Spasmolytic Effect of the NK_2-Receptor-Selective Antagonist MEN 10,627 in Rat Small Intestine

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ABSTRACT—The effect of activation of tachykinin NK_2-receptors on gastrointestinal propulsion was studied in vivo in conscious rats. The selective NK_2-receptor agonist [βAla^8]NKA-(4–10) produced an atropine-resistant specific increase of the small intestinal transit measured by the charcoal method. This effect was restricted to the small intestine since gastric emptying was not affected by [βAla^8]NKA-(4–10). The newly developed polycyclic peptide NK_2-receptor antagonist MEN 10,627 produced a dose-dependent inhibition of this stimulated transit. The spasmolytic effect of MEN 10,627 was highly selective because it did not affect stimulated intestinal transit induced by equieffective doses of carbachol and reserpine. These findings indicate that MEN 10,627 is a valuable tool for assessing the role of NK_2-receptors in intestinal propulsive activity.

Keywords: Intestinal transit, Neurokinin $(NK)_2$-receptor, Tachykinin

Tachykinins are a family of peptides that share the common C-terminal sequence Phe-X-Gly-Leu-Met NH_2. In mammals, three peptides of this family, namely, substance P (SP), neurokinin (NK)-A and NK-B, are believed to play a role as neurotransmitters, either in the peripheral or the central nervous system (1). Tachykinins act as excitatory transmitters in the mammalian gut, where they exert potent spasmogenic effects via specific receptors, termed NK_1, NK_2 and NK_3; and they are located on muscle cells or indirectly generated by activating intramural neurons (2, 3). In vitro studies showed that activation of NK_2-receptors produces potent spasmogenic effects in several segments of the intestine (3, 4). In the last few years, new selective and potent NK_2-receptor antagonists have been discovered (1), allowing researchers to define the effect of tachykinins in various organs. The characterization of a new highly selective NK_2-receptor antagonist, MEN 10,627 (cyclo-MetAspTrpPheDapLeu) (5), prompted us to investigate its activity on an in vivo model of stimulated intestinal transit.

Male Sprague-Dawley rats, weighing 225–250 g, were used. Food was withheld 24 hr before the experiments, but the rats were allowed free access to drinking water. Rats were injected in the tail vein with MEN 10,627 (10–100 μg/kg, i.v.) or the vehicle (10% DMSO) 5 min before the administration of a selective NK_2-receptor agonist, [βAla^8]NKA-(4–10) (5–100 μg/kg, i.p.), carbachol (10 μg/kg, i.p.) or saline. In other experiments, reserpine was administered at a dose (5 mg/kg, i.p., 24 hr before) that produced an increase of intestinal transit comparable to the other stimulants. The meal, a 10% charcoal suspension in 1% carboxymethylcellulose, was given orally in a volume of 10 ml/kg immediately after the stimulant, and the animals were killed 15 min later by cervical dislocation. The gastrointestinal tract was removed, and the distance travelled by the marker was measured in the small intestine and expressed as a percentage of the total length of the intestine from the pylorus to the cecum.

Gastric emptying was measured in 24-hr fasted male Sprague-Dawley rats, weighing 220–250 g. Rats were injected with MEN 10,627 (100 μg/kg, i.v. into the tail vein), [βAla^8]NKA-(4–10) (15 μg/kg, i.p.) or saline 5 min before 1.5 ml, p.o. of a solution containing 1.5% methylcellulose and phenol red (0.5 mg/ml). Fifteen minutes later, the animals were killed by cervical dislocation. In each experiment, non-treated rats, receiving the phenol red solution, were sacrificed immediately thereafter to serve as standards (reference stomachs, 0% emptying rate). The stomachs were clamped at the pylorus and cardia, taken

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out and then homogenized in 100 ml of 0.1 N NaOH. Proteins in 5-ml aliquots of homogenate were precipitated with 0.5 ml of trichloroacetic acid (20% wt./vol.) and centrifuged. The supernatant was mixed with 4 ml of 0.5 N NaOH, and the absorbance of the sample was read at a wavelength of 560 nm (A560) with a Perkin-Elmer spectrophotometer. Gastric emptying was calculated according to the following formula: gastric emptying % = (1 − A560 sample/mean of A560 reference) × 100.

The following drugs were used: [βAla^8]NKA-(4−10) (Peninsula, Belmont, CA, USA), carbachol (Sigma, St. Louis, MO, USA), charcoal activated (Merck, Darmstadt, FRG), phenol red (Fluka, Buchs, Switzerland) and reserpine (Sigma). MEN 10,627 (cyclo-MetAspTrpPheDapLeu) was synthetized at Menarini Laboratories (Firenze, Italy) by solid phase methods. For i.v. injection, all peptides were dissolved in saline and injected in volume of 1 ml/kg. For MEN 10,627, a stock solution (1 mM) was prepared in dimethyl sulfoxide and then diluted in saline.

Data were expressed as means ± S.E., and the statistical analyses were performed by analysis of variance followed by Dunnett's test.

The activation of NK_2-receptors through the selective agonist [βAla^8]NKA-(4−10) produced an increase in intestinal transit that was significant at a dose of 15 μg/kg, i.p. (73 ± 3% vs. 63 ± 3% of controls; $P < 0.05$, n = 8−10 for each group). Higher doses of [βAla^8]NKA-(4−10) did not further increase the values of stimulated transit (72 ± 3% for 50 μg/kg, i.p. and 74 ± 5% for 100 μg/kg, i.p. of [βAla^8]NKA-(4−10) vs. 63 ± 3% of controls; $P < 0.05$, n = 8−10 for each group).

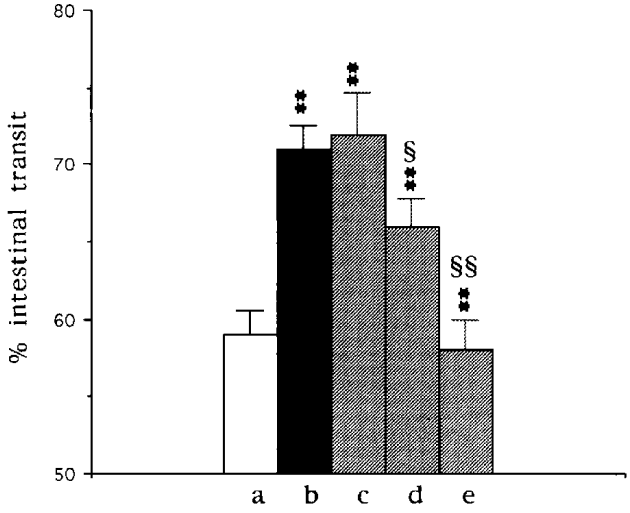

Fig. 1. Effect of a selective NK_2-receptor antagonist MEN10,627 (10−100 μg/kg, i.v.) on intestinal transit stimulated by 15 μg/kg, i.p. of [βAla^8]NKA-(4−10). Groups were: a = vehicle + saline; b = vehicle + [βAla^8]NKA-(4−10); c = MEN10,627, 10 μg/kg, i.v. + [βAla^8]NKA-(4−10); d = MEN10,627, 50 μg/kg, i.v. + [βAla^8]-NKA-(4−10); and e = MEN10,627, 100 μg/kg, i.v. + [βAla^8]NKA-(4−10). **: $P < 0.01$ as compared to the vehicle + saline treated group (white column). § and §§: $P < 0.05$ and $P < 0.01$, respectively, as compared to the vehicle + [βAla^8]NKA-(4−10)-treated group (black column). n = 8−10 for each group.

As shown in Fig. 1, MEN10,627 (10−100 μg/kg, i.v.) dose-dependently inhibited the increase in intestinal transit induced by [βAla^8]NKA-(4−10) without affecting the unstimulated transit. MEN10,627, at a dose of 100 μg/kg, i.v. did not significantly affect the basal values of intestinal transit (58 ± 3% vs. 59 ± 2% of controls, n = 8−10 for each group), while it completely reversed the stimulant effect of [βAla^8]NKA-(4−10).

Intestinal transit stimulated by [βAla^8]NKA-(4−10) was unaffected by atropine at a dose that completely reversed the transit stimulated by carbachol (Table 1). On the other hand, transit induced by equieffective doses of carbachol and reserpine was unaffected by administration of MEN10,627 (Table 1).

The same doses of [βAla^8]NKA-(4−10) or MEN 10,627 used in previous experiments to stimulate or to inhibit the NK_2 stimulated intestinal transit, respectively, were unable to produce a significant change in gastric emptying (48 ± 6% for MEN10,627 (100 μg/kg, i.v.) and 57 ± 9% for [βAla^8]NKA-(4−10) (15 μg/kg, i.p.), as compared to 56 ± 6% for the vehicle; n = 8 for all groups).

The present findings show that activation of NK_2-receptors by a selective agonist produces an increase in intestinal transit in conscious rats. This effect reached a plateau at a dose of 15 μg/kg since increasing the dose of the NK_2-receptor agonist did not result in a further increase of small intestinal transit. The NK_2-receptor-mediated increase in intestinal transit is restricted to the small intes-

Table 1. Effect of MEN10,627 or atropine on intestinal transit stimulated by carbachol, [βAla^8]NKA-(4−10) or reserpine

Treatment and dose	Intestinal transit (%)
Vehicle + saline	59 ± 2
Vehicle + [βAla^8]NKA-(4−10), 15 μg/kg, i.p.	71 ± 2*
MEN 10,627, 100 μg/kg, i.v. + [βAla^8]NKA-(4−10), 15 μg/kg, i.p.	58 ± 2
Atropine, 100 μg/kg, i.p. + [βAla^8]NKA-(4−10), 15 μg/kg, i.p.	68 ± 1*
Vehicle + carbachol, 15 μg/kg, i.p.	75 ± 4*
MEN 10,627, 100 μg/kg, i.v. + carbachol, 15 μg/kg, i.p.	73 ± 3*
Atropine, 100 μg/kg, i.p. + carbachol, 15 μg/kg, i.p.	59 ± 3
Vehicle + reserpine, 5 mg/kg, i.p.	83 ± 3**
MEN 10,627, 100 μg/kg, i.v. + reserpine, 5 mg/kg, i.p.	83 ± 3**

* and **: $P < 0.05$ and 0.01, respectively, as compared to the vehicle + saline group. n = 8−10 for each group.

tine since gastric emptying was not affected by the same dose of NK_2-receptor agonist. Conversely, Holzer (6) showed that SP and NKA influenced gastrointestinal propulsion primarily by their effect of the stomach and pylorus. Methodological differences and the notion that NKA is not a selective NK_2-receptor agonist since it possesses a certain affinity for NK_1-receptors could account for these discrepancies.

The observation that atropine was unable to affect this stimulated transit suggests that tachykinin NK_2-receptors might play an important role in the non-cholinergic excitatory transmission to the small intestine. These data are in agreement with those of Maggi et al. (4) who showed that non-cholinergic contraction induced by nerve stimulation in vitro of human ileum was mainly mediated by activation of NK_2-receptors. On the other hand, biochemical and autoradiographic studies have shown an abundant tachykinin-like immunoreactivity localized into nerve fibers of the muscular layers of rat and human small intestine and the presence of NK_2-receptors at this level (7, 8). Taken together, these pieces of evidence suggest that tachykinins released from nerve endings may mediate a potent spasmogenic effect in the small intestine. The involvement of NK_2-receptors in the action of [βAla^8]NKA-(4–10) was demonstrated by the selective blockade achieved with the polycyclic peptide NK_2-receptor antagonist MEN 10,627 (5), which completely reversed the action of the NK_2-receptor agonist without affecting the increase of intestinal transit produced by other stimulants such as carbachol and reserpine.

In conclusion, the development of a new generation of receptor-selective tachykinin antagonists such as MEN 10,627 allows the definition of the effects induced by the release of endogenous tachykinins on specific targets in the gut and might represent a new class of drugs for controlling intestinal propulsive activity.

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