁹, Met(O₂)¹¹]-SP (100 nM) was reduced from 33% to 5% of the carbachol response by TTX. TTX reduced the contraction evoked by septide (100 nM) from 61% to 31% of the carbachol response. In the ileum, the response to [Sar⁹, Met(O₂)¹¹]-SP (500 nM) was depressed from 48% to 17% of the carbachol response by TTX, while the response to septide (100 nM) was reduced from 53% to 19%.

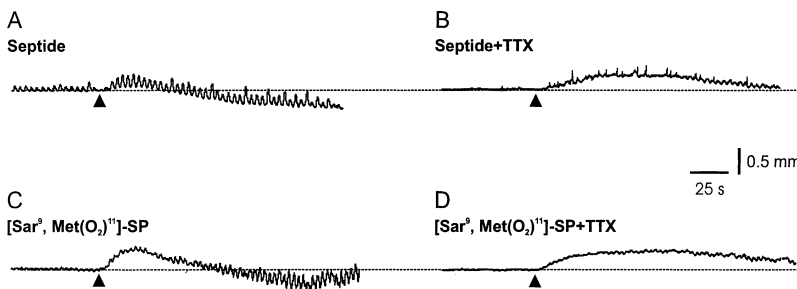
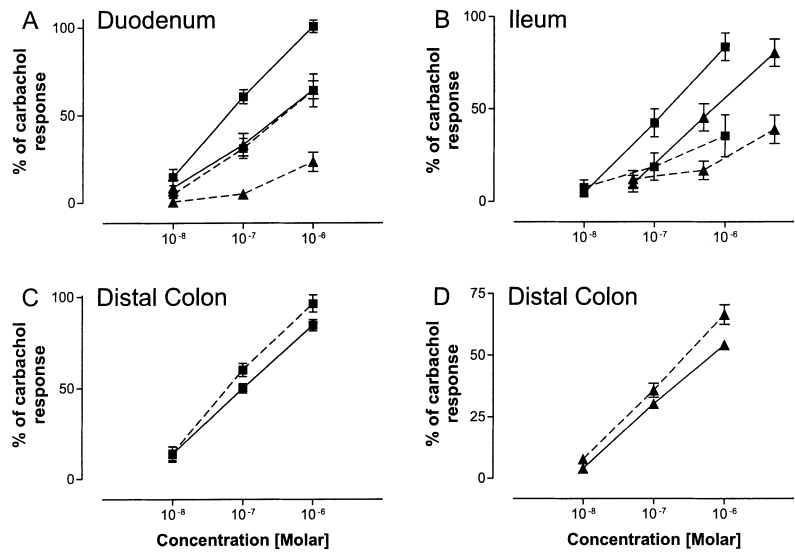


Figure 2 Response of circular muscle strips from the proximal colon of the guinea-pig to septide (A, B) and [Sar⁹, Met(O₂)¹¹]-SP (C, D), each at 100 nM. Each pair of traces shows the mechanical responses of the strips in the absence (A, C) and in the presence of TTX (B, D). ▲ shows the point of application of the agonists.

Figure 3 Effects of TTX (300 nM) on contractions evoked by NK₁ receptor agonists when applied to circular muscle strips from the duodenum (A), the ileum (B) and the distal colon (C, D). ■, mean responses to septide; ▲, mean responses to [Sar⁹, Met(O₂)¹¹]-SP; solid lines are control concentration-effect curves while dashed lines are the equivalent curves in the presence of TTX. The effects of the agonists were significantly depressed in the duodenum and ileum ($P < 0.0001$, two-way ANOVA in each case), but significantly enhanced in the distal colon ($P < 0.05$, two-way ANOVA). Each value is the mean \pm SEM of 5–6 experiments.



By contrast with the small intestine, TTX in the distal colon produced a small, but significant, enhancement of the contractions evoked by the two agonists [Sar⁹, Met(O₂)¹¹]-SP, $P = 0.001$; two-way ANOVA; septide, $P = 0.02$; Fig. 3C,D. The enhancement of the contractile responses by TTX was greater at the higher concentrations of the agonists (Fig. 3C,D). The contraction evoked by [Sar⁹, Met(O₂)¹¹]-SP (1 μ M) increased from 54% to 66%, while that evoked by septide (100 nM) increased from 50% to 60% in the presence of TTX.

Contractions evoked by either [Sar⁹, Met(O₂)¹¹]-SP or septide in the duodenum and ileum were unaffected

by pretreatment with the nicotinic antagonist hexamethonium (100 μ M), see Figure 4.

Effects of septide on ileum in the presence of indomethacin In contrast to the results described above, Johnson *et al.*,¹² found that TTX enhanced the contractions of the circular muscle of guinea-pig ileum evoked by septide, but not those evoked by [Sar⁹, Met(O₂)¹¹]-SP. These earlier experiments were performed in the presence of the prostaglandin synthesis inhibitor, indomethacin. We repeated these experiments in the presence of indomethacin (10 μ M) to determine whether the effects of septide are modified

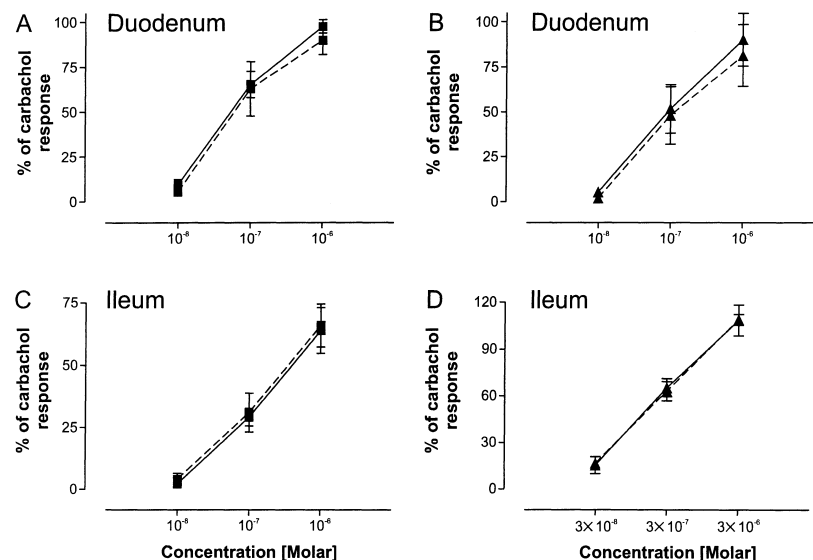


Figure 4 Effects of hexamethonium on the contractions evoked in duodenum (A, B) and ileum (C, D) by septide (A, C, ■) and [Sar⁹, Met(O₂)¹¹]-SP (B, D, ▲). Solid lines are control concentration-effect curves for the agonists; the dashed lines are the equivalent concentration-effect curves in the presence of hexamethonium (100 μ M). The curves are not significantly different ($P > 0.05$, two-way ANOVA).

in the absence of prostaglandins. Under these conditions, circular muscle preparations of ileum developed rhythmic contractile activity (at about 15 times min^{-1}) (Fig. 5A,B), while identical preparations in the absence of indomethacin were quiescent. Septide-evoked contractions in both control and indomethacin-treated preparations, but the potency of this agonist was markedly higher when prostaglandin synthesis was blocked ($P < 0.01$, two-way ANOVA). In all experiments, TTX increased the septide evoked contractions in the presence of indomethacin ($P < 0.05$, two-way ANOVA), while it depressed the effects of septide in the controls (Fig. 5C). This enhancement was more substantial at the lower concentrations of the agonist, e.g. the contraction evoked by 10 nM septide increased from 12% to 58% of the response to carbachol, while that evoked by 100 nM septide increased from 76% to 84%. These results suggest that NK₁ receptor agonists may activate inhibitory motor neurones supplying the circular muscle in the ileum.¹

Effects of [Sar⁹, Met(O₂)¹¹]-SP and septide on the duodenum in the presence of NOLARG In the duodenum, exposure to indomethacin appeared to abolish the change in contractions produced by TTX pretreatment (not illustrated). This suggested that indomethacin may have increased the relative contribution of inhibitory pathways to the duodenal response to NK₁ receptor agonists, i.e. that functional NK₁ receptors are found on neurones of the inhibitory pathways in the duodenum. This possibility was tested by examining the effects of inhibition of nitric oxide synthase on the contractions evoked by both agonists. NOLARG (30 μM) enhanced the contractions evoked by both [Sar⁹, Met(O₂)¹¹]-SP ($n = 6$) and septide ($n = 5$),

see Fig. 6, and this effect was significant for both agonists ($P < 0.05$; two-way ANOVA).

Immunohistochemistry

NK₁ receptor immunoreactivity in duodenum and distal colon NK₁ receptor immunoreactivity (NK₁r-IR) was observed in a minority of nerve cell bodies of both myenteric and submucosal plexuses of the duodenum, and in the myenteric plexus of the distal colon. Two morphological types of NK₁r-IR nerve cell bodies were observed in the myenteric plexus of the duodenum. One group consisted of medium to large neurones that were usually located at the edges of the ganglia or at the entry points of internodal strands. These neurones stained only weakly so their membranes were not always clearly delineated by the NK₁r-IR; such immunoreactivity could sometimes also be seen in the cell cytoplasm. However, where their shapes could be distinguished, the NK₁r-IR neurones had Dogiel type I morphologies with numerous lamellar dendrites protruding from the cell body (Fig. 7).

The cell bodies of the second type of NK₁r-IR neurones were, on average, smaller than those of the first type, but were much more strongly stained (Fig. 8). These neurones tended to be in the middle of the ganglia. They resembled a population of NK₁r-IR neurones that were found by Lomax *et al.*¹ to be secretomotor neurones. It was usually possible to distinguish short dendrites and sometimes a single long axon (Fig. 8). This group of neurones made up about 25% of the NK₁r-IR nerve cells identified.

In the submucosal plexus of duodenum, most NK₁r-IR cells were in the ganglia, although a few were in

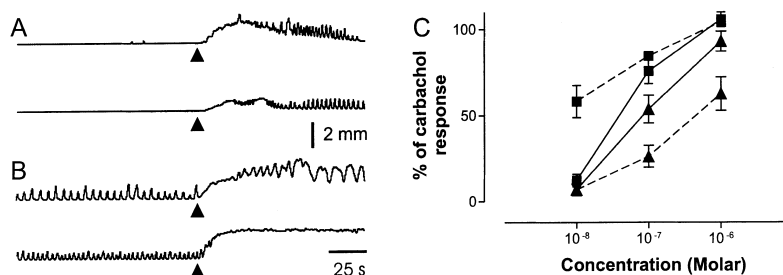


Figure 5 The effects of TTX on NK₁ receptor mediated contractions of circular muscle strips from the ileum in the presence and absence of indomethacin (10 μM). Panel (A) shows control responses to septide applied at the \blacktriangle ; the upper trace is in the control solution while the lower trace is in TTX. Panel (B) shows the responses to septide in the presence of indomethacin (upper trace) and indomethacin plus TTX (lower trace). Panel (C) shows concentration–effect curves in control (\blacktriangle , solid line) and in TTX (\blacktriangle , dashed line), and in indomethacin (\blacksquare , solid line) and indomethacin plus TTX (\blacksquare , dashed line). The effect of TTX was to decrease responses to septide in control solutions, but to significantly increase responses to low concentrations of septide in the presence of indomethacin ($P < 0.05$, two-way ANOVA).

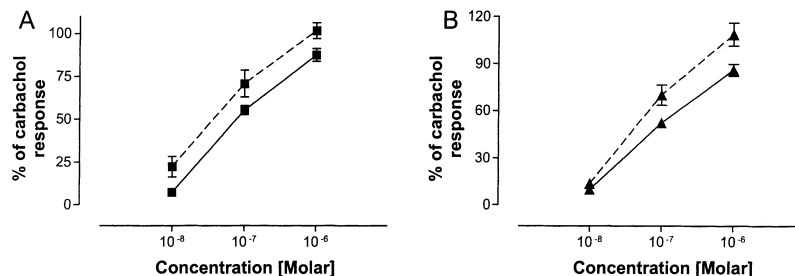


Figure 6 Effect of inhibition of nitric oxide synthase on the contractions of duodenal circular muscle evoked by NK₁ receptor agonists. Panel (A) shows contraction-effect curves for septide (■) in control (solid line) and in the presence of NOLARG (30 μM). Panel (B) shows concentration-effect curves for [Sar⁹, Met(O₂)¹¹]-SP (▲) in control (solid line) and in the presence of NOLARG (30 μM). In each case, the effects of the agonists were significantly enhanced in the presence of NOLARG (*P* < 0.05).

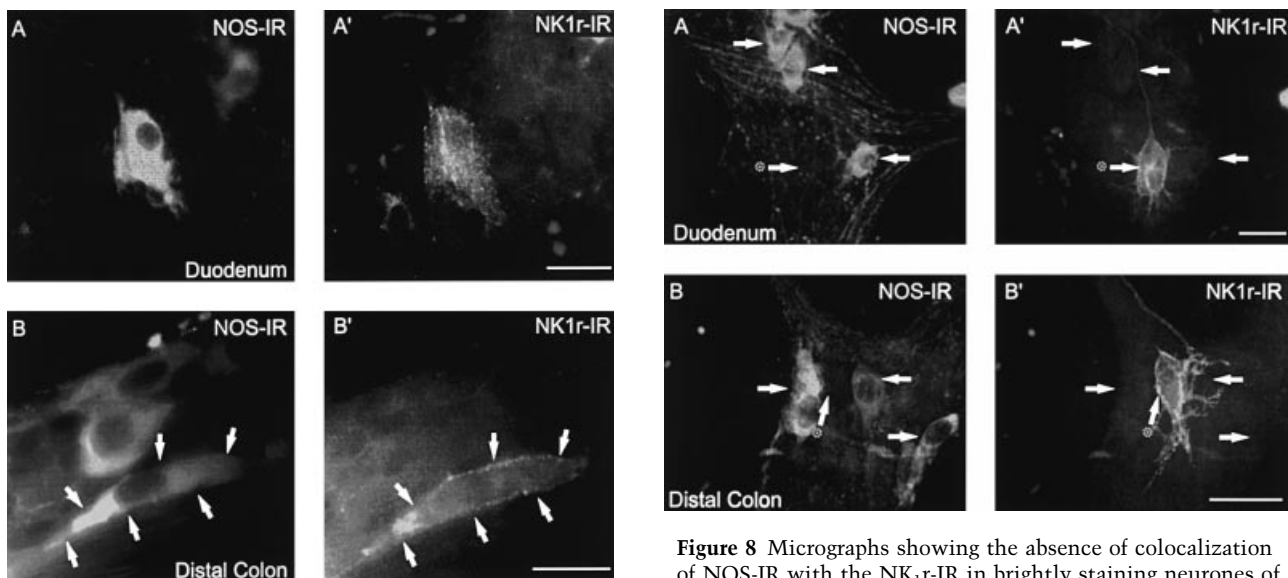


Figure 7 Micrographs showing colocalization of NOS-IR and NK₁r-IR in Dogiel type I neurons of the myenteric plexus of the duodenum (panels A and A', respectively) and the distal colon (panels B and B', respectively) of the guinea-pig. Only one of the three NOS-IR neurons in panel B was also immunoreactive for NK₁r (arrows). Scale bar: 20 μm.

internodal strands. These nerve cell bodies were of small to medium size and the immunoreactivity was normally confined to the surfaces of the cell bodies. The clear distinction between two types of NK₁r-IR cells seen in the myenteric plexus was not observed in the submucosal plexus of duodenum.

Fewer NK₁r-IR nerve cells were identified in the myenteric plexus of the distal colon than in the duodenum. The morphologies of the cells were identical to those of the neurons seen in the duodenum, although the proportion of the second type of NK₁r-IR cells in the distal colon was greater than in the duodenum. However, the background staining was very much

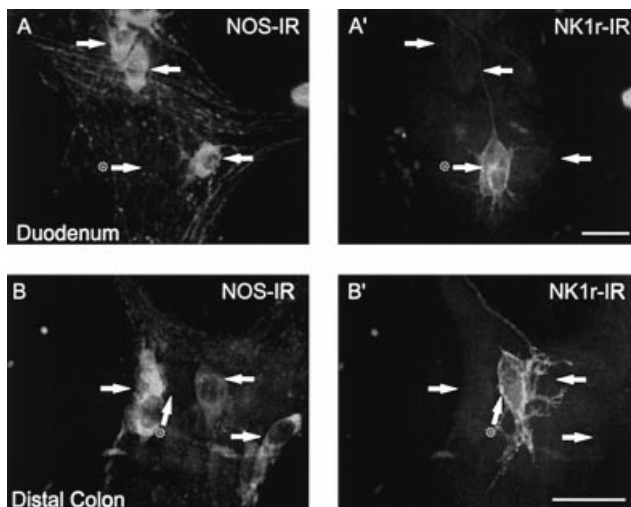


Figure 8 Micrographs showing the absence of colocalization of NOS-IR with the NK₁r-IR in brightly staining neurons of the myenteric plexus of the duodenum (A, A') and the distal colon (B, B'). The arrows show the location of NOS-IR neurons, while the arrows with asterisks show the locations of NK₁r-IR neurons. Scale bar: 20 μm.

higher in the distal colon than in duodenum and this made it difficult to distinguish the weakly staining NK₁r-IR neurons of the first type.

Colocalization of NK₁r-IR and NOS-IR In both the duodenum and distal colon, the weakly reactive NK₁r-IR myenteric neurons with Dogiel type I morphology were often NOS immunoreactive (NOS-IR) (Fig. 7). In contrast, the strongly staining type of NK₁r-IR cells were never NOS-IR in either region. To determine the proportion of NOS-IR neurons that were also reactive for NK₁r, 50 NOS-IR neurons were identified in preparations of myenteric plexus from the duodenum and the distal colon from each of six animals. Once a neurone had been identified as NOS-IR, the

microscope filters were switched and its immunoreactivity for NK₁r was determined. In the duodenum, 27 ± 6% (mean ± SEM) of the NOS-IR neurones were also immunoreactive for NK₁r, but in the distal colon only 4 ± 1% of the NOS-IR neurones appeared to contain the tachykinin receptors. A similar procedure, but with the order reversed, was undertaken to determine the number of neurones expressing NK₁ receptor immunoreactivity that also express NOS. In the duodenum, 55 ± 9% of NK₁r-IR neurones were immunoreactive for NOS, while in the colon 21 ± 5% of the NK₁r-IR neurones were NOS-IR.

DISCUSSION

The studies of agonist effects in the present work indicate that excitatory NK₁ tachykinin receptors are on neurones throughout the small and large intestines. Furthermore, they indicate that functional NK₁ receptors activate both excitatory and inhibitory motor neurones in the small intestine, while in the colon, NK₁ receptor agonists activate inhibitory neurones. The results also highlight a mismatch between the numbers of immunohistochemically identified neurones expressing NK₁ receptors and the predominant responses evoked by activation of such receptors.

In each of the four intestinal regions examined, blockade of neuronal action potentials with TTX produced a significant change in the contractions produced by either septide or [Sar⁹, Met(O₂)¹¹]-SP. In each region, TTX-resistant contractions were observed, which is consistent with the widespread finding that there are NK₁ tachykinin receptors within the smooth muscle layers,^{2,3} although these may be on interstitial cells of Cajal.⁶ In the ileum, TTX depressed the responses to the agonists, as had already been described by others,¹¹ and similar results were also obtained in the duodenum. These results suggest that, in the small intestine, a primary site of action of the NK₁ receptor agonists is on neurones of the excitatory reflex pathways. The specific site of action is likely to be on the excitatory motor neurones, because the effects of the agonists were not altered by the nicotinic blocker, hexamethonium, which would be expected to block fast synaptic input to these neurones.¹³ This result is somewhat surprising because previous immunohistochemical studies of the location of the NK₁ receptors in the myenteric plexus of the guinea-pig ileum^{1,6} and the current immunohistochemical study of these receptors in the duodenum, indicate that most neurones that express NK₁ receptors are inhibitory motor neurones. Furthermore, only a small fraction of the NK₁r-IR

neurones in the guinea-pig ileum can be characterized as excitatory motor neurones and the receptors are only found on a small proportion of such motor neurones.¹ An explanation for this apparent inconsistency may come from the observation that the tachykinin receptors are not strongly expressed in the inhibitory motor neurones. The more strongly staining neurones are likely to be secretomotor neurones,¹ rather than excitatory motor neurones. A second possibility comes from the observation that neurones responsive to NK₁ receptor agonists are not necessarily revealed by immunohistochemistry for the NK₁ receptor.¹⁴ Thus, excitatory motor neurones may be under represented in the immunohistochemical studies thus far.

Although the predominant effect of the NK₁ receptor agonists in the small intestine was on excitatory motor neurones, evidence for an effect on inhibitory motor neurones was also obtained in both the ileum and the duodenum. For example, blockade of the synthesis of nitric oxide enhanced the contractions evoked by each agonist, while the depression of the effect of septide seen in TTX was turned into a facilitation when indomethacin was present in the bathing solution. The presence of NK₁ receptors on the inhibitory motor neurones is consistent with the observation that NK₁ antagonists depress descending inhibitory reflexes in the ileum when applied to a region in which synapses between interneurones and inhibitory motor neurones would be specifically affected.¹²

In the large bowel, functional NK₁ receptors appeared to be confined to the inhibitory pathways. TTX enhanced the circular muscle contractions of the distal colon and blocked the circular muscle relaxations of the proximal colon evoked by each agonist. The immunohistochemical data indicate that some of these receptors may be on inhibitory motor neurones. However, the vast majority of NOS-IR neurones in the distal colon do not express immunohistochemically identifiable NK₁ receptors and many neurones expressing these receptors do not express NOS. Thus, it is possible that the inhibition evoked by the NK₁ receptor agonists is due to activation of neurones other than motor neurones in the inhibitory reflex pathways. Unfortunately, no known antagonist or combination of antagonists can completely block transmission between neurones of the descending inhibitory reflex pathway in the small intestine^{12,13} and presumably the same applies in the distal colon. Thus, no pharmacological test of the idea that the NK₁ receptors might be on interneurones or intrinsic primary afferent neurones was possible in the present study.

In the ileum, NK₁ receptors can be identified immunohistochemically on some intrinsic primary

afferent neurones.¹ Furthermore, NK₁ receptor agonists depolarize such neurones and, in some cases, NK₁ receptor antagonists depress or block electrically evoked slow excitatory synaptic potentials in these neurones.¹⁵ Thus, it is possible that some of the neurogenic inhibition evoked by septide and [Sar⁹, Met(O₂)¹¹]-SP in this study is due to activation of reflex pathways by an action on intrinsic primary afferent neurones. However, blockade of NK₁ receptors on the intrinsic primary afferent neurones excited by a physiological stimulus does not modify descending inhibitory reflexes.¹²

Thus, the results of the present study taken together with those of a variety of electrophysiological, pharmacological and immunohistochemical studies confirm that there are NK₁ receptors on neurones throughout both the small and large intestines. However, both the pharmacological and immunohistochemical results described here indicate that there are substantial regional variations in the distribution of these receptors between different functional groups of neurones. The functional significance of these variations remains to be determined.

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