

# Mediation of irregular spiking activity by multiple neurokinin-receptors in the small intestine of the rat

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**1** We have studied the small intestinal myoelectric response to the natural tachykinins substance P (SP), neurokinin A (NKA), neurokinin B (NKB), and the neurokinin-receptor selective agonists substance P methyl ester (SPME), [ $\beta$ -Ala<sup>8</sup>]neurokinin A 4–10, and senktide in conscious rats.

**2** The effects of the agonists were studied before and after administration of the selective neurokinin<sub>2</sub> (NK<sub>2</sub>)-receptor antagonist MEN 10,627.

**3** Under basal conditions SP, NKA, NKB, as well as the selective NK<sub>1</sub>-receptor agonist SPME, the NK<sub>2</sub>-receptor agonist [ $\beta$ -Ala<sup>8</sup>]NKA 4–10, and the NK<sub>3</sub>-receptor agonist senktide, disrupted the interdigestive rhythm with regularly recycling migrating myoelectric complexes and induced a phase II-like irregular spiking activity.

**4** MEN 10,627 given alone did not affect the interdigestive rhythm.

**5** MEN 10,627 inhibited the response to [ $\beta$ -Ala<sup>8</sup>]NKA 4–10 but not to SP, SPME, NKA, NKB or senktide.

**6** It is concluded that not only NK<sub>2</sub> receptors, but also other receptors, such as NK<sub>1</sub> and NK<sub>3</sub> receptors, may mediate the motility-stimulating action of different tachykinins *in vivo*.

**7** It is further concluded that MEN 10,627 exerts a selective NK<sub>2</sub>-receptor antagonism, and may be a valuable tool for assessing the functional role of NK<sub>2</sub>-receptors in gastrointestinal physiology.

**Keywords:** Tachykinins; tachykinin receptor agonists; tachykinin receptor antagonists; small intestinal motility

## Introduction

The mammalian tachykinins, substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), are found in the gastrointestinal tract of several species and are proposed to play a role in the regulation of gastrointestinal motility.

NKA (Maggio & Hunter, 1984; Theodorsson-Norheim *et al.*, 1984; Tateishi *et al.*, 1990; Takeda *et al.*, 1990) and SP (McGregor & Bloom, 1983; Takeda *et al.*, 1990) have been demonstrated in the small intestine of the rat with radioimmunoassay methods. However, there is some uncertainty whether NKB exists in the small intestine of rats (Sternini, 1991) and only small amounts of this tachykinin have been detected. However, immunohistochemistry has localized NKA, NKB (Tateishi *et al.*, 1990) and SP (Schultzberg *et al.*, 1980; Ekblad *et al.*, 1987) to intrinsic neurones of the myenteric plexus and peripheral endings of capsaicin-sensory nerves (Barthó & Holzer, 1985).

Investigations with radiolabelled tachykinins (Souquet *et al.*, 1985; Mantyh *et al.*, 1989; Gates *et al.*, 1989), receptor protection techniques (Kim & Hellström, 1993; Hellström *et al.*, 1994), as well as functional studies *in vitro* (Regoli *et al.*, 1991; Hellström *et al.*, 1994; Rahman *et al.*, 1994; Tolessa *et al.*, 1996), indicate the presence of three distinct tachykinin receptors in rat duodenum, the NK<sub>1</sub>, NK<sub>2</sub> and NK<sub>3</sub> receptors with the preferred agonists SP, NKA and NKB, respectively. Immunofluorescence and confocal microscopy have disclosed NK<sub>1</sub> receptors in the plasma membranes of myenteric and submucosal neurones and interstitial cells of Cajal, NK<sub>2</sub> receptors at the surface of longitudinal and circular muscle cells, and NK<sub>3</sub> receptors on myenteric and submucosal neurones (Grady *et al.*, 1996).

Motility of the small intestine serves to mix and transport its contents through the gut. The motor patterns of the small intestine are grossly divided into the fed and the interdigestive state. In the rat, the fed state is characterized by irregular contractions up to two hours following each meal. The characteristic pattern of the interdigestive state is the migrating myoelectrical complex (MMC) (Szurszewski, 1969) occurring in a cyclic sequence. The activity front, or phase III of the MMC, is identified as a period of clearly distinguishable intense electrical spiking, propagating distally over all recording sites, followed by a period of quiescence, phase I of MMC. Phase II of MMC is characterized as a period of irregular spiking preceding phase III. Intestinal myoelectric activity is considered to correlate to contractile activity (Ambache, 1947).

In the rat, mainly NKA, but also SP and NKB, disrupt the MMC pattern of the small intestine and induce irregular myoelectric activity (Lördal *et al.*, 1993). This response was attenuated by the non selective NK-receptor antagonist spantide, from which is cannot be deduced which of the receptor subtype(s) mediates the tachykinin-induced disruption of the MMC. However, in the same study, NKA and NKB, but not SP, was shown to hasten the transit of the contents in the small intestine (Lördal *et al.*, 1993).

The development of selective NK-receptor agonists and antagonists provides useful tools for studies of the importance of individual receptor subtypes in the physiology of tachykinins. MEN 10,627 (cyclo(Met-Asp-Trp-Phe-Dap-Leu)-cyclo(2 $\beta$ -5 $\beta$ )) is a polycyclic peptide tachykinin antagonist endowed with high affinity for the NK<sub>2</sub> receptor. The antagonism is of the competitive type. A 100 to 10,000 fold selectivity is documented compared to NK<sub>1</sub> or NK<sub>3</sub> receptors in different species (Maggi *et al.*, 1994).

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The aim of the present study was to disclose functions mediated by the three different NK receptors in the control of interdigestive motility of the small intestine in the rat, and to study the specificity of the NK<sub>2</sub> receptor antagonist MEN 10,627 as an inhibitor of tachykinin-induced motility in the gut.

## Methods

### Surgical procedure

A total of 46 male Sprague-Dawley rats (B&K, Sollentuna, Sweden) weighing 200–300 g were used. The animals were kept under standardized conditions (room temperature 22°C, humidity 60% and automatically regulated lighting in 12 h cycles).

The rats were anaesthetized with pentobarbitone (50 mg kg<sup>-1</sup>, i.p.) and their abdomens were opened via a midline incision. The animals were supplied with three bipolar insulated stainless steel electrodes (SS-5T, Clark Electromedical Instr., Reading, U.K.) implanted into the muscular wall of the small intestine 15 (J<sub>1</sub>), 25 (J<sub>2</sub>) and 35 (J<sub>3</sub>) cm distal to the pylorus. All animals were supplied with two catheters, one in each jugular vein, for administration of the test substances. The electrodes and catheters were tunnelled subcutaneously to exit at the back of the animals's neck. After surgery the animals were housed individually and allowed to recover for at least seven days before experiments were undertaken. During recovery, the rats were trained daily to accept experimental conditions. Experiments were then carried out in conscious animals after a 24 h fasting period with free access to water in wire-bottomed cages.

During the experiments, the rats were placed in Bollman cages. The electrodes were connected to an EEG pre-amplifier (7P5B) operating a Grass Polygraph 7 B (Grass Instr., Quincy, MA, U.S.A.), with a time constant set at 0.015 s, and the low and high cut-off frequencies at 10 Hz and 35 Hz, respectively. To enable detailed analysis of

spiking activity, the amplified myoelectric signal was connected to a computerized system for processing, as described earlier (Bränström & Hellström, 1996).

### Analysis of myoelectrical activity

The following elements were included in the analysis of the motility pattern during the control period and during infusion of either test substance: presence or absence of MMC, the interval, duration and propagation velocity of phase III if present, as well as the frequency of electric spiking during phase II or phase II-like activity, 20 min before and after the start of either test substance.

### Experimental protocol

The animals were divided into eleven groups, each group receiving different test substances. Twelve animals participated in two experiments and four animals participated in three experiments each. The interval between repeated experiments in one rat was at least three days. All experiments began with a control period, of approximately 80 min duration, for recording of basal myoelectric activity with four propagated activity fronts. After the fifth activity front had vanished at the first electrode site, infusion of each respective test substance was started with a microinjection pump (CMA 100, Carnegie Medicine, Stockholm, Sweden).

### Infusion of natural tachykinins

To study the effects of the peptide alone either SP (*n*=6), NKA (*n*=6) or NKB (*n*=6) was infused for 20 min. The doses chosen have in earlier studies been shown to disrupt the MMC and induce irregular spiking (Lördal *et al.*, 1993). After cessation of the infusion, the next activity front was awaited and after its propagation through the study segment, an infusion of MEN 10,627 was started. Five minutes later, one of the natural tachykinins was given again for a 20 min period.

**Table 1** Characteristics of MMC during infusion of natural tachykinins

Group	Interval (min)			Duration (min)			Velocity (cm min <sup>-1</sup> )	
	J <sub>1</sub>	J <sub>2</sub>	J <sub>3</sub>	J <sub>1</sub>	J <sub>2</sub>	J <sub>3</sub>	J <sub>1</sub> -J <sub>2</sub>	J <sub>2</sub> -J <sub>3</sub>
<b>SP</b>								
Control period	20.4 (16.3–24.5)	19.1 (16.4–21.8)	19.4 (16.6–22.2)	3.7 (2.5–4.9)	3.7 (3.1–4.3)	4.3 (3.3–5.2)	1.0 (0.8–1.2)	1.2 (1.1–1.3)
Infusion of SP	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS
SP/MEN 10,627	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS
<b>NKA</b>								
Control period	17.9 (13.6–22.2)	18.4 (13.9–22.9)	18.4 (13.9–22.9)	3.8 (2.7–4.9)	4.0 (3.3–4.7)	4.0 (3.1–4.9)	1.5 (1.2–1.8)	1.2 (0.9–1.6)
Infusion of NKA	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS
NKA/MEN 10,627	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS
<b>NKB</b>								
Control period	19.1 (14.2–24.1)	19.4 (13.9–25.0)	20.0 (14.1–25.8)	3.5 (2.9–4.1)	3.5 (2.8–4.1)	3.7 (3.3–4.2)	1.8 (1.1–2.5)	1.2 (0.9–1.6)
Infusion of NKB	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS
NKB/MEN 10,627	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS
<b>MEN 10,627</b>								
Control period	19.1 (14.2–24.1)	19.4 (13.9–25.0)	19.9 (14.1–25.8)	3.5 (2.9–4.1)	3.5 (2.9–4.1)	3.7 (3.3–4.1)	1.8 (1.1–2.5)	1.2 (0.9–1.5)
Infusion MEN 10,627	17.9 (9.3–26.5)	18.4 (13.0–23.8)	19.3 (13.7–24.9)	3.9 (2.5–5.3)	3.7 (3.0–4.3)	3.5 (2.6–4.4)	1.1 (0.8–1.4)	1.4 (1.0–1.7)

Values are mean and 95% confidence interval for all MMC cycles during the respective period. IRS=irregular spiking activity.

Table 2 Characteristics of MMC during infusion of NK receptor selective agonists

Group	Interval (min)			Duration (min)			Velocity (cm min <sup>-1</sup> )		
	J <sub>1</sub>	J <sub>2</sub>	J <sub>3</sub>	J <sub>1</sub>	J <sub>2</sub>	J <sub>3</sub>	J <sub>1</sub> -J <sub>2</sub>	J <sub>2</sub> -J <sub>3</sub>	
SPME									
Control period	21.2(13.6-28.8)	20.4(12.4-28.3)	21.4(14.1-28.7)	3.9(3.1-4.7)	3.8(2.9-4.7)	4.0(3.4-4.6)	1.3(0.9-1.7)	3.1(-1.5-7.7)	
Infusion of SPME	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS	
SPME/MEN 10,627	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS	
[β-Ala <sup>8</sup> ]-NKA(4-10)									
Control period	20.3(14.8-25.8)	21.6(16.7-26.5)	21.9(16.8-27.0)	3.9(1.6-6.3)	3.8(3.3-4.3)	4.3(3.6-5.1)	1.1(0.3-1.9)	1.3(0.9-1.8)	
Infusion of [β-Ala <sup>8</sup> ]-NKA(4-10)	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS	
[β-Ala <sup>8</sup> ]-NKA(4-10)/MEN 10,627	15.2(7.6-22.8)	15.1(10.6-19.6)	14.8(9.1-20.6)	3.0(2.1-3.9)	3.0(2.7-3.3)	3.4(2.7-4.1)	1.0(0.6-1.3)	1.5(0.7-2.2)	
[β-Ala <sup>8</sup> ]-NKA(4-10)									
Control period	18.0(15.3-20.7)	18.1(15.2-21.0)	18.3(15.0-21.7)	3.9(3.1-4.7)	3.5(3.0-4.1)	3.7(3.3-4.2)	1.1(0.9-1.3)	1.4(1.0-1.9)	
Infusion of senktide	17.2(12.5-22.0)	18.1(12.3-23.8)	19.0(12.2-25.8)	3.5(2.8-4.2)	3.2(2.4-4.1)	3.4(2.6-4.2)	1.5(0.9-2.0)	1.4(1.2-1.6)	
[β-Ala <sup>8</sup> ]-NKA(4-10) low dose									
Control period	20.4(14.0-26.7)	20.2(13.8-26.6)	21.4(13.0-29.8)	4.1(3.5-4.7)	4.0(3.2-4.7)	4.4(3.1-5.6)	1.7(0.3-3.1)	1.0(0.6-1.4)	
Infusion of senktide	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS	
Senktide/MEN 10,627	IRS	IRS	IRS	IRS	IRS	IRS	IRS	IRS	

Values are mean and 95% confidence interval for all MMC cycles during the respective period. IRS=irregular spiking activity.

### Infusion of selective neurokinin receptor agonists

The dose of each selective agonist was titrated to the lowest possible dose capable of replacing the MMC with irregular spiking.

SPME ( $n=6$ ), a NK<sub>1</sub> receptor agonist, or senktide ( $n=6$ ), a NK<sub>3</sub> receptor agonist, was administered for 20 min, to document the effects of the agonist alone. Thereafter, an activity front was awaited and after its propagation through the segment of the small intestine under study, infusion of MEN 10,627 was started. Five minutes later, one of the receptor agonists was given again for a period of 20 min.

The NK<sub>2</sub> receptor agonist [β-Ala<sup>8</sup>]NKA 4-10 was given according to the following four different regimens.

One group of animals ( $n=6$ ) received [β-Ala<sup>8</sup>]NKA 4-10 during the first 20 min after the control period. After the next activity front, an infusion of MEN 10,627 was started, followed after 5 min by an infusion of [β-Ala<sup>8</sup>]NKA 4-10 for 20 min.

A second group of animals ( $n=6$ ) received [β-Ala<sup>8</sup>]NKA 4-10 during the first 20 min after the control period. After the subsequent propagated activity from MEN 10,627 was administered, followed after 5 min by infusion of [β-Ala<sup>8</sup>]NKA 4-10 for 45 min to study its influence on the MMC.

A third group of animals ( $n=6$ ) received [β-Ala<sup>8</sup>]NKA 4-10 for 20 min after the control period. After an activity front reappeared and was propagated through the study segment, [β-Ala<sup>8</sup>]NKA 4-10 was given again for a period of 20 min to verify that no tachyphylaxis was obtained upon repeated administration of the peptide.

A fourth group of animals ( $n=6$ ) received a continuous infusion of [β-Ala<sup>8</sup>]NKA 4-10 alone at a low dose after the control period for a period of 45 min, to study whether the peptide might change the MMC pattern.

### Infusion of MEN 10,627

After the control period, MEN 10,627 alone was administered for a period of 45 min to study the effects of the antagonist itself on myoelectrical activity ( $n=6$ ). The dose of MEN 10,627 was chosen on the basis of earlier experiments in rats (Tramontana *et al.*, 1994).

### Chemicals

NKA, NKB, SP, SPME, [β-Ala<sup>8</sup>]-NKA 4-10 and senktide were purchased (Peninsula, Merseyside, U.K.). MEN 10,627 was a generous gift from Dr Carlo Maggi (Menarini Pharmaceuticals, Florence, Italy). NKA, SP, SPME, [β-Ala<sup>8</sup>]-NKA 4-10 and senktide were dissolved and diluted in 0.15 M NaCl solution. NKB was dissolved in 1 ml 6 M HCOOH before further dilutions in 0.15 M NaCl. MEN 10,627 was dissolved in dimethylsulphoxide (Sigma, St Louis, MO, U.S.A.) and further diluted in 0.15 M NaCl.

### Statistics

Results are expressed as means with 95% confidence intervals within parentheses. Differences between two groups were evaluated by the Mann-Whitney U-test, whereas differences between three or more groups were evaluated with the Kruskal-Wallis test. Differences within groups were evaluated by the Wilcoxon signed rank test.  $P<0.05$  was considered significant.

## Results

### Control conditions

During the control period, a regularly recycling MMC pattern was registered in all animals. The characteristics of the MMC are presented in Tables 1 and 2.

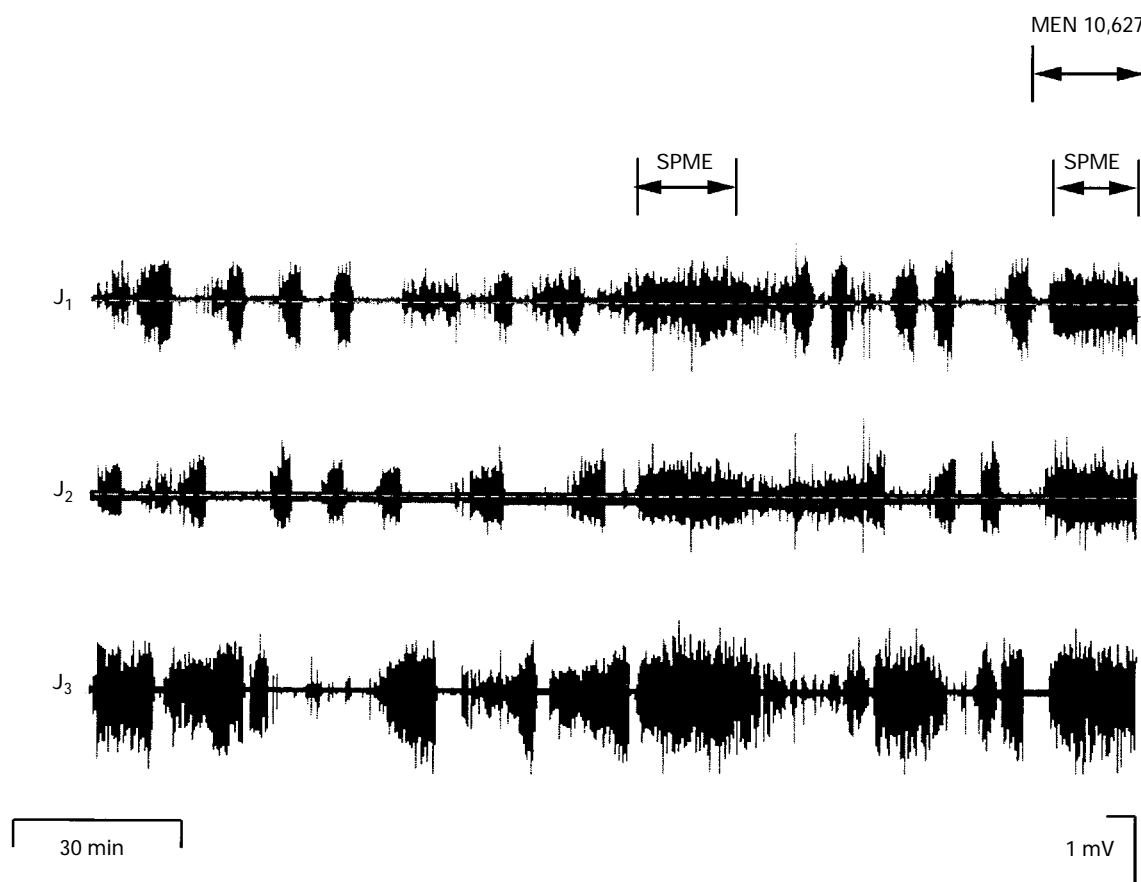
### Infusion of natural tachykinins

Infusion of SP, NKA or NKB disrupted the MMC pattern in all animals and induced intense irregular spiking activity resembling phase II of the MMC (Table 3). There were no significant differences in the intensity of spiking activity between the three groups. However, SP and NKB had to be

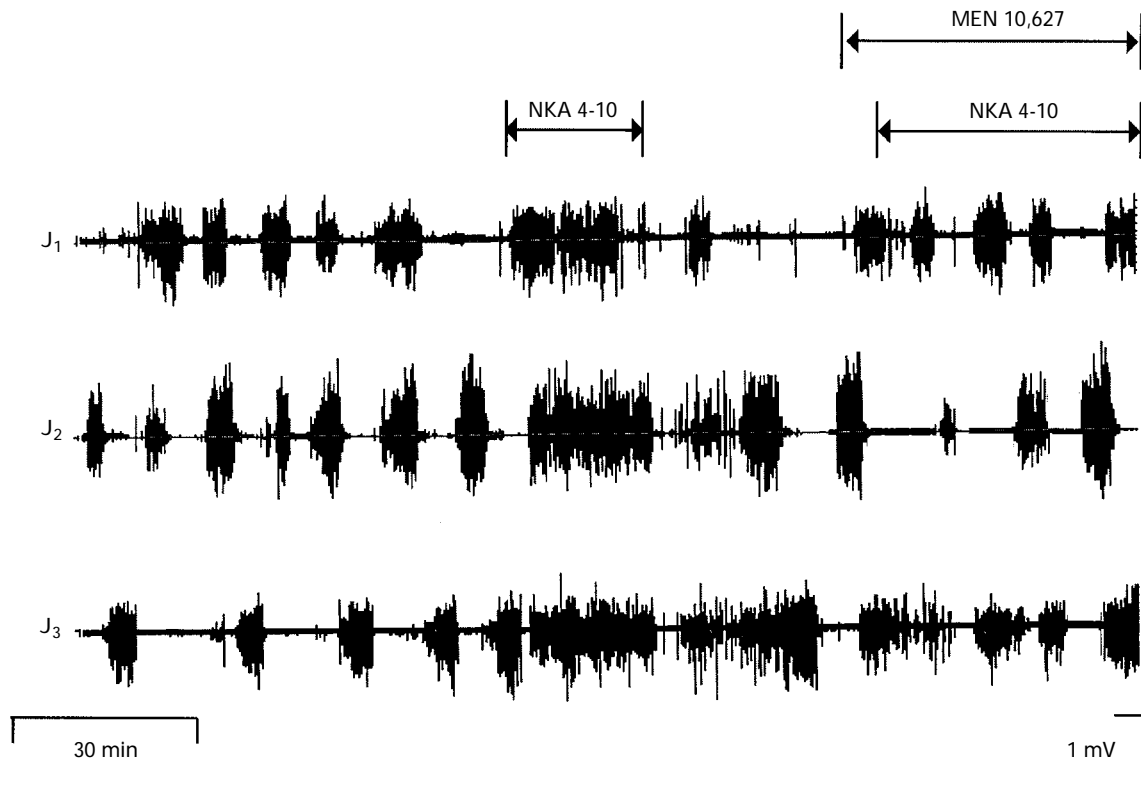
**Table 3** Spiking activity, expressed as spikes  $\text{min}^{-1}$ , induced by SP, NKA and NKB

Group	Electrode site (spikes $\text{min}^{-1}$ )		
	$J_1$	$J_2$	$J_3$
<b>SP</b>			
Control period	5.3(0.1–10.4)	5.5(–0.1–11.8)	4.7(1.4–8.1)
Infusion of SP	27.8(12.5–43.3)*	51.6(2.6–100.5)*	42.9(14.7–71.2)*
SP/MEN 10,627	27.8(14.2–41.3)*	54.0(8.5–99.6)*	55.6(24.4–86.7)*
<b>NKA</b>			
Control period	5.6(1.4–9.7)	5.1(0.8–9.3)	11.8(2.9–20.6)
Infusion of NKA	50.4(22.3–78.5)*	82.3(22.9–141.8)*	87.6(21.9–153.2)*
NKA/MEN 10,627	42.3(8.4–76.2)*	76.1(29.3–122.9)*	94.2(20.5–167.9)*
<b>NKB</b>			
Control period	5.0(–8–18)	7.4(–8.5–23.3)	11.7(–24.2–47.5)
Infusion of NKB	27.4(–15–70.1)*	40.1(–0.2–80.3)*	67.0(–1–135.0)*
NKB/MEN 10,627	21.7(–27.9–71.3)*	32.0(–3–66.9)*	70.4(–31.6–172.5)*

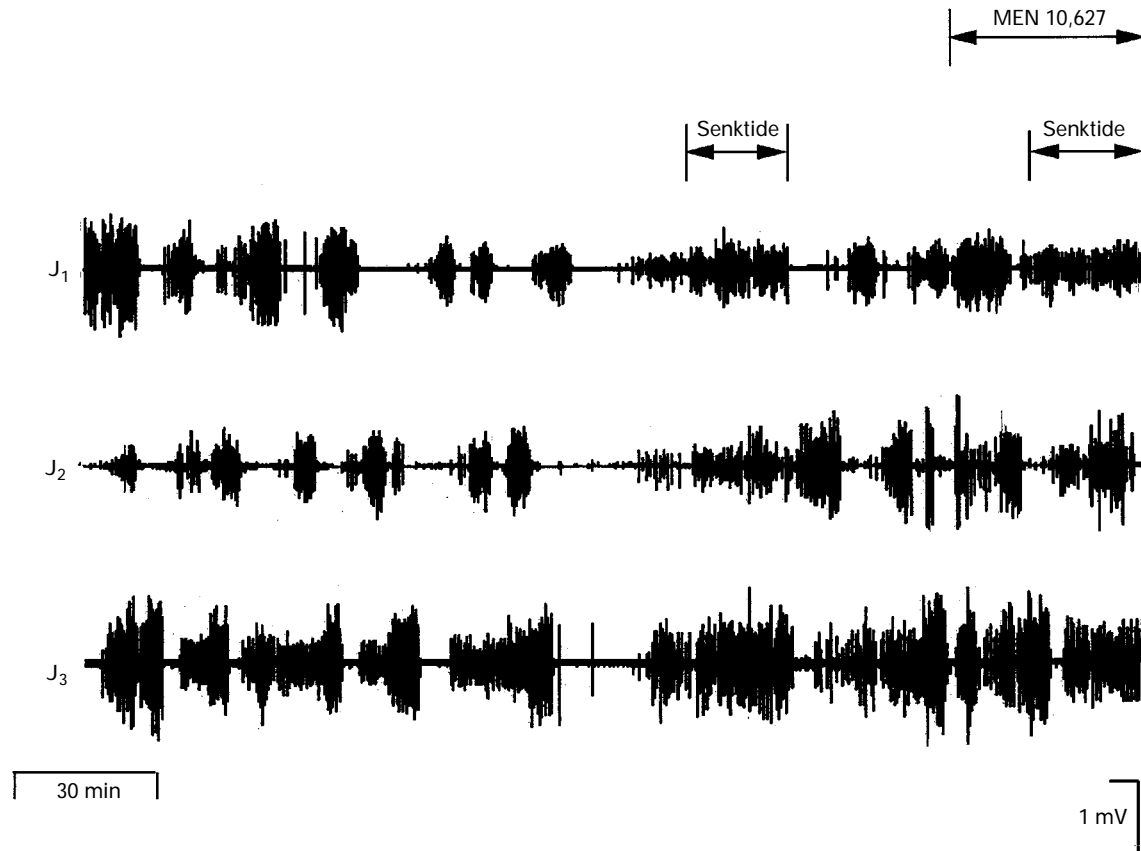
The results are shown for the last 20 min of the control period and for infusion of the respective test substance before and after administration of the  $\text{NK}_2$ -receptor selective antagonist MEN 10,627. \* $P < 0.05$  compared to the control period.



**Figure 1** Electromyographic recording illustrating the effect of substance P methyl ester (SPME), which disrupted the MMC pattern and induced a phase II-like spiking activity, not prevented by MEN 10,267.  $J_1$ : electrode site 15 cm from the pylorus;  $J_2$ : 25 cm from the pylorus;  $J_3$ : 35 cm from the pylorus.



**Figure 2** Electromyographic recording illustrating the effect of  $[\beta\text{-Ala}^8]\text{-NKA}$  (4–10) on small intestinal motility. Given alone,  $[\beta\text{-Ala}^8]\text{-NKA}$  (4–10) disrupted the MMC pattern and induced a phase II-like spiking activity. These effects were effectively prevented by pretreatment with MEN 10,627. J<sub>1</sub>: electrode site 15 cm from the pylorus; J<sub>2</sub>: 25 cm from the pylorus; J<sub>3</sub>: 35 cm from the pylorus.



**Figure 3** Electromyographic recording illustrating the effect of senktide, which disrupted the MMC-pattern and induced a phase II-like pattern with spiking activity, not prevented by MEN 10,267. J<sub>1</sub>: electrode site 15 cm from the pyloric ring; J<sub>2</sub>: 25 cm from the pyloric ring and J<sub>3</sub>: 35 cm from the pyloric ring.

**Table 4** Spiking activity, expressed as spikes  $\text{min}^{-1}$ , induced by SPME,  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$  and senktide

Group	Electrode site (spikes $\text{min}^{-1}$ )		
	$J_1$	$J_2$	$J_3$
<b>SPME</b>			
Control period	3.1(0.3–5.9)	4.1(0.2–8.0)	10.9(2.0–19.9)
Infusion of SPME	17.1(11.1–19.9)*	37.1(10.1–64.0)*	61.1(6.7–115.6)*
SPME/MEN 10,627	18.6(9.2–28.0)*	51.1(12.1–90.0)*	87.6(6.9–168.3)*
<b><math>[\beta\text{-Ala}^8]\text{-NKA}(4-10)</math></b>			
Control period	2.5(–0.3–5.3)	4.4(1.4–7.4)	7.9(1.3–14.5)
Infusion of $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$	26.0(5.3–46.7)*	46.1(22.9–69.3)*	62.3(29.6–95.1)*
$[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ /MEN 10,627	4.0(1.2–6.8)	9.5(4.4–14.5)	15.6(1.7–29.5)
<b>Control period</b>			
Infusion of $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ I	4.6(2.0–7.2)	4.5(2.2–6.8)	15.4(4.7–26.2)
Infusion of $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ II	25.3(11.9–36.6)*	39.3(32.6–46.0)*	68.9(47.2–90.7)*
	19.1(4.4–31.9)*	32.3(17.1–47.5)*	63.5(40.9–86.2)*
<b>Senktide</b>			
Control period	1.6(0.6–2.6)	8.2(–10.4–26.7)	11.0(–11.7–33.6)
Infusion of senktide	15.6(3.1–28.0)*	22.4(1.3–43.4)*	24.7(–2.5–51.9)*
Senktide/MEN 10,627	8.8(–4.6–22.3)*	20.5(–1.4–42.5)*	23.3(–3.4–50.0)*

The results are shown for the last 20 min of the control period and for infusion of the respective test substance before and after administration of the  $\text{NK}_2$ -receptor selective antagonist MEN 10,627. \* $P < 0.05$  compared to the control period.

administered at a higher dose of  $200 \text{ pmol kg}^{-1} \text{ min}^{-1}$  to induce spiking activity, compared to  $100 \text{ pmol kg}^{-1} \text{ min}^{-1}$  for NKA. Pretreatment with MEN 10,627 at a dose of  $4 \text{ nmol kg}^{-1} \text{ min}^{-1}$  failed to block the motility-stimulating effect of SP, NKB or NKA (Table 3).

#### Infusion of selective neurokinin receptor agonists

Infusion of SPME,  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ , or senktide disrupted the MMC pattern and induced an irregular spiking activity resembling phase II-activity (Figures 1–3, Table 4). There were no significant differences between the intensity of spiking activity induced by the different agonists. Compared to the natural tachykinins, the selective agonists were given at considerably higher doses to obtain an effect comparable to that of natural tachykinins. The lowest effective dose was  $1000 \text{ pmol kg}^{-1} \text{ min}^{-1}$  for SPME and senktide, and  $5000 \text{ pmol kg}^{-1} \text{ min}^{-1}$  for  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ .

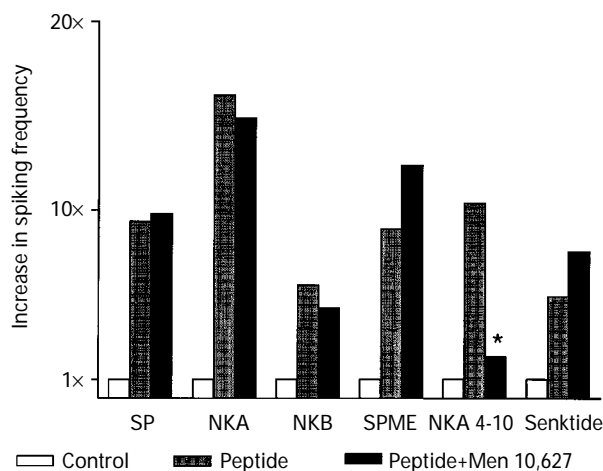
Infusion of MEN 10,627 at  $4 \text{ nmol kg}^{-1} \text{ min}^{-1}$  effectively prevented the irregular spiking induced by  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ , and regularly recycling MMCs were preserved during the infusion of the agonist (Figures 2 and 4). Pretreatment with MEN 10,627 at the same dose had no effect on the response to SPME or senktide (Figures 1, 3 and 4).

Infusion of  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$  at two subsequent occasions during the same experiment resulted in disruption of MMC and induction of irregular spiking activity, resembling phase II activity on both occasions. The intensity of the irregular spiking was similar during both infusion periods (Figure 5 and Table 4).

Infusion of  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$  at a rate of  $500 \text{ pmol kg}^{-1} \text{ min}^{-1}$  for 45 min did not affect the MMC pattern. Neither did the interval, duration, nor propagation velocity of the MMC change during infusion of  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$  at this low dose (Table 2).

#### Infusion of MEN 10,627

Infusion of MEN 10,627 at a dose of  $4 \text{ nmol kg}^{-1} \text{ min}^{-1}$  for 45 min did not change the myoelectrical pattern, and the recycling MMC pattern was preserved in all animals. The



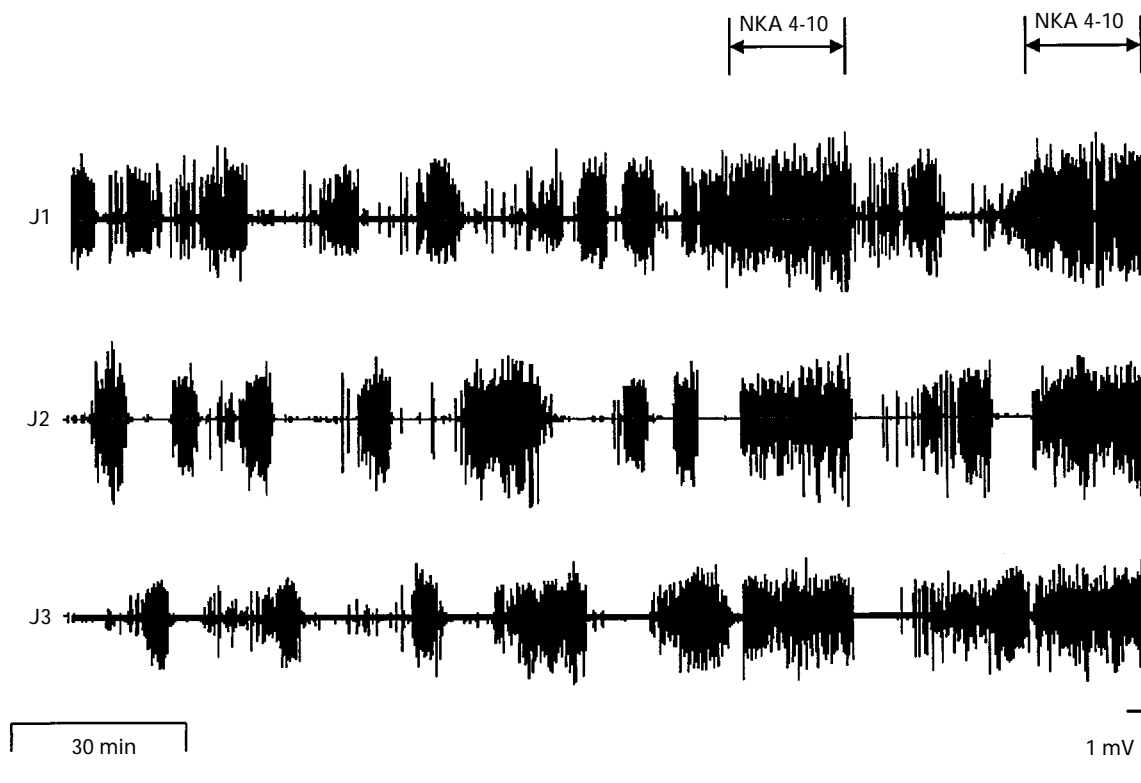
**Figure 4** The relative increase in spiking frequency during infusion of each test substance alone and after pretreatment with MEN 10,627, compared with the control period for each substance. Pretreatment with MEN 10,627 antagonized the effects of  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ . \* $P < 0.05$  compared to infusion of  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$  alone.

characteristics of the MMC before and after administration of the antagonist are presented in Table 1.

## Discussion

The present study demonstrated that the natural tachykinins NKA, NKB and SP, as well as selective agonists to all three neurokinin receptors, are capable of disrupting the MMC pattern and inducing irregular spiking activity of the small intestine in the rat. In addition, MEN 10,627 seems to prevent selectively the effects of  $\text{NK}_2$  receptor-mediated stimulation of myoelectrical activity in the small intestine.

In the present study, the  $\text{NK}_2$  receptor antagonist MEN 10,627 did not change the basal interdigestive motility pattern, which indicates that the generation of the interdigestive rhythm is not dependent on  $\text{NK}_2$  receptors. This finding is in



**Figure 5** Electromyographic recording illustrating the effect of repeated administration of  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ , which disrupted the MMC-pattern and induced a phase II-like spiking activity on both occasions. J<sub>1</sub>: electrode site 15 cm from the pylorus; J<sub>2</sub>: 25 cm from the pylorus; J<sub>3</sub>: 35 cm from the pylorus.

line with the demonstration of NK<sub>2</sub> receptors on muscle cells, but not on the nerve cells in the enteric nervous system (Grady *et al.*, 1996) believed to be responsible for the rhythmic myoelectrical activity in the gut.

SP, NKA and NKB all disrupted the MMC pattern and induced phase II-like activity, an effect that was not inhibited by MEN 10,627. NKA exhibited the highest potency regarding effects on the myoelectric pattern. This is in accordance with our previous experience with tachykinin-stimulated motility in rats (Lördal *et al.*, 1993) and in man (Lördal *et al.*, 1997).

The finding that the natural tachykinins SP, NKA, and NKB, as well as the selective agonists SPME,  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ , and senktide, all changed the motility pattern, gives functional support for the existence of all three NK receptor types in the rat small intestine. The result of NK receptor stimulation is evidently disruption of MMC and induction of irregular spiking activity. One explanation for this finding is that NK receptor stimulation can cause uncoupling of the oscillators thought to be responsible for the generation of phase III of the MMC, i.e. that NK receptor stimulation has an inhibitory influence on an ongoing MMC activity. The latter can explain why blockade with MEN 10,627 did not change the basal interdigestive pattern, given that the peptidergic (tachykinin) tone is low under basal conditions. Another explanation might be that the contractions of phase III of MMC are not dependent on peptidergic (tachykinin) stimulation, but rather are obscured by the tachykinin-induced contractions mediated by all three receptor types, and are therefore not possible to discern.

The selective agonists had to be given at much higher doses than the natural tachykinins to achieve a similar motility-stimulating effect. These findings are at variance with results

from a study on muscle strips from the rat duodenum. In the latter study the selective agonists SPME,  $\text{Nle}^{10}\text{-NKA}[4-10]$  and senktide were found to be equally potent in contracting circular and longitudinal muscle (Kim & Hellström, 1993). A possible explanation for the difference in potency between *in vivo* and *in vitro* conditions may be differences in the elimination of the natural tachykinins and the NK receptor-selective agonists.

MEN 10,627 inhibited the response to the NK<sub>2</sub> receptor-selective agonist  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ , but not to SPME or senktide which are selective for NK<sub>1</sub> and NK<sub>3</sub> receptors, respectively. These findings provide further evidence that MEN 10,627 is a selective NK<sub>2</sub> receptor antagonist, and the results are in accordance with earlier experiments in rats showing that MEN 10,627 inhibits duodenal contractions induced by  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$  (Maggi *et al.*, 1994) and inhibits transit in the small intestine of the rat stimulated by  $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$  (Tramontana *et al.*, 1994). In the present study the different agonists, as well as the antagonist MEN 10,627, were used at a single dose level. Against this background there is a potential risk that the selectivity noted may have arisen because the different agonists were used at doses producing different levels of stimulus to the system. However, the doses of the different agonists were titrated to give clear and predictable results, minimizing the risk that the 'selectivity' was due to a variable stimulus.

The finding that MEN 10,627 did not inhibit the response to NKA suggests that the effect of the natural NK<sub>2</sub> receptor preferring agonist is not mediated solely by this receptor subtype, but also by the NK<sub>1</sub> and NK<sub>3</sub> receptor subtypes. Our finding *in vivo* is supported by earlier observations in studies *in vitro*, demonstrating that NKA binds not only to NK<sub>2</sub>

receptors but also to NK<sub>1</sub> and NK<sub>3</sub> receptors (Regoli *et al.*, 1987).

The present finding that the response to [ $\beta$ -Ala<sup>8</sup>]-NKA(4–10) did not change upon repeated stimulation indicates that the NK<sub>2</sub> receptors were not desensitized in our experiments and that the effect of MEN 10,627 is the result of receptor antagonism and not merely a reduced sensitivity to the agonist. The fact that MEN 10,627 did not prevent the effects of SPME or senktide further indicates that the effect of MEN 10,627 is not a nonspecific depressant action on the target function studied.

It was concluded that not only NK<sub>2</sub> receptors, but also other receptors, such as NK<sub>1</sub> and NK<sub>3</sub> receptors, may mediate

the motility-stimulating action of different tachykinins *in vivo*. Furthermore, it was concluded that MEN 10,627 exerts a selective NK<sub>2</sub> receptor antagonism. Thus, MEN 10,627 seems a valuable tool for assessing the role of NK<sub>2</sub> receptors in gastrointestinal physiology.

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