Peristalsis regulation by tachykinin NK₁ receptors in the rabbit isolated distal colon


In the mammalian gastrointestinal tract, the tachykinins (TKs), substance P (SP) and neurokinin A (NKA) are cotransmitters in several functional classes of myenteric neurons and are concomitantly released in response to depolarizing stimuli (see Ref. 14 for review). In particular, TKs and ACh are coexpressed by certain intrinsic primary afferent (sensory) neurons (IPANs) and by many ascending interneurons and motoneurons, which are part of the circuits regulating excitatory peristaltic reflexes (4, 10, 28). Conversely, descending pathways do not contain TKs (i.e., cholinergic/noncholinergic descending interneurons and inhibitory motoneurons), although TK receptors are expressed in a portion of these neurons (4, 5). This makes it difficult to explain the contribution of NK₁, NK₂, and NK₃ receptors to the development of descending inhibitory reflexes (18, 19, 44).

Of the three tachykinin receptors, NK₁ receptors show a higher distribution in the intestine, because they are expressed by a number of neuronal and nonneuronal cells involved in gut motor activity. In the myenteric plexus of the guinea pig ileum, NK₁-receptor immunoreactivity (NK₁r-IR) is present in a large population of neurons containing nitric oxide (NO) synthase (NOS), a marker of inhibitory neurons to the muscle and in a small portion of IPANs, descending nitrergic interneurons, and excitatory neurons to the circular muscle (27, 34). In the rat ileum, NK₁r-IR occurs in cell bodies and in the proximal processes of IPANs and myenteric interneurons (31, 41). In nonneuronal cells, NK₁r-IR is present in the interstitial cells of Cajal (ICC) and smooth muscle cells of the rat and guinea pig small and large intestine (22, 34, 39, 41). Therefore, tachykinergic NK₁ transmission to the circular muscle may be mediated by receptors located both on ICCs and smooth muscle cells.

Information on the role of NK₁ receptors on propulsive activity mainly comes from studies on ileal and colonic peristalsis in the guinea pig. In the ileum, SP and selective NK₁-receptor agonists exert an inhibitory effect, which can be interpreted either as an NK₁-mediated inhibitory feedback mechanism on ACh release from cholinergic motoneurons or as an activation of a nitrergic pathway leading to a direct or an indirect depression of circular muscle activity (13, 16, 26, 35). Surprisingly, in the guinea pig ileum, even NK₁-receptor blockade was found to inhibit peristalsis (15, 42). This effect is probably mediated by the inhibition of postjunctional NK₁ receptors, which facilitate propulsion by enhancing the excitatory drive to the circular muscle. This hypothesis is supported by the finding that the inhibitory effect of the NK₁-receptor antagonism is unveiled or amplified by the concomitant blockade of the predominant excitatory transmission (i.e the cholinergic muscarinic transmission) to intestinal muscles (15, 42). By contrast, in the guinea pig colon propulsion, NK₁ receptors appear to play a scarce pro-

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kinetic role, mainly observed in the presence of a cholinergic muscarinic receptor blockade, indicating a predominant postjunctional location of NK1 receptors (8, 24, 43).

The rabbit isolated distal colon represents a suitable model to investigate the contribution of noncholinergic excitatory transmitters at both the neuroneuronal and neuromuscular level during propulsion, because it shows a high resistance to the cholinergic (either nicotinic or muscarinic) transmission blockade (25, 29). In this preparation, we recently demonstrated that NK2 and NK3 receptors exert an inhibitory effect as well as an excitatory role on propulsion, depending on their cellular distribution and the concentration of selective TK antagonists used (32, 33). In the present study, we investigated the potential role of NK1 receptors on rabbit colonic propulsion by using a selective agonist, septide, endowed with a high affinity for both neuronal and muscular NK1 receptors, and two selective antagonists, SR-140333 and MEN-10930 (1, 6, 18, 30). The first, is a widely used, nonpeptidic, NK1-receptor antagonist, devoid of any selectivity for both neuronal and muscular NK1 receptors, and two selective antagonists, SR-140333 and MEN-10930 (1, 6, 18, 30). The first, is a widely used, nonpeptidic, NK1-receptor antagonist, devoid of any significant interspecies selectivity for NK1 receptors. The second belongs to a class of cyclic NK1 antagonists showing a greater affinity for human, guinea pig, and rabbit NK1 receptors than for NK1 receptors expressed in rodent tissues (1, 6).

MATERIALS AND METHODS

Male New Zealand White rabbits of either sex, weighing 2,000–2,400 g were used in this study. Animal care and procedures were in accordance with the National Institutes of Health recommendations and the European Union directives for the humane use of animals. Rabbits were killed by stunning and bleeding. A 10-cm segment of distal colon was excised with the aboral end cut nearly 1 cm above the pubis and transferred to a Petri dish containing prewarmed Tyrode’s 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 NaH2PO4, and 5.5 glucose. Drugs and substances were SR-140333 (Sanofi Recherche, Montpellier, France; courtesy of Dr. X. Edmond-Alt); MEN-10930 (Menarini, Florence, Italy, courtesy of Dr. C. A. Maggi); and atropine, septide, and l-noradrenaline (l-NNA; 100 µM), an inhibitor of NOS (20), and in the presence of atropine (1 µM) plus l-NNA (100 µM). The effect of l-NNA was evaluated after 20 min of contact and remained stable for at least 60 min of observation. The effect of atropine was evaluated after 60 min exposure, when the reduction of propulsion underwent a time-dependent partial recovery and the propulsion evoked by both stimuli became stable, as described in detail in a previous work (33).

The effects of SR-140333 and MEN-10930 on the velocity of propulsion were evaluated 15 min after antagonist administration, a timespan sufficient to obtain the maximum antag-
onistonic effect, which remained stable for at least 60 min. In each preparation, a cumulative concentration-response curve of SR-140333 (0.3–100 nM) and MEN-10930 (0.3–100 nM) was obtained. For each antagonist concentration (usually 2 antagonist concentrations were tested in each experiments), propulsions were evaluated at 0.3- and 1.0-ml balloon distension. In different experimental sets, the effects of SR-140333 (0.3 and/or 1 nM) were evaluated in the presence of atropine (1 µM) or l-NNA (100 µM) and in the presence of atropine (1 µM) plus l-NNA (100 µM) by using the pharmacological approach mentioned above.

Measurement and Pharmacological Characterization of Peristalsis

The method for peristalsis induction has been described previously (9). Briefly, whole segments of distal colon, 8 cm long, were transferred to an organ bath containing 100 ml Tyrode’s 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 (load 2 g) via a pulley. The propulsion was elicited every 10 min using a thin rubber balloon, which was inserted intraluminally 1 cm below the oral end of the colon and distended with either 0.3 or with 1.0 ml of water (external balloon diameter, 6 and 13 mm, respectively). In each experiment, the velocity of balloon propulsion was considered as 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. The distensions of 0.3 and 1.0 ml caused a submaximal and maximal velocity of propulsion, respectively. The mean velocity of propulsion was calculated by considering two consecutive propulsions.

To study the role of NK1 receptors in propulsive activity in the rabbit isolated distal colon, septide, SR-140333, and MEN-10930 have been used. To avoid NK1-receptor desensi-
tization to septide (31), the agonist was left in contact with the tissue for 1 min before inducing propulsion with a single distension (of 0.3 or 1.0 ml). The preparations were then repeatedly washed and left to reequilibrate for at least 20 min before adding the same or a different septide concentration. Under these conditions, the effect of a subsequent septide concentration on propulsion was reproducible, and a noncumulative concentration-response curve to the agonist (3–100 nM) was created. The effects of a low septide concentration (3 nM), which induced a significant antipropulsive effect, were then assessed in the presence of Nω-nitro-l-arginine (l-NNA; 100 µM), an inhibitor of NOS (20), and in the presence of atropine (1 µM) plus l-NNA (100 µM). The effect of l-NNA was evaluated after 20 min of contact and remained stable for at least 60 min of observation. The effect of atropine was evaluated after 60 min exposure, when the reduction of propulsion underwent a time-dependent partial recovery and the propulsion evoked by both stimuli became stable, as described in detail in a previous work (33).

RESULTS

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.

Effects of SR-140333 and MEN-10930

In preparations in which peristalsis was elicited at a 0.3-ml distension, SR-140333 caused an acceleration of
the velocity of propulsion at 0.3 and 1 nM (Figs. 1 and 2). At 10 and 100 nM, SR-140333 was ineffective (Fig. 1), whereas at 300 and 600 nM it caused a slight (<15%) antipropulsive effect (data not shown). At a 1.0-ml distension, SR-140333 did not significantly affect the velocity of propulsion at any concentration (Fig. 1).

Similarly, MEN-10930 (0.3–10 nM) concentration-dependently accelerated the velocity of propulsion induced by the submaximal (0.3 ml) distension (Fig. 3). The maximal effect was observed at 10 nM, whereas at 100 nM and at higher concentrations (300 nM; data not shown), MEN-10930 was ineffective. MEN-10930 did not affect the velocity of propulsion elicited at a 1.0-ml distension.

Effects of SR-140333 in the Presence of L-NNA or Atropine and Atropine Plus L-NNA

Effects of SR-140333 in the presence of L-NNA. As demonstrated previously (3), L-NNA (100 μM) significantly enhanced the velocity of propulsion by ~20% (at both 0.3- and 1.0-ml balloon distension), thus confirming the involvement of NO in mediating the inhibition of colonic propulsion in the rabbit. In the presence of L-NNA, SR-140333 (0.3 and 1 nM) did not affect the velocity of propulsion to 0.3 ml distension (Fig. 4).

Effects of SR-140333 in the presence of atropine. Atropine (1 μM), after 10 min of incubation, induced a profound inhibition of the velocity of propulsion elicited at 0.3- and 1.0-ml balloon distension, which recovered progressively over 50–60 min until a steady-state value of inhibition of the propulsion was established (Fig. 4). A second administration of atropine (1 μM) did not further modify the velocity of propulsion.

In the presence of the stable effect of atropine, SR-140333 (1 nM) caused a significant reversal of the
inhibition at both distension stimuli (Fig. 5). The enhancing effect induced on the propulsion due to the submaximal stimulus was comparable in extent with that obtained in the absence of atropine [39.2 ± 2.25 (n = 100) vs. 39.5 ± 5.7% (n = 8)]. In the presence of atropine, SR-140333 (1 nM) induced a significant acceleration (~30%) of the propulsion induced by the maximal stimulus (Fig. 5), an effect not observed in the absence of atropine (see Fig. 1).

**Effects of SR-140333 in the presence of atropine and L-NNA.** In the presence of atropine (1 μM), L-NNA (100 μM) induced a prokinetic effect comparable with that observed in the absence of the drug (Fig. 5). In the presence of atropine and L-NNA, SR-140333 (1 nM) induced a significant inhibitory effect on the velocity of propulsion evoked by the submaximal stimulus (Fig. 5), leaving unaffected that evoked by maximal stimulation.

**Effects of Septide**

Septide (concentration range: 3–10 nM) induced a concentration-dependent inhibition of the propulsion velocity induced by both stimuli. This effect faded at a higher concentration (100 nM; Fig. 6). At 300 nM, septide caused a propulsion blockade (data not shown). On visual inspection, the arrest of propulsion was associated with nonpropulsive circular muscle contractions occurring at both sides of the bolus.

**Effects of septide in the presence of L-NNA.** As mentioned above, septide (3 nM) induced a significant inhibition of the velocity of propulsion (Fig. 7). In the presence of 100 μM L-NNA, which per se accelerated propulsion, septide induced a significant enhancement of the velocity of propulsion, independently of the stimulus applied (Fig. 7).

**Effects of septide in the presence of L-NNA plus atropine.** In the presence of 100 μM L-NNA plus 1 μM atropine, the prokinetic effect of septide (3 nM) was not different (total propulsion enhancement by 52.5 and 39.5% at a 0.3- and 1.0-ml distension, respectively) from that observed in the absence of atropine (n = 6).

**DISCUSSION**

The main conclusion of this study is that NK1-receptor agonist inhibits, whereas NK1-receptor antagonism enhances the velocity of propulsion in the rabbit isolated distal colon. The inhibitory modulation oper-
ated by NK1 receptors is mainly sustained by the activation of sites expressed by or connected to descending nitrergic pathways. Indeed, in the presence of L-NNA, a blocker of NOS activity, the prokinetic effect of SR-140333, a selective NK1-receptor antagonist, was abolished, whereas the inhibitory effect of sepiptide, a selective NK1-receptor agonist, was reverted into that of facilitating propulsion.

As also demonstrated previously, the blockade of NOS activity induced a small but significant prokinetic effect on the rabbit isolated distal colon, presumably due to the removal of a nitrergic inhibitory mechanism regulating circular muscle activity (3, 32, 33). In this study, the L-NNA accelerating effect was not modified by atropine, which per se inhibited propulsion evoked by submaximal and maximal stimuli. This suggests that in this preparation, endogenous NO does not modulate cholinergic muscarinic transmissions, as opposed to findings obtained in gastrointestinal tissues of other species (17). It is of note that even the NK1-receptor-mediated inhibition of propulsion does not involve an appreciable impairment of cholinergic muscarinic drive subserving peristalsis. This is inferred by the evidence that the prokinetic effect of SR-140333 was not modified by atropine, at least when the evoking stimulus was submaximal. Similarly, the prokinetic role of NK1 receptors did not appear to be due to activation of cholinergic muscarinic transmission. Indeed, the sepiptide’s enhancing effect on propulsion in the presence of L-NNA was not modified by the simultaneous blockade of cholinergic muscarinic receptors. The hypothesis that postjunctional NK1 receptors facilitate colonic propulsive activity is corroborated by the finding that, in the presence of L-NNA and atropine, SR-140333 exerted a marked inhibitory effect on peristalsis. This suggests that the facilitating role of muscular NK1 receptors is observed when the predominant muscarinic excitatory neuromuscular transmission is abolished.

The Complex Influence of NK1 Receptors on Rabbit Distal Colon Propulsion Deserves Additional Detailed Comments

Effects of NK1-receptor antagonism by SR-140333 and MEN-10930. SR-140333, in a narrow range of subnanomolar concentrations (0.1–1 nM), and MEN-10930, in a wider range of concentrations (0.1–10 nM), both enhanced the velocity of propulsion when evoked by a submaximal stimulus. The apparent lack of participation of the NK1 receptors in propagations evoked by maximal stimulation is difficult to explain. The most simple explanation is that the negative tachykinergic modulation of colonic propulsion can be unveiled more easily when the velocity of propulsion is submaximal, as observed previously with selective NK2- and NK3-receptor antagonists in the same preparation (32, 33). An alternative hypothesis may consist in the fact that larger stimuli might enhance the release of TKs acting on different receptors/targets. In the guinea pig ileum and colon circular muscle, there is evidence that the TK component of the ascending reflex contraction is relatively larger when the stimulus is maximal or near maximal (11, 12). On a molar basis, the effect of SR-140333 and MEN-10930 was roughly equipotent. At a slightly higher concentration (100 nM), the effect of both antagonists faded, whereas it disappeared within the submicromolar range of concentrations (300, 600 nM). This suggests the existence of NK1 receptors on functionally different colonic structures; so NK1 receptors may play an opposite role on the velocity of propulsion. To our knowledge, a net prokinetic effect induced by an NK1-receptor antagonist has not previously been observed in any model of intestinal peristalsis. In fact, in the guinea pig ileum and colon, NK1-receptor antagonists inhibit peristalsis. Recently, however, SR-140333 at 10 nM was found to induce a nonsignificant facilitation (20% enhancement) of the velocity of propulsion in the guinea pig distal colon (43). It is noteworthy that even in the present study, the same concentration of SR-140333 induced a nonsignificant enhancement of propulsion velocity.

Effects of SR-140333 in the presence of L-NNA. To assess whether nitrergic inhibitory pathways are involved in the prokinetic effect of SR-140333, experiments were carried out in the presence of L-NNA. As observed previously, L-NNA induced a slight but significant prokinetic effect in the rabbit colon, independent of the degree of stimulus (3, 32, 33). In the presence of L-NNA, SR-140333 was ineffective. This suggests that the prokinetic effect of SR-140333 (as observed in the absence of L-NNA) is largely due to a blockade of NK1 receptors located on nitrergic neurons, which modulate propulsion negatively. This may occur in inhibitory motoneurons or in descending interneurons inhibiting descending excitatory and inhibitory pathways underlying the peristaltic reflex in the colon (36, 40).

Effects of SR-140333 in the presence of atropine. To evaluate a potential impairment of cholinergic muscarinic transmissions in the NK1-receptor-mediated inhibition of propulsion, we tested the effect of SR-140333 in the presence of atropine. As previously described in detail, a full concentration of atropine (1 μM) induced an inhibitory effect on the velocity of propulsion of −55 and 40% at submaximal and maximal stimulus, respectively (33). In the presence of atropine, the propulsion evoked by the submaximal stimulus was enhanced by SR-140333 to an extent similar to that found in the absence of atropine. This indicates that the NK1 receptor-mediated inhibition of propulsion is not due to an appreciable impairment of cholinergic muscarinic drive subserving this motor pattern. Surprisingly, in the presence of atropine, SR-140333 induced a slight but significant facilitation of propulsion, also when induced by a maximal degree of balloon distension. This suggests that in the presence of atropine, the blockade of the NK1 receptor-mediated activation of nitrergic pathways preferentially favors the propulsion evoked by the maximal stimulus. In these conditions, the latter may activate a noncholinergic excitatory transmission. Theoretically, this may occur via tachy-
kinin NK₂ (33) or bombesin receptors. Indeed, in the rabbit distal colon, bombesin is released by electric field stimulation and induces a marked contraction of circular muscle cells (2, 38), which may sustain propulsion when the prominent excitatory transmissions are abolished.

**Effects of SR-140333 in the presence of atropine and L-NNA.** The effect of SR-140333 in the presence of atropine plus L-NNA was tested to unveil any additional role of NK₁ receptors after the simultaneous abolition of cholinergic muscularic and nitrergic transmissions. In the presence of atropine, L-NNA induced a prokinetic effect similar in extent to that observed in the absence of the drug. This finding indicates that, at least in this preparation, endogenous NO does not substantially affect cholinergic muscularic excitatory transmission. However, in the presence of atropine plus L-NNA, the velocity of propulsion, independently of the degree of stimulus applied, was lower than that of controls, because the inhibitory effect of atropine was relatively larger than the prokinetic effect of L-NNA. In the guinea pig distal colon, on the other hand, it is significant to note that endogenous NO plays a very complex modulating role on cholinergic ascending and descending interneurons and cholinergic motoneurons involved in peristaltic reflexes (36, 37).

In the presence of both atropine and L-NNA, SR-140333 induced a significant inhibitory effect on the velocity of propulsion evoked by the submaximal stimulus. This effect is probably due to blockade of postjunctional NK₁ receptors, whose activation mainly contributes to the low-stimulus propulsion when the cholinergic excitatory neuromuscular transmission is abolished by atropine. It is well known that in the guinea pig ileal and colonic peristalsis, atropine unveils or enhances the antipropriulsive effect of NK₁- and NK₂-receptor antagonists (8, 15, 24, 42, 43). The evidence that, in the rabbit distal colon, atropine exerted a similar effect only after suppression of NOS activity further strengthens the predominance of an NO-mediated inhibitory role of NK₁ receptors on propulsion in this preparation.

**Effects of NK₁-receptor activation by septime.** Within a narrow range of nanomolar concentrations (3–10 nM), septime induced a concentration-dependent inhibition of velocity propulsion, independent of the degree of stimulus. This effect faded at higher (100 nM) concentrations. A blockade of propulsion was obtained when submicromolar agonist concentrations were used. On visual inspection, nonpropulsive circular muscle contractions appeared to accompany the blockade and apparently disrupted the analy propagating wave of contraction during propulsion. These septime effects are consistent with the antipropriulsive effect of the NK₁-receptor agonism in the guinea pig ileum (13, 16, 35) and with the general notion that SP and selective NK₁-receptor agonists inhibit intestinal motility in various animal species at concentrations much lower than those required to induce an excitatory effect via the activation of postjunctional receptors (7, 23).

**Effects of septime in the presence of L-NNA.** In the presence of L-NNA, septime [at a concentration (3 nM) that inhibited the velocity of propulsion in control conditions] further increased the velocity of propulsion in response to the submaximal stimulus. This finding confirms the involvement of an NO mechanism in the NK₁ receptor-mediated inhibition of colonic propulsion and demonstrates a prokinetic role played by a different population of NK₁ receptors (probably located on excitatory pathways) when the NO pathway is suppressed. In another set of experiments, this prokinetic effect of septime was not modified by the concomitant presence of L-NNA and atropine in the solution bath. This finding suggests that septime could enhance the velocity of propulsion by activating postjunctional NK₁ receptors located on the effector cells. In this respect, a previous paper provided evidence that the SP-induced circular muscle contraction in the rabbit distal colon is myogenic in nature and probably mediated by the activation of postjunctional NK₁ receptors (21).

In summary, to our knowledge, the present findings offer the first evidence that TKs inhibit rather than enhance colonic propulsion via NK₁ receptors. This action is apparently mediated by neuronal NK₁ receptors that may activate nitrergic pathways, which, in turn, lead to the inhibition of propulsion via a direct NO-mediated inhibitory restraint on the circular muscle and probably on descending excitatory or inhibitory pathways. Furthermore, a different population of NK₁ receptors, probably located at a postjunctional level, plays a prokinetic role. The NK₁ receptor-mediated facilitation of propulsion is unveiled only after the suppression of NOS activity and is more easily observed when these NK₁ receptors are activated by exogenous agonists rather than when inhibited by antagonists. Therefore, the facilitating role of NK₁ receptors in rabbit distal colon propulsion is unlikely to occur in the physiological condition.

**REFERENCES**