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A novel tachykinin NK2 receptor antagonist prevents motility-stimulating effects of neurokinin A in small intestine

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1 MEN 11420 (nepadutant) is a potent, selective and competitive antagonist of tachykinin NK2 receptors.

2 The objective of the present study was to assess the capability of the drug to antagonize the stimulatory effects of neurokinin A (NKA) on gastrointestinal motility, as well as to change the fasting migrating motor complex (MMC).

3 Thirty-four male volunteers were randomized to treatment with either placebo or MEN 11420 in a double-blinded manner. Effects of MEN 11420 (8 mg intravenously) were evaluated as changes in phases I, II and III of MMC, as well as contraction frequency, amplitude and motility index during baseline conditions and during stimulation of motility using NKA (25 pmol kg^{-1} min^{-1} intravenously).

4 NKA preceded by placebo increased the fraction of time occupied by phase II, increased contraction frequency, amplitude and motility index.

5 MEN 11420 effectively antagonized the motility-stimulating effects of NKA. MEN 11420 reduced the phase II-stimulating effect of NKA. In addition, the stimulatory effect of NKA on contraction frequency and amplitude, as well as motility index were inhibited by MEN 11420. MEN 11420 did not affect the characteristics of MMC during saline infusion.

6 Plasma levels of MEN 11420 peaked during the first hour after infusion and decreased to less than half during the first 2 h.

7 In conclusion, intravenous MEN 11420 effectively inhibited NKA-stimulated, but not basal gastrointestinal motility, and was well tolerated by all subjects.
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Abbreviations: ECG, electrocardiography; HIV, human immunodeficiency virus; HPLC, high pressure liquid cromatography; MMC, migrating motor complex; NK, neurokinin; NK1, neurokinin 1; NK2, neurokinin 2; NKA, neurokinin A; PEF, peak expiratory flow; SP, substance P

Introduction

Of the two tachykinins, substance P (SP) and neurokinin A (NKA), considered as transmitters in the enteric nervous system (Barthó & Holzer, 1985) we have previously found NKA to be the dominating tachykinin in stimulating gut motility in man (Lördal *et al*., 1997). This finding is supported by studies in the guinea-pig and rat (Maggi *et al*., 1990; 1994; Holzer *et al*., 1998; Kim & Hellström, 1993; Rahman *et al*., 1994; Tolessa *et al*., 1996; Lördal *et al*., 1993; 1998; Lördal & Hellström, 1999) showing that NKA is more potent than SP in stimulating intestinal motility.

The responses of the two peptides are preferably mediated through tachykinin NK1 receptors for SP and tachykinin NK2 receptors for NKA, although the selectivity of natural ligands for each receptor is not absolute (Regoli & Nantel, 1991). The functional importance of the different tachykinin receptors resides in their ability to mediate different biological actions. In our recent study in man, we found that NKA had a selective and dose-dependent stimulatory effect on gut motility, while SP had a prominent vasodilatory and blood pressure-lowering effect that limited its use (Lördal *et al*., 1997). As a consequence of its ability to stimulate intestinal smooth muscle, we considered NKA to be specifically active in regulating intestinal motility by its action on NK2 receptors. In contrast, SP showed a higher specificity for vascular smooth muscle and is therefore more likely to be of greater importance for the regulation of local blood flow in tissues.

In the gut, tachykinins are synthesized by different subsets of enteric neurons, including 'cholinergic' motor neurons and are co-released with acetylcholine to stimulate the longitudinal and circular smooth muscle layers. In the human intestine, the potent spasmogenic effect of tachykinins is mainly mediated by NK2 receptors, as shown by *in vitro* studies (Maggi *et al*., 1989). In most animal smooth muscle preparations both NK1 and NK2 receptor blockers are needed for a complete antagonism to occur, but in the human isolated intestine NK2 receptor antagonists are fully effective in blocking nonadrenergic noncholinergic excitatory transmission, as well as the effect of exogenously applied tachykinins (Giuliani *et al*., 1991; Maggi *et al*., 1992; Zagorodnyuk *et al*., 1997).

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Based on these findings potent and selective NK2 receptor antagonists have been developed. The acetylaminoglycosidated bicyclic hexapeptide MEN 11420 (Nepadutant) competitively binds with high affinity and specificity to the human NK2 receptor expressed in CHO cells. It selectively blocks NK2 receptors on isolated smooth muscle preparations from animal and human tissues with pK_B values in the range 8.1–10.2 (Catalioto *et al*., 1998a, b).

MEN 11420 has been demonstrated to be a potent, selective and competitive antagonist of tachykinin NK2 receptors, both in animal and human preparations. In *in vivo* animal models, MEN 11420 produces an effective and long-lasting blockade of the NK2 receptors expressed in the smooth muscle of the intestinal, genito-urinary and respiratory tract (Catalioto *et al*., 1998a). In rats this antagonist activity is observed after several routes of administration, including intraduodenal, and the mean plasma half-life was found to be 44 min (Lippi *et al*., 1998).

Hence, the present study was conducted in a randomized double-blinded manner in order to study the importance of NK2 receptors for the regulation of intestinal motility in man. The primary aim of this study was to evaluate the ability of MEN 11420 in antagonizing NKA-stimulated small bowel motility. The secondary aim was to assess the effects of MEN 11420 on basal fasting motility and to determine plasma concentrations to which inhibition of NKA-stimulated gut motility occurred.

Methods

Subjects

Thirty-four healthy male subjects 18–45 years of age were studied. Subjects were screened for inclusion in the study by physical examination, electrocardiography (ECG), respiratory test, measurement of full blood count, liver function tests, blood urea, creatinine, sodium, potassium, albumin, calcium, urate, glucose, cholesterol, triglycerides and antibodies to hepatitis B and C, and HIV. The subjects were included in the study only if all tests were normal. The subjects were within 20% of ideal body weight for height and were taking no medication. They were requested to abstain from alcohol and caffeinated beverages for 4 days before and until the end of study. At a follow-up visit within 14 days after the experiment the same procedures as at the screening visit were repeated.

All subjects gave written informed consent. The study was approved by the Swedish Medical Products Agency and the Ethics Committee of the Karolinska Hospital.

Study design

The study was carried out as a single centre randomized placebo-controlled study. The study design is shown in Figure 1.

The experiments were undertaken after an overnight fast during a period of 8 h with subjects in the recumbent position. Each experiment consisted of two consecutive motility recording periods of 4 h each.

The subjects were divided in two groups. In group one saline was administered for the first 4 h, then subjects were challenged with a 10 min intravenous infusion of either MEN 11420 (group 1A, $n=8$) or placebo (group 1B, $n=10$) followed by an infusion of NKA for 4 h. In group two, saline was given intravenously during both the first and second 4-h periods after a 10 min infusion of either MEN 11420 (group 2A, $n=8$) or placebo (group 2B, $n=8$).

Monitoring of test subjects

For safety reasons the following tests were performed immediately before the start of the experiment, at 4 h, and at the end of the experiment: measurement of full blood count, liver function tests, blood urea, creatinine, sodium, potassium, albumin, calcium, urate, glucose, cholesterol, triglycerides, ECG, peak expiratory flow (PEF), and urine analysis (Figure 1). Throughout the experiment heart rate and blood pressure were monitored every 15 min and PEF every 30 min.

Adverse events occurring during the study were documented and followed until resolved.

Plasma concentration of neurokinin A

Blood samples were collected in cold heparinized tubes (10 ml) every hour throughout the experiment. Samples were immediately centrifuged ($1500\times g$, 4°C, 10 min) and the supernatants were stored at −20°C until analysis. Radioimmunoassay for determination of plasma concentration of NKA was performed as previously described (Lördal *et al*., 1997). Detection limit was 7.8 pmol l^{-1} and the coefficient of variation 7%.

Determination of MEN 11420 in plasma and urine

Blood samples were collected and treated as described above. A solid-liquid extraction procedure using Bond Elut C18 columns was carried out on 0.5 ml of plasma. Twenty-five μl of the 50 μl reconstituted volume for plasma (or of the 250 μl reconstituted volume for urine extraction) was injected into the C18 100×2.1 mm, 5 μm reverse-phase HPLC column. Separation was carried out under gradient conditions using a flow rate of 200 μl min^{-1} and samples were split with 1/5 being injected into a mass-spectrometer.

Intestinal motility recordings

The motility pattern of the proximal small intestine was monitored by means of a multichannel polyvinylchloride tube (William Cook, Bjaeverskov, Denmark). The tube was 250 cm in length, 4.7 mm in outer diameter, and had eight channels 0.7 mm in width, ending as side-holes at different levels. Three of these spaced 1.5 cm apart located orally, another three side-holes located 2 cm apart were positioned 11.5 cm further aborally, followed by another separate side-hole 13 cm further aborally and the last side-hole 15 cm further beyond, for recording of antro-duodeno-jejunal motility. The tube was passed through a nostril and positioned with its most distal recording site aboral to the angle of Treitz using fluoroscopy. Each channel was continuously perfused with degassed water at a rate of 0.25 ml min^{-1} from a low-compliance pneumohydraulic system (Arndorfer Medical Specialities, Greendale, WI, U.S.A.). The channels were connected to external pressure

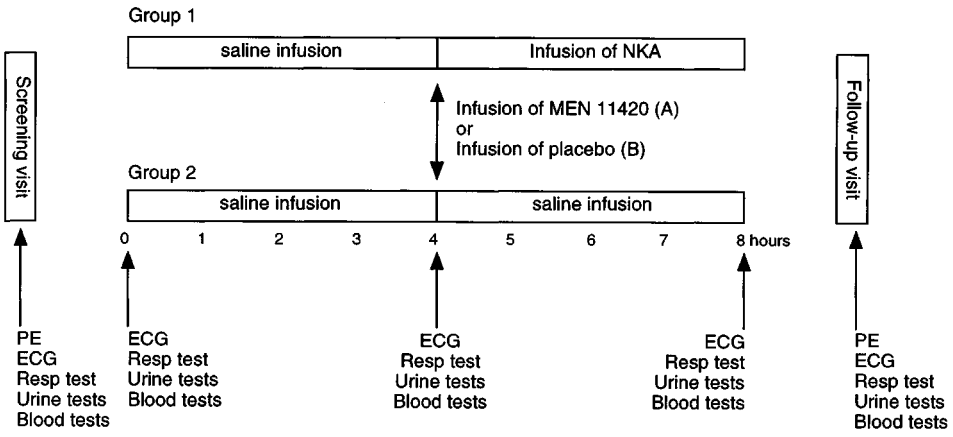

Figure 1 Summary of study design and procedures performed. PE = physical examination, ECG = electrocardiogram, Resp test = peak expiratory flow.

transducers (Synectics, Stockholm, Sweden). Digital recordings were obtained by connecting the pressure transducers *via* a PC Polygraph HR (Synectics) to a personal computer (486D/66 MHz, Dell Corporation, Austin, TX, U.S.A.). The software program used was Polygram Lower GI 6.31C3 (Synectics) with a sampling frequency of 4 Hz. The pressure rise velocity upon sudden occlusion of the recording system exceeded 200 mmHg s^{-1} in each channel.

Analysis of motility recordings

Recordings were inspected by two independent observers who agreed upon the presence or absence of motor patterns. Contractions exceeding a cut-off amplitude of 10 mmHg at the angle of Treitz were included in the analysis. MMCs were identified according to criteria of Vantrappen *et al.* (1977): (1) appearance of uninterrupted bursts of pressure waves with a frequency of 11–12 contractions per minute (phase III); (2) aboral migration of phase III activity passing at least the distal two registration points; and (3) a period of complete quiescence following phase III activity. Phase III of MMC (activity front) was defined as the presence of uninterrupted phasic pressure changes for at least 2 min at the maximum frequency for that locus. The duration of phase III at the angle of Treitz was measured as the time from the onset of regular contractions to quiescence. The propagation velocity of phase III was calculated by dividing the traversed distance from the onset of phase III by the time interval from one registration point to the next. Phase II was defined as $\geqslant 2$ phasic contractions per min, whereas phase I was defined as <2 phasic contractions per min.

The fraction of time occupied by phase I, II or III of the MMC cycle was calculated during the control period and during infusion of NKA or saline. The number of contractions and their amplitude, as well as the overall motility index [ln $(1+(\Sigma(\text{amplitude*duration})/\text{min}))$] during the control period and infusion periods were calculated.

Compounds

NKA was purchased from Peninsula laboratories (Merseyside, U.K.), dissolved and diluted in sterile 0.9% NaCl and then filtered (Millex-Micropore, pore size 0.22 μm). Ten-ml vials were prepared containing a sterile stock solution of NKA (180 nmol ml^{-1}) and 0.25 ml of albumin (200 mg ml^{-1}, Kabi Pharmacia, Uppsala, Sweden). Saline (0.154 M NaCl) for intravenous infusions, 0.154 M NaCl, was purchased from Pharmacia & Upjohn (Stockholm, Sweden).

MEN 11420 and matching placebo were supplied by the study sponsor in ampoules of 12 mg in 30 ml. For our study a dose of 8 mg in 20 ml was administered *via* a syringe driver (P4000 IVAC syringe pump, P.M.S. Instruments Ltd, Maidenhead, Berkshire, U.K.).

Statistics

Results are presented as mean values $\pm$95% confidence interval. Kruskal-Wallis one-way analysis of variance or Mann-Whitney *U*-test was used where appropriate. $P<0.05$ was considered significant.

Results

Effects on small intestinal motility

Baseline motility

During the first 4-h period MMCs were registered in all test subjects. The number and characteristics of MMCs are shown in Tables 1 and 2.

Infusion of neurokinin A

Treatment with placebo In the placebo group (group 1B) intravenous infusion of NKA at a dose of 25 pmol kg^{-1} min^{-1} disrupted the MMC pattern in six of 10 subjects. The MMC pattern changed to phase II-like activity with continuous irregular contractile activity (Figure 2).

Infusion of NKA changed the time proportions between phase I and II. Compared with the control period, phase I decreased and phase II increased, while phase III was unchanged (Table 1).

Table 1 Motility data for subjects given NKA after either MEN 11420 or placebo. Values are mean with 95% confidence interval between parenthesis

	NKA after MEN 11.420		*NKA after placebo*	
	1st 4 h	*2nd 4 h*	*1st 4 h*	*2nd 4 h*
Phase III				
Total duration	14.0 (8.2–19.8)	5.4 (0–10.8)	10.7 (6.9–14.5)	4.2 (−0.5–8.9)
No of activity fronts	2.0 (1.4–2.6)	0.9 (0.0–1.7)	1.7 (1.2–2.2)	0.7 (0.0–1.4)
Propagation velocity	26.9 (5.5–48.2)	23.9 (4.0–43.8)	34.8 (−3.6–73.3)	11.8 (−1.1–24.8)
Contraction frequency	11.4 (10.4–12.4)	11.5 (9.5–13.5)	11.9 (11.1–12.8)	10.9 (9.2–12.6)
Amplitude	29.6 (19.2–40.1)	35.7 (14.6–56.9)	28.6 (23.3–34.1)	28.8 (19.9–37.7)
Motility index	6.9 (6.6–7.2)	7.2 (6.6–7.7)	7.0 (6.8–7.2)	7.0 (6.5–7.5)
Phase II				
Total duration	152.8 (126.4–179.2)	188.6 (154.4–222.8)	127.7 (89.2–166.3)	216.4 (193.4–239.4)*
Phase I				
Total duration	68.2 (40.0–96.4)	28.4 (−3.2–60.1)	104.0 (63.8–144.3)	0.8 (−0.5–2.2)
No of discrete clustered contractions	2.6 (−0.2–5.5)	5.8 (1.8–9.7)	0.7 (0.2–1.2)	2.8 (0.1–5.5)
Fraction time				
Phase I	31.6 (23.2–40.1)	12.5 (−1.3–26.4)	41.4 (25.5–57.3)	0.3 (−0.2–0.8)*
Phase II	62.4 (53.7–71.1)	85.0 (70.3–99.7)	54.1 (37.9–70.3)	97.6 (95.2–100)*
Phase III	5.9 (3.5–8.2)	2.4 (0–4.8)	4.5 (2.8–6.2)	2.1 (−0.2–4.4)

*$P<0.05$, 1st 4 h compared to 2nd 4 h.

Table 2 Motility data for subjects given NKA after either MEN 11420 or placebo. Values are mean with 95% confidence interval between parenthesis

	Saline after MEN 11.420		*Saline after placebo*	
	1st 4 h	*2nd 4 h*	*1st 4 h*	*2nd 4 h*
Phase III				
Total duration	10.7 (6.9–14.5)	13.3 (6.2–20.5)	9.2 (5.3–13.1)	9.1 (5.8–12.4)
No of activity fronts	2.1 (1.3–3.0)	2.8 (1.5–4.0)	2.1 (1.2–3.1)	2.1 (1.2–3.1)
Propagation velocity	13.4 (9.0–17.8)	25.9 (0.4–51.4)	23.1 (2.6–43.6)	26.3 (10.6–42.2)
Contraction frequency	11.5 (10.9–12.1)	11.7 (11.3–12.1)	115. (10.4–12.5)	12.3 (11.7–12.8)
Amplitude	29.1 (22.8–35.4)	26.0 (21.9–30.0)	25.4 (20.6–30.2))	27.4 (21.9–32.8)
Motility index	7.0 (6.7–7.2)	6.9 (6.7–7.0)	6.8 (6.5–7.1)	6.9 (6.6–7.2)
Phase II				
Total duration	148 (106.8–190.1)	163.5 (119.2–207.7)	174.6 (158.2–191.0)	171.5 (124.5–218.4)
Contraction frequency	1.9 (1.1–2.7)	1.8 (1.4–2.1)	2.0 (1.4–2.6)	2.0 (1.4–2.5)
Amplitude	23.8 (21.0–26.6)	24.8 (22.7–26.8)	22.2 (20.1–24.2)	21.7 (20.3–23.1)
Motility index	4.8 (4.3–5.3)	4.8 (4.5–5.1)	4.8 (4.4–5.2)	4.8 (4.5–5.1)
Phase I				
Total duration	73.2 (38.8–107.7)	71.4 (26.4–116.5)	53.6 (34.0–73.2)	60.8 (18.2–103.3)
No of discrete clustered contractions	3.2 (0.6–5.9)	2.1 (0.8–3.4)	2.9 (1.0–4.8)	3.1 (1.1–5.1)
Fraction time				
Phase I	31.8 (16.7–46.8)	28.0 (10.7–45.3)	22.6 (14.4–30.8)	25.2 (7.1–43.4)
Phase II	63.6 (47.1–80.2)	66.8 (46.9–86.6)	73.5 (66.1–80.9)	71.0 (52.5–89.8)
Phase III	4.5 (2.8–6.2)	5.2 (2.4–8.1)	3.9 (2.1–5.6)	3.8 (2.4–5.1)

*$P<0.05$, 1st 4 h compared to 2nd 4 h.

During phase II the contraction frequency, contraction amplitude and motility index all increased during infusion of NKA (Figures 3–5).

Treatment with MEN 11420 The motility-stimulating effects of NKA (25 pmol kg^{-1} min^{-1}) were effectively inhibited when a short term intravenous infusion with MEN 11420 (8 mg) was administered prior to NKA infusion (Tables 1 and 2, Figures 2–5).

The inhibitory effect of MEN 11420 on NKA-induced changes was most pronounced during the first 2 h of infusion (Table 3), coincident with the plasma concentration peak of MEN 11420 (Figure 6).

Infusion of saline

Treatment with placebo In studies where saline was infused during the second 4-h period, no changes in baseline motility characteristics from the first to the second 4-h period were observed following treatment with placebo.

Treatment with MEN 11420 In studies where saline was infused during the second 4-h period, no changes in baseline motility characteristics from the first to the second 4-h period were observed following treatment with MEN 11420. In fact, no change in the motility pattern was

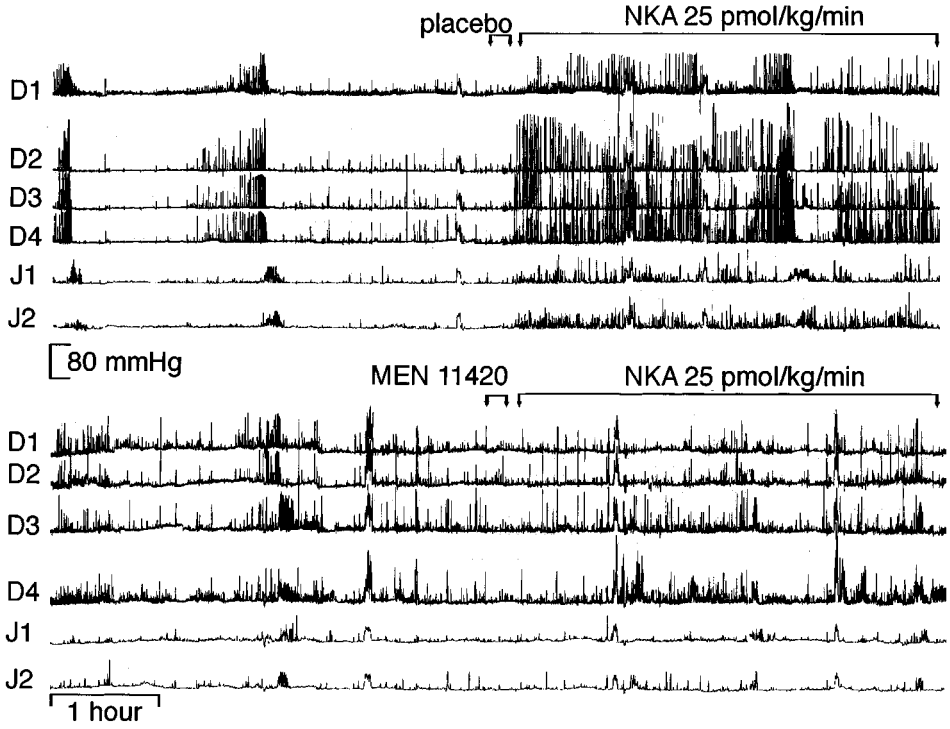

Figure 2 Manometry recordings from duodenum and upper jejunum. D_1-D_4: recordings from duodenum. J_1-J_2: recordings from upper jejunum. Upper half: Recording from a test subject receiving placebo followed by NKA at a dose of 25 pmol kg^{-1} min^{-1}. During infusion of NKA the MMC pattern is replaced by phase II-like activity. Lower half: Recording from a test subject receiving MEN 11420 followed by NKA at a dose of 25 pmol kg^{-1} min^{-1}. During infusion of NKA the MMC pattern is preserved.

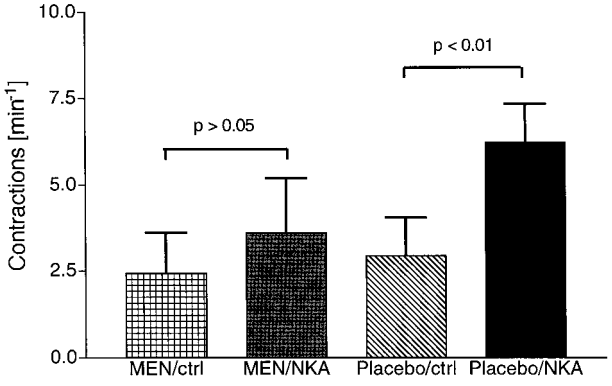

Figure 3 Diagram showing contraction frequency of phase II contractions during infusion of NKA after pretreatment with either MEN 11420 or placebo. Infusion of NKA preceded by placebo (black bar, $n=8$) increased phase II contraction frequency significantly while infusion of NKA preceded by MEN 11420 (grey bar, $n=8$) did not. Values are mean with 95% confidence interval.

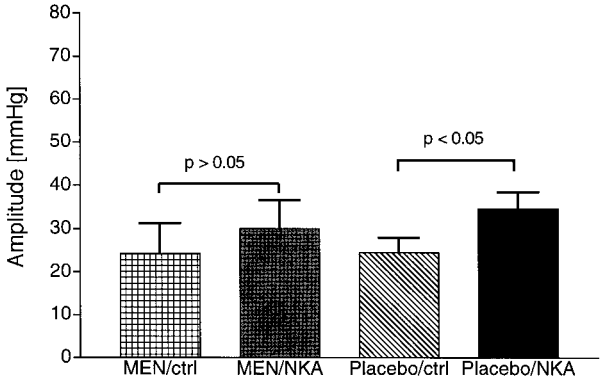

Figure 4 Diagram showing amplitude of phase II contractions during infusion of NKA after pretreatment with either MEN 11420 or placebo. Infusion of NKA preceded by placebo (black bar, $n=8$) increased phase II contraction amplitude significantly while infusion of NKA preceded by MEN 11420 (grey bar, $n=8$) did not. Values are mean with 95% confidence interval.

observed with MEN 11420 as compared to placebo (Table 2, Figure 7).

Plasma concentrations of MEN 11420

Plasma levels of MEN 11420 peaked during the first hour after infusion with a maximum concentration of 335 (263–407) ng ml^{-1} and then decreased to less than half during the first 2 h (Figure 6).

Plasma concentration of neurokinin A

In experiments with saline infusion over 8 h as well as in experiments with saline during the first 4-h period alone, the plasma concentrations of NKA were below the detection limit in all subjects. Intravenous infusion of NKA at a dose of 25 pmol kg^{-1} min^{-1} during the second 4-h period resulted in plasma concentrations of NKA between 118 and 228 pmol l^{-1} (Table 4). There were no differences in plasma levels of NKA in subjects pretreated

with MEN 11420 (group 1A) or subjects pretreated with placebo (group 1B).

Vital signs and adverse events

Systemic arterial blood pressure, heart rate and PEF did not change during infusion of NKA either in the group treated with MEN 11420 or in the placebo-treated group. No changes were seen in ECGs taken at the screening visit, during the experiment and at the follow-up visit.

Forty adverse events were reported in 20 subjects. Of these, 34 occurred in group 1 in which test subjects were given NKA preceded by either MEN 11420 or placebo. Only six adverse events occurred in group 2 given saline plus MEN 11420 or placebo. Of these, three occurred in the subjects receiving MEN 11420. In group 1 the dominating adverse event (17 cases) was flush, of either the face or whole body, with a clear temporal relationship to administration of NKA. In those treated with placebo before NKA, two cases of borborygmi, three cases of abdominal pain, two cases of headache, three cases of nausea and two cases of vomiting were observed, involving eight test subjects. In the group receiving NKA after placebo (1B) two experiments were interrupted due to side effects of NKA. The plasma concentration of NKA in these two cases did not differ from the levels in other subjects receiving NKA after placebo or MEN 11420.

In the subjects receiving NKA after MEN 11420 the only adverse event was flush which occurred in seven cases (Table 5).

Laboratory safety data

No abnormalities in haematology or clinical chemistry tests were seen. No significant abnormalities were found in the urine tests during the experiments. The only abnormality noted was a high frequency of a mild proteinuria, present already at screening and before administration of the test substance.

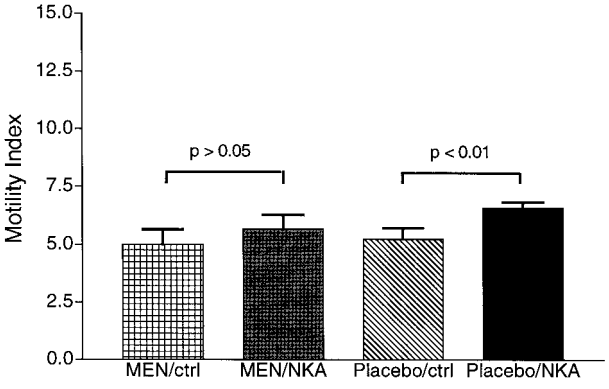

Figure 5 Diagram showing motility index for phase II contractions during infusion of NKA after pretreatment with either MEN 11420 or placebo. Infusion of NKA preceded by placebo (black bar, $n=8$) increased phase II contraction frequency significantly while infusion of NKA preceded by MEN 11420 (grey bar, $n=8$) did not. Values are mean with 95% confidence interval.

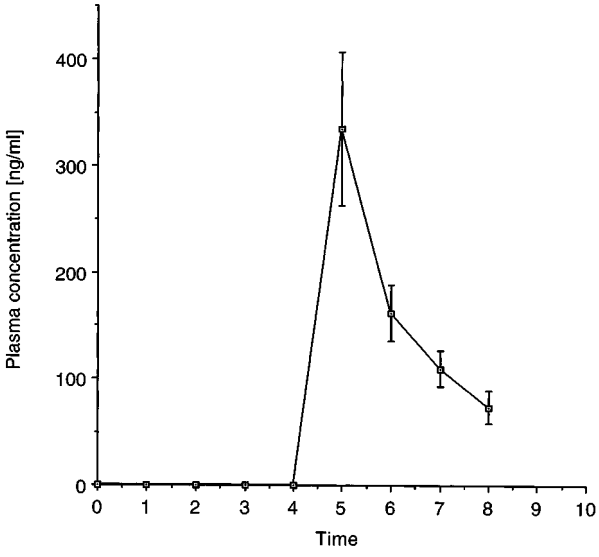

Figure 6 Diagram illustrating the plasma levels of MEN 11420, reaching a maximum during the first hour after infusion and decreasing to less than half during the first 2 h. Values are mean with 95% confidence interval.

Table 3 Data for test subjects given either MEN 11420 or placebo followed by infusion of neurokinin A. Phase II contraction frequency, contraction amplitude and motility index are shown hour by hour and by 2-h periods

	Motility index	*Contraction frequency* (min^{-1})	*Contraction amplitude* (mmHg)
0–1 h placebo	7.8 (6.6–8.9)	6.8 (6.6–7.0)	35.1 (31.7–38.4)
0–1 MEN 11420	3.2 (1.6–4.7)*	5.4 (4.6–6.1)*	28.4 (19.3–37.5)
1–2 h placebo	6.6 (5.0–8.1)	6.6 (6.2–6.9)	33.7 (29.2–38.1)
1–2 MEN 11420	3.6 (2.4–4.8)	5.7 (5.3–6.1)*	28.7 (23.7–33.6)
2–3 h placebo	6.2 (4.7–7.8)	6.5 (6.0–7.0)	34.0 (27.5–40.6)
2–3 MEN 11420	3.9 (1.8–6.0)	5.6 (4.9–6.4)	29.8 (22.8–36.8)
3–4 h placebo	5.9 (4.5–7.3)	6.4 (6.0–6.8)	33.7 (27.3–40.2)
3–4 MEN 11420	3.7 (2.0–5.5)	5.8 (5.0–6.5)	32.4 (24.8–40.0)
0–2 h placebo	7.2 (5.9–8.4)	6.7 (6.5–6.9)	34.6 (31.3–38.0)
0–2 MEN 11420	3.4 (2.1–4.6)	5.6 (5.1–6.0)	28.4 (21.4–35.5)
3–4 h placebo	5.8 (4.2–7.3)	6.4 (5.8–6.8)	33.0 (26.7–39.4)
3–4 MEN 11420	3.8 (1.9–5.8)	5.7 (5.0–6.4)	30.1 (23.1–37.1)

*$P<0.05$, comparison between placebo and MEN 11420.

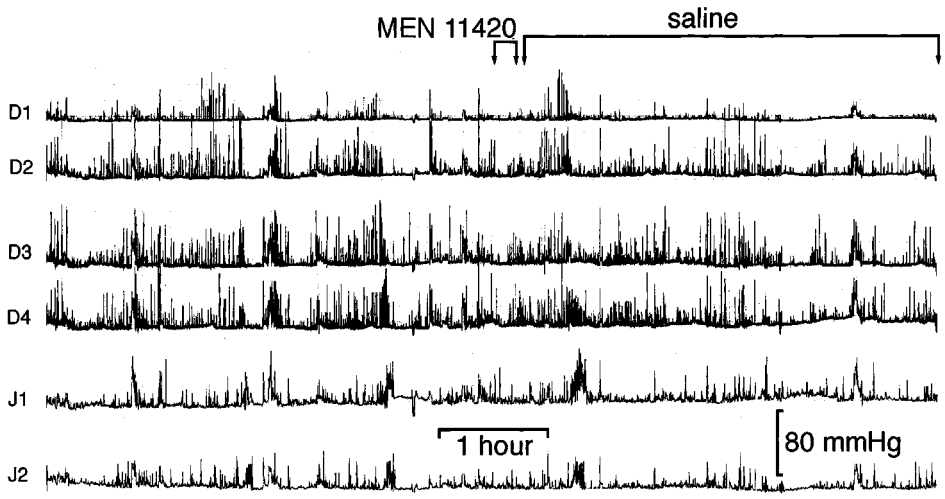

Figure 7 Manometry recording from duodenum and upper jejunum. D_1-D_4: recordings from duodenum. J_1-J_2: recordings from upper jejunum. Infusion of MEN 11420 followed by infusion of saline did not change the MMC pattern.

Table 4 Plasma concentration of NKA. Group 1A and 1B received i.v. infusion of NKA during the second 4-h period while subjects in group 2A and 2B received i.v. saline infusion during the same period

	Plasma concentration of neurokinin A (pmol L^{-1})			
Time	*Group 1A*	*Group 1B*	*Group 2A*	*Group 2B*
0–4 h	<7.8	<7.8	<7.8	<7.8
5 h	133 (119–146)	148 (111–185)	<7.8	<7.8
6 h	177 (22–333)	159 (127–191)	<7.8	<7.8
7 h	118 (64–172)	179 (24–334)	<7.8	<7.8
8 h	168 (31–306)	228 (0.52–509)	<7.8	<7.8

Values are mean values with 95% confidence interval within parenthesis.

Table 5 Adverse events recorded during infusion of NKA or saline after MEN 11420 or placebo respectively

	MEN 11420/NKA	*Placebo/NKA*	*MEN 11420/Saline*	*Placebo/Saline*
Flush	7	10	0	0
Borborygmi	0	2	0	0
Abdominal pain	0	3	0	0
Headache	0	2	0	0
Nausea	0	2	0	0
Nausea and vomiting	0	2	0	0
Discomfort	0	1	0	0
Flatulence	0	0	1	0

Discussion

The present study shows that NKA changes motility from a fasting to a fed-type of motor pattern with an increased motility index as verified by increased contraction frequency and amplitude. After administration of MEN 11420 the response to NKA was blocked and the normal fasting motility pattern with recurring MMCs was preserved. MEN 11420 alone had no effect on the basal MMC pattern. Our data suggest that endogenous tachykinins, at least *via* the NK2 receptor, are not involved in regulating the MMC in the fasted state. Since NKA itself induces a fed-like pattern of motility it would be of interest in future studies to check whether NK2 antagonists have any effect on the fed pattern.

Because NKA seems to specifically stimulate intestinal smooth muscle without affecting blood flow or blood pressure in man (Lördal *et al*., 1997), and data from animal studies indicates a dominating role for NKA in the regulation of gut motility (Barthó & Holzer, 1985; Maggi *et al*., 1990; 1994., Holzer *et al*., 1998; Kim & Hellström, 1993; Rahman *et al*., 1994; Tolessa *et al*., 1996; Lördal *et al*., 1993; 1998; Lördal & Hellström, 1999), it is likely that NKA is of major importance in the regulation of motor activity in the human gut. This refers not only to normal physiology with propulsion of contents aborally, but also to different disease states where abdominal pain and intestinal cramping with colicky pain, with or without diarrhoea, should be the expected symptoms of disease. Hitherto, only a few diseases

with gastrointestinal symptoms have been mentioned in the context of an increased tachykinin release, the major one being carcinoid syndrome. In this disease entity, high circulating plasma levels of tachykinins are reported, indicating a pathogenetic role of these peptides (Pearse *et al*., 1974; Alumets *et al*., 1977; Skrabanek *et al*., 1978; Wilander *et al*., 1977a, b; Gamse *et al*., 1981; Ratzenhofer *et al*., 1981; Emson *et al*., 1984; Norheim *et al*., 1986) The role of tachykinins has been postulated in other diseases such as irradiation bowel disease (Esposito *et al*., 1998), *Clostridium difficile* enteritis (Pothoulakis *et al*., 1998; Castagliuolo *et al*., 1998), inflammatory bowel disease (IBD) (Holzer, 1998; Watanabe *et al*., 1998), and irritable bowel syndrome (IBS) due to their characteristic action of mediating contraction of intestinal musculature (Mclean *et al*., 1998; Holzer & Holzer-Petsche, 1997).

Since pathological alterations may be limited to mechanisms within the enteric nervous system, the only way to obtain knowledge of disease mechanisms is to use specific antagonists, alone or in combination, in order to establish the importance of a neuropeptide for a specific disease. The present study employing a highly selective tachykinin NK2 receptor antagonist did not show any effect on the basal MMC pattern. This speaks in favour of a negligible role of tachykinins, at least *via* NK2 receptors in basal unstimulated motility. In support of a pathophysiological role for tachykinins, two of the subjects examined in this study developed symptoms similar to IBS with abdominal cramping during infusion of NKA. However, in cases with increased concentrations of NKA, as achieved by infusion of the peptide, the stimulated motility responses were prevented. This speaks in favour of a reliable usefullness for MEN 11420 in diseases where local NKA levels within the gut wall are increased.

The effectiveness of MEN 11420 as tachykinin blocker is due to the fact that the receptor stimulation is specific. In the present study, we used NKA, rather than specific NK receptor agonists, with the possibility of additional drug actions of NKA on NK1 receptors. This problem seems to be limited to theoretical considerations as we registered no general vasodilatory effects or fall in blood pressure in conjunction with NKA administrations. However, in some subjects the motility-inhibiting action of MEN 11420 was less effective which may be explained by the fact that some individuals may have a greater proportion of tachykinin NK1 receptors free for low-grade stimulation with NKA. Indeed, the contractile effect of tachykinins *via* NK1 receptors in the circular muscle of human small intestine has been documented in a previous *in vitro* study (Zagorodnyuk *et al*., 1997).

Previous data obtained in the rat data indicate that the compound blocks NKA at times when plasma levels of the NK2 receptor antagonist are very low (Catalioto *et al*., 1998a). Kinetic data also show that, despite having a relatively short half-life, the elimination of the compound is very slow and a 'tail' in plasma levels of the compound can be measured for several hours (Lippi *et al*., 1998). It is possible – owing to the high affinity for NK2 receptors – that tissues rich in NK2 receptors, such as the gut, may form a compartment from which the compound is slowly released and yet pharmacological effects are still produced. Concerning the results of the present study, the duration of action was estimated through the duration of blockade of NKA action. Since MEN 11420 is a competitive antagonist for NK2 receptors, the estimate of the duration of action indicates that when plasma levels of the compound fall below approximately 150 ng ml^{-1}, about 2 h from injection, then the agonist effect of NKA is resumed due to the steady state concentrations of the agonist in plasma.

Taken together, the present study shows that the NK2 receptor antagonist MEN 11420 is capable of alleviating intestinal motor responses in humans evoked by infusion of NKA. Under basal conditions, MEN 11420 had no effect on motility indicating that tachykinins are not involved in the physiological regulation of the MMC *via* NK2 receptors in man, but may be of importance in different disease states where increased motility is a predominating symptom.

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