Contribution of NK₂ tachykinin receptors to propulsion in the rabbit distal colon

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Onori, L., A. Aggio, G. Taddei, and M. Tonini. Contribution of NK₂ tachykinin receptors to propulsion in the rabbit distal colon. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G137–G147, 2000.—The role of the tachykinin neurokinin (NK)₂ receptors on rabbit distal colon propulsion was investigated by using two selective NK₂-receptor antagonists, MEN-10627 and SR-48968. Experiments on colonic circular muscle strips showed that contractile responses to [β-Ala²]NKA-(4–10) (1 nM–1 µM), a selective NK₂-receptor agonist, were competitively antagonized by MEN-10627 (1–100 nM), whereas SR-48968 (0.1–10 nM) caused an insurmountable antagonism, thus confirming the difference in the mode of action of the two compounds. Colonic propulsion was elicited by distending a mobile rubber balloon with 0.3 ml (submaximal stimulus) or 1.0 ml (maximal stimulus) of water. The velocity of anadisplacement of the balloon (mm/s) was considered the main propulsion parameter. At low concentrations (1.0–100 nM and 1–10 nM, respectively), MEN-10627 and SR-48968 facilitated the velocity of propulsion, whereas at high concentrations (100 nM and 1 µM, respectively) they decelerated propulsion. The excitatory and inhibitory effects of both antagonists were observed only with submaximal stimulus. We focused on the hypothesis that the facilitatory effect on propulsion may result from blockade of neuronal NK₂ receptors and the inhibitory effect from suppression of the excitatory transmission mediated by NK₂ receptors on smooth muscle cells. In the presence of N⁵⁰-nitro-L-arginine (300 µM), a nitric oxide synthase inhibitor, MEN-10627, at a concentration (10 nM) that was found to accelerate propulsion in control experiments inhibited the velocity of propulsion. In the presence of threshold (1–10 nM) or full (1 µM) concentration of atropine, which inhibited to a great extent the velocity of propulsion, the inhibitory effect of MEN-10627 (1 µM) was markedly increased. In conclusion, in the rabbit distal colon NK₂ receptors may decelerate propulsion by activating a nitric oxide-dependent neuronal mechanism and may accelerate it by a postjunctional synergistic interaction with cholinergic muscarinic receptors.

IN MAMMALIAN GASTROINTESTINAL tract, the tachykinins (TKs) substance P (SP) and neurokinin (NK) A are cotransmitters in several functional classes of myenteric neurons and are concomitantly released in response to depolarizing stimuli (31). They are implicated in both neuroneuronal and neuromuscular transmission. In the guinea pig ileum, tachykinergic neurons have adequate projections and chemical coding to be considered excitatory motor neurons to both longitudinal and circular muscle layers, ascending interneurons, and intrinsic sensory neurons in the enteric circuits subserving peristaltic reflexes and intestinal propulsion (7). The vast majority of TK-containing neurons also costore and corelease acetylcholine (8).

The best-documented actions of both TKs in the enteric nervous system are consistent with their status as noncholinergic neuromuscular excitatory transmitters (for review, see Ref. 20). The TK postjunctional receptors are identified as NK₁ (NKA preferring) and NK₂ (SP preferring) receptors located on smooth muscle cells (3, 38, 45, 48, 52). NK₁ receptors are also located on enteric neurons and interstitial cells of Cajal. Thus NK₁ receptors may directly or indirectly participate in the activation of intestinal circular muscle (26, 51). Recently, a subset of NK₂ receptors has been identified using immunohistochemical procedures on terminals of descending interneurons in the guinea pig myenteric plexus expressing nitric oxide (NO) or bombesin (44). Similarly, neuronal NK₂ receptors are located on nerve terminal varicosities in the rat gastrointestinal tract (16). There is also pharmacological evidence that NK₂ receptors are implicated in the activation of inhibitory motorneurons in the guinea pig colon circular muscle, mainly releasing NO, which is one of the most important inhibitory transmitters at the neuromuscular level and an important neuromodulatory agent of sensory and excitatory enteric neurons (47, 54, 55, 56).

Endogenous TKs serve as neuromuscular transmitters of the ascending reflex contraction of the circular muscle and contribute to the propulsive motility both in ileal and colonic preparations through activation of NK₂ receptors (11, 14, 25). However, only when cholinergic neuromuscular transmission is blocked by atropine/hyoscine is it possible to demonstrate a significant contribution of endogenous TKs in both the ascending reflex contraction and propulsive activity. Furthermore, at least in the guinea pig ileum, a comparison of the effects of atropine and NK₂-receptor antagonists suggests that acetylcholine and TKs act synergistically to contract the circular muscle in response to gut distension and to subserve peristalsis (22, 23).
consistent with the coexistence and corelease of acetylcholine and TKs from the enteric motor neurons (5, 35).

Recently, it has been shown that blockade of the NK₂ receptor increases peristaltic activity in the guinea pig colon in vivo, even though only one class of NK₂ receptor antagonist (i.e., the MEN compounds) induced a significant prokinetic action. This action has been tentatively interpreted as due to the preferential blockade of a subset of NK₂ receptors activating inhibitory neuronal pathways, probably located on descending interneurons (29).

In the present study, we investigated the possible role of NK₂ receptors on propulsion of an intraluminally distended balloon in isolated rabbit distal colon segments. Two selective NK₂-receptor antagonists, structurally different and endowed with different mechanisms of blocking NK₂ receptors, MEN-10627 and SR-48968 (10, 33, 41), were used. Previous studies, in which peristalsis was induced by intraluminal fluid distension, reported that rabbit distal colon propulsion was markedly resistant to muscarinic receptor blockade. This suggests that noncholinergic excitatory transmitters play an important role in the neuromuscular transmission subserving the propulsive activity (30, 32). Furthermore, there is evidence that, in circular muscle strips of rabbit distal colon, electrical field stimulation induced a contractile response that was partially cholinergic and that the atropine-resistant response was abolished by SP-receptor desensitization and by spantide, a nonselective TK-receptor antagonist (50). Together, these findings suggest that TKs play a substantial role in the excitatory neuromuscular transmission subserving peristalsis in this intestinal preparation.

MATERIALS AND METHODS

Male New Zealand White rabbits weighing 2,000–2,400 g were killed by stunning and bleeding, in agreement with the National Research Council’s criteria for the care and use of animals. A 10-cm segment of distal colon was excised, with the aboral end cut nearly 1 cm above the pubis symphysis, and transferred to a petri dish containing prewarmed Tyrode solution to remove the intraluminal content. From each specimen two kinds of preparation were obtained. All preparations were equilibrated for at least 60 min before experiments were started.

Circular Muscle Strips

Segments (3 mm long) of distal colon were cut open at the mesenteric border to obtain rectangular circular muscle strips. After removal of mucosa, strips were mounted isometrically (load 0.5 g) in a 20-ml organ bath containing Tyrode solution maintained at 37°C and continuously bubbled with 95% O₂ and 5% CO₂. This preparation was used to investigate the effect of MEN-10627 and SR-48968 as antagonists of contractile responses mediated by the selective NK₂-receptor agonist [β-Ala₈]NKA-(4—10) (46). In a preliminary set of experiments, concentration-dependent contractile effects of [β-Ala₈]NKA-(4—10) (0.1 nM–1 µM) were evaluated in the absence and presence of atropine or N⁵-nitro-L-arginine (L-NNa) plus apamin to characterize the mode of action of this agonist. NK₂-receptor antagonists were also evaluated against the contractile response evoked by a selective NK₁-receptor agonist, SP-methyl ester.

At least two cumulative concentration-response curves to the agonists were performed at intervals of 45–60 min, with repeated washing after each curve and before testing the effect of the antagonists. In each preparation, a single concentration of antagonist was used and left in contact with the tissue for 15 min.

In a separate set of experiments, the concentration of hexamethonium able to inhibit the response of the nicotinic-receptor agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) was determined.

Measurement and Pharmacological Characterization of Peristalsis

The method for peristalsis induction has been described previously (12). Briefly, whole segments of distal colon, 8 cm long, were transferred to an organ bath containing 100 ml of Tyrode solution. Each segment was set up horizontally, with the aboral end tied to a fixed Perspex holder and the free aboral end connected to an isotonic transducer (2-g load) via a pulley.

Propagation was elicited using a thin rubber balloon that was inserted intraluminally 1 cm below the oral end of the colon and distended with either 0.3 or 1.0 ml of water (external balloon diameters of 6 and 13 mm, respectively). In each experiment, the velocity of balloon propulsion was considered the main parameter of peristaltic activity and was evaluated by recording the time (s) required for the balloon to travel the entire length (mm) of the preparation. Stimuli were elicited every 10 min by alternating 0.3- and 1.0-ml balloon distension, which caused submaximal and maximal velocity of propulsion. The mean velocity of propulsion was calculated by considering two consecutive propulsions.

To study the role of NK₂ receptors in the rabbit distal colon propulsion, the NK₂-receptor antagonists MEN-10627 and SR-48968 were used. Propulsion was elicited 20 min after antagonist administration, a time span sufficient to obtain the maximum effect of the concentration tested, which remained stable for at least 60 min. A concentration-dependent effect of both antagonists on velocity of propulsion was evaluated (MEN-10627, 1 nM–1 µM; SR-48968, 0.1 nM–0.1 µM). In each preparation, a single concentration of antagonist was used.

In a separate series of experiments, MEN-10627 at a concentration (1 µM) that caused a slight but significant antipropulsive effect was tested in the presence of 1 µM atropine according to a procedure used to either unmask or enhance the inhibitory effect of NK₂-receptor blockade in both ileal and colonic peristaltic motor activity (14, 29). Finally, to further evaluate the nature of interaction between cholinergic-muscarinic receptors and NK₂ receptors, we tested the effect of MEN-10627 in the presence of threshold concentrations of atropine (10–20 nM) able to inhibit propulsion.

The effects of a low concentration of MEN-10627 (10 nM), which induced a significant enhancement of the velocity of propulsion, were studied. In a separate set of experiments, the concentration of antagonist was varied from 10 to 1000 nM.

Calculations and Statistical Analysis

Antagonist pA₂ value of MEN-10627 (10 and 100 nM) and SR-48968 (1 and 10 nM) was calculated following Schild regression analysis (2), using [β-Ala₈]NKA-(4—10) dose ratios determined at the EC₅₀ levels in control and test curves.
Values are expressed as percentage of control value and were calculated as means ± SE, with n indicating the number of preparations. Statistical significance of mean differences was assessed by applying Student’s t-test for paired or unpaired data. For multiple mean comparisons, one-way ANOVA plus Bonferroni post hoc analysis was used. P values ≤0.05 were regarded as significant; P > 0.05 was referred to as not significant.

Solutions and Drugs
Tyrode solution (pH 7.3) contained the following (in mM): 136.9 NaCl, 2.7 KCl, 1.8 CaCl2, 1.04 MgCl2, 11.9 NaHCO3, 0.4 Na2HPO4, and 5.5 glucose. Drugs and substances included SR-48968 (Sanofi Recherche, Montpellier, France; by courtesy of Dr. X. Edmond-Alt), MEN-10627 (Menarini, Florence, Italy; by courtesy of Dr. C. A. Maggi), [β-Ala8]NKA-(4—10) (Bachem, Bubendorf, Switzerland), atropine, l-NNA (Janssen Chimica, Geel, Belgium), hexamethonium, DMPP, SP-methyl ester, isoproterenol hydrochloride, and apamin (Sigma Chemical, Milan, Italy).

Stock solutions (1–10 mM) of MEN-10627, SP-methyl ester, and [β-Ala8]NKA-(4—10) were prepared in DMSO and diluted in distilled water. The control experiments showed that DMSO alone (0.1–0.3% final concentration) had no effect on colonic motor activity.

RESULTS
Colonic Circular Muscle Strips
Effects of NK2-receptor antagonists on [β-Ala8]NKA-(4—10)-induced contractions. Circular muscle strips usually displayed low basal tone, which was rhythmically interrupted by phasic contractions. This activity persisted unchanged for up to 60 min. [β-Ala8]NKA-(4—10) (1 nM–1 µM) induced a concentration-dependent contractile effect. This effect was not modified by tissue pretreatment with atropine (1 µM) (n = 3) or l-NNA (200 µM) plus apamin (100 nM) (n = 3), even though atropine induced a slight inhibition and l-NNA plus apamin induced a sustained increase in muscular tone. These findings suggest that, in this preparation, functional NK2 receptors are largely located at a postjunctional level. In preparations displaying a higher basal tone, MEN-10627 and SR-48968 induced a concentration-dependent smooth muscle relaxation, which was slow in onset and peaked within 15 min. After this period, the tone remained stable for at least 40 min, indicating that NK2 receptors modulate resting tone in isolated circular muscle (34). MEN-10627 (10 or 100 nM) and SR-48968 (1 or 10 nM) produced concentration-dependent rightward shifts of the concentration-response curves to [β-Ala8]NKA-(4—10) (1 nM–1 µM) (Figs. 1A and 2A). Low antagonist concentrations (1 nM MEN-10627 and 0.1 nM SR-48968) were ineffective. The antagonism of MEN-10627 concentrations (1 nM MEN-10627 and 0.1 nM SR-48968) were ineffective. The antagonism of MEN-10627 was competitive, as indicated by the parallel rightward shift of concentration-response curves to the agonist, without depression of the maximal agonist effect (Emax), by the slope of the Schild plot not significantly different from unity (Fig. 1A), with a pA2 value of 9.1. These results are consistent with what is found in human ileum and colon, where MEN-10627 featured a similar nanomolar affinity for NK2 receptors (42). The antagonistic effect of MEN-10627 was not completely reversed by washout up to 1 h.

By contrast, SR-48968 (1 or 10 nM) noncompetitively antagonized [β-Ala8]NKA-(4—10)-induced contractions, as shown by the nonparallel rightward shift of concentration-response curves to the agonist (Fig. 2A) and by the Schild plot analysis, which resulted in a slope of 1.63 (Fig. 2B) with an “apparent” affinity estimate of 9.4. Furthermore, SR-48968 induced a 25% reduction of the agonist Emax. The depression of [β-Ala8]NKA-(4—10) Emax was not due to a nonspecific
effect on smooth muscle contractility, since the contractile response to 80 mM KCl was similar in amplitude to that caused by \([\beta-Ala^8]NKA-(4—10)\) was not affected by 1 µM SR-48968. The high-affinity noncompetitive nature of SR-48968 antagonism at NK₂ receptors was previously reported on rabbit preparations (37) and guinea pig (41) and human colonic circular muscle strips (9). The antagonistic effect of SR-48968 was largely reversible by washout. The affinity of SR-48968 at NK₂ receptors was ~1.5-fold higher than the affinity of MEN-10627.

MEN-10627 (1 µM, n = 3) and SR-48968 (1 µM, n = 3) did not affect the contractile response induced by a submaximal concentration of the NK₁-receptor agonist SP-methyl ester (0.1 µM), indicating their selectivity of action at NK₂ receptors.

In preparations showing a spontaneous high tone, hexamethonium (200 µM) abolished the relaxation induced by a submaximal concentration of DMPP (10 µM), which was ~80% of the maximal relaxation induced by isoproterenol (5 µM).

Effects of MEN-10627 and SR-48968 on the velocity of propulsion. The mean velocity of propulsion was 2.2 ± 0.2 mm/s (n = 6) and 2.8 ± 0.4 mm/s (n = 6) at 0.3- and 1.0-ml balloon distension, respectively. In preparations in which peristalsis was elicited by 0.3-ml distension, MEN-10627 caused an acceleration of the velocity of propulsion at concentrations from 0.1 nM to 100 nM (Fig. 3). MEN-10627, at 100 nM, was less effective than 1 nM and 10 nM in enhancing the velocity of propulsion, whereas at 1 µM it caused a slight but significant antipropulsive effect (Fig. 3). Higher concentration (3 µM) did not further inhibit the velocity of propulsion (data not shown). At 1.0-ml distension, MEN-10627 did not significantly affect the velocity of propulsion at any concentration (Fig. 3). Similarly, SR-48968 (0.01–10 nM) accelerated the velocity of propulsion induced by submaximal (0.3-ml) distension (Fig. 4). The maximal effect was observed at 0.1 nM. A concentration of 100 nM was ineffective, whereas the highest concentration administered (1 µM) induced a slight but significant inhibitory effect on the velocity of propulsion. At 1.0-ml distension, SR-48968 did not affect the velocity of propulsion at any concentration (Fig. 4).

It is noteworthy that the lowest concentrations of both antagonists able to induce a prokinetic action did not affect the concentration-response curves to \([\beta-Ala^8]NKA-(4—10)\) in contracting the circular muscle strips (Figs. 1 and 2). On the basis of the latter observation and of previous evidence reporting that NK₂-receptor antagonists inhibit guinea pig ileal (22) and colonic (11) peristalsis mainly through blockade of postjunctional NK₂ receptors, we hypothesized that the prokinetic action of low MEN-10627 and SR-48968 concentrations may be due to the blockade of a subset of NK₂ receptors located on descending pathways (56) and exerting a negative modulation of peristalsis. Thus in one set of experiments we evaluated the effects of NO synthase inhibition on the prokinetic action caused by a low concentration (10 nM) of MEN-10627. L-NNA (200 µM) significantly enhanced the velocity of propulsion induced by 0.3- and 1.0-ml distension by ~20–35% (Fig. 5), thus confirming previous results (6). The administration of MEN-10627 (10 nM) in the presence of L-NNA induced an inhibitory effect on the velocity of propulsion, evoked at 0.3-ml balloon distension, rather than a prokinetic action, as observed in the absence of L-NNA (Fig. 5). In the same experimental conditions, MEN-10627 had no effect on velocity of propulsion evoked at 1.0-ml balloon distension.

To further investigate the mode and site of a putative neuronal mechanism, by which endogenous TKs may negatively modulate peristaltic efficiency through NK₂ receptors, we evaluated the effects of the blockade of

![Graph](image-url)
cholinergic-nicotinic receptors on the prokinetic action of MEN-10627 (10 nM). Hexamethonium (200 µM) induced a slight inhibition of the velocity of propulsion, independently of the degree of stimulus applied (Fig. 6). This effect, which lasted for 10–20 min, was followed by a full recovery of the velocity of propulsion elicited at 0.3- or 1.0-ml distension. A second administration of the drug did not induce any further effect on the velocity of propulsion (not shown). The administration of MEN-10627 (10 nM) in the presence of hexamethonium significantly enhanced the velocity of propulsion elicited at 0.3-ml distension, although to a lesser extent than that caused by MEN-10627 alone (Fig. 6). No modification of propulsion elicited at 1.0-ml distension was observed (Fig. 6).

The inhibitory effect on the velocity of propulsion induced by high concentrations of MEN-10627 and SR-48968 may be due to the blockade of a subset of NK2 receptors located at a postjunctional level and favoring propulsion. According to a procedure largely utilized to enhance or unmask the inhibitory effect of NK2 (or NK1)-receptor antagonists on intestinal propulsion, we tested the effects of MEN-10627 in the presence of atropine (14, 22). Atropine (1 µM), after 10-min incubation, induced a profound inhibition of the velocity of propulsion elicited at 0.3-ml and 1.0-ml balloon distension, which recovered progressively over 50–60 min (Fig. 7). The recovery was more pronounced when velocity of propulsion was elicited at 1.0-ml distension. Under these conditions, a second atropine administration did not affect further velocity of propulsion. In the presence of 1 µM atropine (60-min incubation), MEN-10627 (1 µM) invariably blocked peristalsis elicited at 0.3-ml distension (n = 5) but left unchanged peristalsis evoked at 1.0-ml distension (control velocity: 0.3-ml distension, 0.72 ± 0.1 mm/s, n = 6; after treatment: 0.3-ml distension 0 mm/s, n = 6). Furthermore, in the presence of threshold concentrations of atropine (10–20 nM) (n = 5) that induce a slight but significant inhibition of propulsion, the effect of MEN-10627 (1 µM) (n = 5) was significantly more pronounced than that obtained in the absence of atropine (Fig. 8).

**DISCUSSION**

The results of the present study suggest that endogenous TKs contribute to the propulsive activity of the isolated rabbit distal colon through the activation of NK2 receptors located on neuronal pathways and muscular structures subserving peristalsis. The role of NK2 receptors has been investigated using two structurally different selective antagonists, MEN-10627 and SR-48968 (10, 33). MEN-10627 is a poly cyclic peptide antagonist, whereas SR-48968 is a nonpeptide compound possessing high affinity for rabbit smooth muscle NK2 receptors (1) and considered to act through a
different mechanism than MEN-10627, at least in the guinea pig gallbladder and colon (41). The antagonism of SR-48968 at NK2 receptors may be allosteric (9, 13). The different mechanisms of NK2-receptor blockade by SR-48968 and MEN-10627 were also observed in the present study, in which we showed that SR-48968 induced an insurmountable (noncompetitive) antagonism of NK2 receptor-mediated contraction in colonic circular muscle strips, whereas MEN-10627 produced a competitive antagonism. Both types of antagonism were time independent (no change in the antagonistic effect was observed by prolonging the incubation from 15 min to 120 min). The affinity of SR-48968 at NK2 receptors was ~3 times higher than that of MEN-10627.

MEN-10627 (1–100 nM) and SR-48968 (0.1–10 nM) induced a significant increase in the velocity of propulsion. The effects of both antagonists tended to fade at higher concentrations. By contrast, MEN-10627 (1 μM) and SR-48968 (0.1 μM) induced a slight but significant inhibitory effect on the velocity of propulsion. Both excitatory and inhibitory effects induced by the two antagonists were shown only on propulsion induced by submaximal stimulus. The prokinetic action caused by NK2 receptor blockade was rather unexpected on the basis of previous observations obtained in both ileal (25) and colonic (11) preparations of guinea pig, which indicated an inhibitory effect on peristaltic activity evaluated in the absence and presence of atropine (23). We speculated that the facilitatory effect induced by low concentrations of both antagonists on rabbit distal colon propulsion may arise from blockade of inhibitory mechanisms activated by NK2 receptors. It is likely that this effect is mediated by NK2 receptors located in myenteric plexus since the prokinetic concentrations of MEN-10627 (1 nM) and SR-48968 (0.1 nM) did not affect the direct contraction of circular muscle strips induced by the selective NK2-receptor agonist [β-Ala8]NKA-(4—10). This is consistent with the presence of NK2 receptors on a subset of descending myenteric interneurons expressing bombesin and NO (44) and with the evidence that NK2-receptor activation induces NO release, thus leading to depression of circular muscle activity (56). Indeed, L-NNA induced a significant enhancement of the velocity of propulsion (at both 0.3-ml and 1-ml distension), thus confirming the involvement of NO in mediating the inhibition of the velocity of colonic propulsion in the rabbit (6). The inhibition of NO synthase activity was also found to induce a facilitatory effect on peristalsis in the guinea pig ileum (53). More recently, dual excitatory and inhibitory effects of NO on peristalsis have been reported in the guinea pig intestine. The excitatory effect involved cholinergic motorneurons, whereas the inhibitory effect reflected relaxation of intestinal muscle (21).

In contrast to what we observed in the rabbit colon, the inhibition of NO synthase induced an inhibitory effect in the guinea pig colonic propulsion (11). In this
preparation, recent evidence suggests that NO facilitates and depresses the release of acetylcholine from interneurons in ascending and descending pathways, respectively, in addition to directly inhibiting smooth muscle (49). Whatever the mechanism by which endogenous NO negatively modulates the rabbit distal colon propulsion, low concentrations of MEN-10627 and SR-48968 inhibited the propulsion evoked at submaximal stimulus once NO synthase activity was suppressed by L-NNA. This suggests that the facilitatory effect of MEN-10627 recorded in the absence of L-NNA may be due to the blockade of a subset of NK2 receptors activating NO-dependent inhibitory mechanisms on propulsive activity. Similar results were obtained using low concentrations of SR-48968 (not shown).

To further assess the putative prejunctional action of MEN-10627 in enhancing the velocity of propulsion, we investigated the effect of this compound in the presence of hexamethonium, a blocker of cholinergic-nicotinic transmission. Hexamethonium induced a slight transient decelerating effect on the velocity of propulsion that may be due to the occurrence of adaptive responses, since it was not modified by a second administration of hexamethonium. This is in agreement with previous observations in the same preparations in which peristalsis was induced by fluid supply (30, 32). In the presence of hexamethonium, low concentrations of MEN-10627 still induced a significant enhancing effect on the velocity of propulsion, although to a lesser degree than that obtained in the absence of nicotinic-receptor blockade. Partial inhibition of the prokinetic effect of MEN-10627 in the presence of hexamethonium may suggest that descending interneurons activated by endogenous TKs through NK2 receptors can be cholinergic. However, this is not supported by the immunohistochemical localization of NK2 receptors, at least in the guinea pig ileal myenteric plexus (44). Hexamethonium may also inhibit the MEN-10627 effect through other mechanisms, including antagonism of nicotinic receptors distributed along the ascending pathways subserving peristalsis (27). The impairment of such nicotinic transmission may mask, at least in part, the prokinetic effect of NK2 antagonists through inhibition of NO-production/release.

At the highest concentration (1 µM), MEN-10627 significantly inhibited the velocity of propulsion elicited at low distension. It is reasonable to assume that this inhibitory effect is due to the blockade of postjunctional NK2 receptors, which comigrate with muscarinic receptors (and to a minor extent with postjunctional NK1 receptors) the activation of muscular effectors subserving peristalsis (22, 25). Thus the antiperistaltic effect of MEN-10627 was evaluated in the presence of atropine. This procedure has been commonly applied to unmask or to enhance the contribution of excitatory noncholinergic transmitters mainly at a postjunctional
level in sustaining neuromediated muscle contractions, such as those underlying ascending circular muscle reflexes and peristalsis (14, 23). Atropine induced a reduction of the velocity of propulsion and sometimes a transient blockade when peristalsis was evoked by submaximal stimulus. In the presence of atropine, the velocity of propulsion underwent a time-dependent partial recovery, which was significantly more pro-

Fig. 6. Comparison of effects of low concentrations of MEN-10627 on rabbit distal colon propulsion in absence and presence of cholinergic-nicotinic transmission blockade by hexamethonium (C6). Note that MEN-10627 in presence of hexamethonium enhanced velocity of propulsion evoked by submaximal stimulus (open bars), but stimulatory effect was significantly less than that induced by MEN-10627 alone. MEN-10627 did not affect hexamethonium-resistant propulsion evoked by maximal stimulation (closed bars). Values are means ± SE of 7 experiments. Error bars indicate significance (ANOVA plus Bonferroni post hoc analysis) between different treatments. **P < 0.01 vs. control velocity.

Fig. 7. Time course of effect of atropine (1 µM) on velocity of propulsion of isolated rabbit distal colon. Atropine induced a marked inhibition of propulsion that was significantly more pronounced when propulsion was evoked by submaximal stimulus (open bars) compared with maximal stimulus (closed bars). In continuous presence of atropine, propulsion underwent a time-dependent partial recovery. Values are means ± SE of 6 experiments. *P < 0.05 or **P < 0.01 vs. control velocity.
nounced when the propulsion was evoked by maximal stimulus. These findings suggest the existence of adaptive mechanisms, probably consisting of activation/enhancement of noncholinergic excitatory transmission, leading to a partial substitution of the cholinergic drive. Apparently, this noncholinergic excitatory mechanism plays a major role when the propulsion is evoked by maximum degree of stimulus. These results are in agreement with previous observations suggesting that the atropine-resistant ascending reflex contraction of the circular muscle in both ileal and colonic preparations is more pronounced with maximal or supramaximal stimuli (18, 23).

In the presence of muscarinic receptor blockade, MEN-10627 (1 µM) produced a marked and persistent inhibition and often a blockade of propulsion evoked by submaximal distension. Similarly, in the presence of threshold concentrations of atropine (10–20 nM), able to consistently reduce the velocity of propulsion, the inhibitory effect of MEN-10627 was significantly increased. These findings suggest a synergistic interaction between muscarinic and NK₂ receptors. A similar interaction between muscarinic and NK₂ receptors has been previously described in guinea pig ileal peristalsis (23).

The apparent lack of participation of NK₂ receptors in the atropine-resistant propulsion evoked by maximum stimulus is more difficult to explain. There is evidence that tachykinergic contribution, via NK₁ and NK₂ receptors, to atropine-resistant ascending reflex contraction of the circular muscle is directly related to the extent of stimulation (14, 17, 19). These findings indicate the presence of a low-threshold cholinergic transmission and a high-threshold tachykinergic transmission mediated by NK₁ and NK₂ receptors. However, it was recently demonstrated that the contribution of acetylcholine and TKs in circular muscle contraction to electrical field in the guinea pig colon is relatively independent of the intensity of the stimulus and/or the mechanism of transmitter release. Therefore, postjunctional factors seem predominant in determining the contribution in producing excitation of the circular muscle (35). This observation may be consistent with the important role exerted by NK₁ receptors on atropine-resistant propulsion evoked by the submaximal stimulus. Nevertheless, the atropine-resistant, non-NK₂ receptor component of propulsion evoked by maximal stimulation may be mediated by different TK receptors (mainly NK₂) or by other noncholinergic excitatory transmitters. Our previous findings indicate that the atropine-resistant component of peristalsis evoked by maximal distension was sensitive to a concomitant blockade of NK₁ and NK₂ receptors by SR-140333 and MEN-10627, respectively (39). Under this condition, an ~65% inhibition of the velocity of propulsion was obtained. However, it is interesting to point out that the administration of SR-140333 alone failed to significantly affect peristalsis. This indicates that TKs contribute to the atropine-resistant component of peristalsis to high stimulation and that a synergistic interaction between the two TK receptor systems may intervene.

The atropine- and TK-resistant component may be mediated by excitatory transmitters such as bombesin, which was found to be released by electrical field stimulation in the rabbit distal colon and to induce circular muscle contraction (4, 50). Therefore, in the present study, a noncholinergic transmitter(s) other than TKs may contribute to the atropine-resistant peristalsis induced by maximal gut wall distension.

Finally, the large difference in concentration range (100- to 1000-fold) at which the NK₂-receptor antagonists exert their dual effect on colonic propulsion suggests the existence of different subtypes of NK₂ receptors in the rabbit distal colon, which are probably located on distinct structures, such as nerves and smooth muscle cells. Recently, the existence of two distinct NK₂-receptor subtypes on human colonic muscle strips has been proposed (37, 52). In addition, a high degree of pharmacological homology has been described between human colon and rabbit NK₂ receptors (36, 43). However, our data did not allow us to establish clear-cut evidence for the existence of NK₂-receptor heterogeneity in the rabbit colon.

In conclusion, our findings provide evidence that NK₂-receptor antagonists accelerate or inhibit the velocity of propulsion in the rabbit isolated distal colon according to concentration. These effects are probably mediated by indirect neuronal (leading to acceleration) and direct postjunctonal (leading to deceleration) mechanisms, due to inhibition of NO production and blockade of muscular NK₂ receptors, respectively.

Preliminary results of this study were presented at Digestive Disease Week 1999, Orlando, FL (40).

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