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Characterization of tachykinin NK_2 receptor on dog proximal colon. Antagonism by MEN 10,627 and SR 48,968

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Abstract

The nature of the tachykinin receptors involved in the contraction of the circular muscle of dog colon has been investigated. The following rank order of potency for agonists was obtained: [Sar^9,Met(O_2)^{11}]substance P ≥ neurokinin A > [β-Ala^8]neurokinin A-(4–10) ≫ [MePhe^7]neurokinin B. The efficacy of the tachykinin NK_2 receptor agonists was significantly greater than that of the tachykinin NK_1 receptor agonists and of carbachol. A concentration-dependent rightward shift of the motor response to neurokinin A (obtained in the presence of (±)-CP 96,345) was induced by peptide and non-peptide tachykinin NK_2 receptor antagonists with this rank order: MEN 10,627 = SR 48,968 ≫ L 659,877 > MEN 10,376 > MDL 28,564. MEN 10,627 and SR 48,968 affinities were similar to those measured in human tissues. In conclusion, the tachykinin NK_2 receptor plays a predominant role in tachykinin-induced contraction of the canine colonic circular muscle and this tissue could be useful to predict the pharmacological actions of MEN 10,627 and SR 48,968 in human colon.

Keywords: Smooth muscle; Intestinal motility; Tachykinin receptor agonist; Tachykinin receptor antagonist

1. Introduction

Tachykinins are a family of peptides widely distributed both in the central and peripheral mammalian nervous system (Otsuka and Yoshioka, 1993 for review) whose biological responses are mediated by three kinds of tachykinin receptors named NK_1, NK_2 and NK_3 (Regoli et al., 1987). Tachykinins exert powerful spasmogenic actions in visceral smooth muscle from various species, including humans. They are found in abundance in nerve terminals innervating smooth muscle layers of human stomach, colon, duodenum and gallbladder (Maggi et al., 1990a, 1992a; Giuliani et al., 1991; Kishimoto et al., 1991). Stimulation of tachykinin NK_2 receptors is mainly linked to tachykinin-induced motor responses in visceral smooth muscle (Advenier et al., 1987; Parlani et al., 1990; Maggi et al., 1993; Astolfi et al., 1994).

In the past few years, studies on the distribution and physiological roles of the tachykinin NK_2 receptor have profited from the discovery of potent and selective antagonists belonging to several chemical classes: linear peptides (MEN 10,376 or R 396; Maggi et al., 1990b, 1991a), cyclic peptides (L 659,877 or MEN 10,627; Williams et al., 1988; Pavone et al., 1994; Maggi et al., 1994a), pseudopeptide (MDL 28,564; Harbeson et al., 1990) and non-peptide (SR 48,968; Emonds-Alt et al., 1992). An unforeseen consequence of testing these selective antagonists was the recognition of a marked pharmacological heterogeneity of the tachykinin NK_2 receptor, with relevant species-related differences in the rank order of potency of these antagonists (Maggi et al., 1992b, 1993; Maggi, 1994).

Tachykinin NK_2 receptor antagonists have been proposed as a new class of spasmolytic agents to be used for gastrointestinal spastic disorders (Maggi et al., 1992a, 1993 for review) such as irritable bowel syndrome (Mantyh et al., 1988). Assessment of drug action on colonic motility is generally performed in the dog because of its histological and physiological similarity with the human colon. In

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this species, autoradiographic studies indicate the presence of both tachykinin NK_1 and NK_2 receptors in the muscle layers of the colon (Mantyh et al., 1988). In particular, tachykinin NK_2 receptors were identified mainly in the circular muscle layer while tachykinin NK_1 receptors were present in the circular and longitudinal muscle layers as well as on the endothelium of the arterioles and venules and on the epithelial layer (Mantyh et al., 1988).

The aim of this study was to pharmacologically characterize the tachykinin receptors mediating the contraction of the dog isolated proximal colon (circular muscle) by using the natural tachykinin neurokinin A and selective tachykinin receptor agonists. Since a predominant role for the tachykinin NK_2 receptor was observed, further experiments were performed to compare the antagonistic properties of the various prototypes of peptide and non-peptide receptor NK_2 antagonists.

2. Materials and methods

2.1. Dog isolated colon

Beagle dogs (8–10 kg) were killed by i.v. overdose of sodium pentobarbital and the tissue was rapidly dissected out. A segment of proximal colon was obtained from 13 dogs. The specimens were pinned flat on a Petri dish containing Krebs solution and the mucosa was carefully dissected. From each colonic segment a series (10 to 12) of small strips of muscle (0.5–0.8 cm long, 2–3 mm wide) were cut along the circular axis. The Krebs solution was composed as follows (mM): NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, KCl 4.7, $CaCl_2$ 2.5, glucose 11 and was gassed with 95% O_2 and 5% CO_2 (pH 7.4) and maintained at 37°C. An optimal resting load of 1 g was applied to the preparations, which were allowed to equilibrate for 60 min. Changes in motor activity were recorded with a Basile 7050 polygraph. Isometric contractile responses are expressed as percentage of the maximal response to carbachol (1 μM) obtained in the same preparation. All experiments were carried out in the presence of a cocktail of peptidase inhibitors (bestatin, captopril and thiorphan; 1 μM each). Agonist-induced motor response was measured as the increase in resting tone or, when rhythmic motor activity was superimposed, as the maximal amplitude of rhythmic contraction.

Concentration–response curves for [β-Ala^8]neurokinin A-(4–10), neurokinin A and [MePhe^7]neurokinin B were made in a cumulative manner, the next concentration being added when the effects of the preceding one had reached the steady state. Preliminary experiments had shown that no significant desensitization of the response to these tachykinin agonists occurred. The concentration-response curves for the selective tachykinin NK_1 receptor agonist [Sar^9,Met(O_2)^{11}]substance P were obtained in a non-cumulative manner. Increasing concentrations were added at 25-min intervals and left in the bath until maximal responses developed.

The effect of tachykinin NK_2 receptor antagonists was evaluated against the contractile responses induced by neurokinin A. These experiments were carried out in the presence of the selective tachykinin NK_1 receptor antagonist (±)-CP 96,345 (1 μM, added to the bath 60 min before each agonist concentration-response curve) to avoid the activation of tachykinin NK_1 receptors by neurokinin A. All the antagonists were added to the bath 60 min before of the concentration–response curve for neurokinin A. In each preparation only one concentration of antagonist was tested. Each antagonist concentration was evaluated in specimens obtained from at least three different animals.

2.2. Data analysis

Statistical analysis of the data was performed by using Student's *t*-test for unpaired data when applicable; *P* values lower than 0.05 were considered significant. The pD_2 of the concentration-response curves for tachykinin agonists were calculated as the negative log of the peptide concentration that caused 50% of the maximal effect. Schild plots were constructed from experimental data (3–4 different concentrations of each antagonist and at least three experiments for each concentration) to calculate pA_2 values by using a computer program for the Apple IIe (Tallarida and Murray, 1981).

2.3. Drugs

The tachykinin NK_2 receptor-selective antagonist L 659,877 [cyclo-(Met-Gln-Trp-Phe-Gly-Leu)] was purchased from Cambridge Research Biochemicals, (Cambridge, UK). [β-Ala^8]Neurokinin A-(4–10), (±)-CP 96,345 [(2*S*,3*S*)-*cis*-2-(diphenyl-methyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo-[2.2.2]octan-3-amine], MDL 28,564 [H-Asp-Ser-Phe-Val-Gly-Leu-¥[CH_2NH]Leu-NH_2, MEN 10,376 [H-Asp-Tyr-DTrp-Val-dTrp-DTrp-Lys-NH_2], SR 48,968 [(*S*)-*N*-methyl-*N*[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorphenyl)butyl]benzamide] and MEN 10,627 [cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo(2β-5β)] were synthesized in the Chemistry Department, Menarini Pharmaceuticals (Florence, Italy), by conventional solid-phase methods. Neurokinin A, [MePhe^7]neurokinin B, [Sar^9,Met(O_2)^{11}]substance P, bestatin, captopril and tiorphan were obtained from Peninsula Laboratories (Belmont, CA, USA). Stock solutions (1–10 mM) of L 659,877, MEN 10,376, [β-Ala^8]neurokinin A-(4–10), MEN 10,627 and MDL 28,564 were prepared in dimethyl sulfoxide (DMSO) and diluted in water. Control experiments showed that DMSO alone (0.1–0.3% final concentration) had no effect on the response under study. The other peptides were dissolved in water.

3. Results

3.1. Contractile effects of tachykinin receptor agonists

In resting conditions, dog proximal colon strips were either quiescent (about 50%) or exhibited rhythmic activity with amplitude ranging between 90 and 2400 mg. The addition of neurokinin A or [β-Ala^8]neurokinin A-(4–10) elicited a concentration-dependent increase in resting tension and in the amplitude of the rhythmic contractions (Fig. 1).

Concentration-response curves for the contractile effects on the circular muscle of the dog proximal colon (expressed as percentage of the maximal response to carbachol) of neurokinin A, [β-Ala^8]neurokinin A-(4–10), [Sar^9,Met(O$_2$)^{11}]substance P and [MePhe^7]neurokinin B are shown in Fig. 2. Neurokinin A had the lowest threshold concentration (3 nM) and the highest efficacy (140 ± 9% of maximal response to carbachol, $n = 6$) and its pD$_2$ was 7.1 ± 0.05. Also the tachykinin NK$_1$-selective receptor agonist [Sar^9,Met(O$_2$)^{11}]substance P (Drapeau et al., 1987) was remarkably potent (threshold concentration of 30 nM and pD$_2$ of 7.3 ± 0.1) but its efficacy was weaker (58 ± 4% of the carbachol response, $n = 6$). The proposed

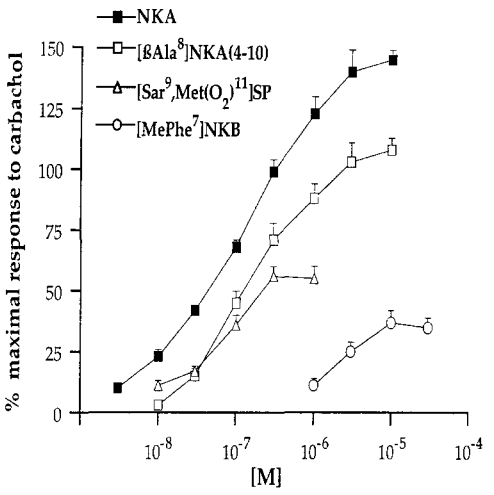

Fig. 2. Concentration-response curves for the contractile effect on the circular muscle of the dog proximal colon of natural neurokinin A and synthetic selective agonists of tachykinin NK$_2$ receptors. Concentration-response curves for neurokinin A, [β-Ala^8]neurokinin A-(4–10) and [MePhe^7]neurokinin B were obtained in a cumulative manner. Concentration-response curves for [Sar^9,Met(O$_2$)^{11}]substance P were obtained in a non-cumulative manner. Responses are expressed as percentage of the maximum carbachol (1 μM) response. Each value represents the mean ± S.E.M. of at least six experiments.

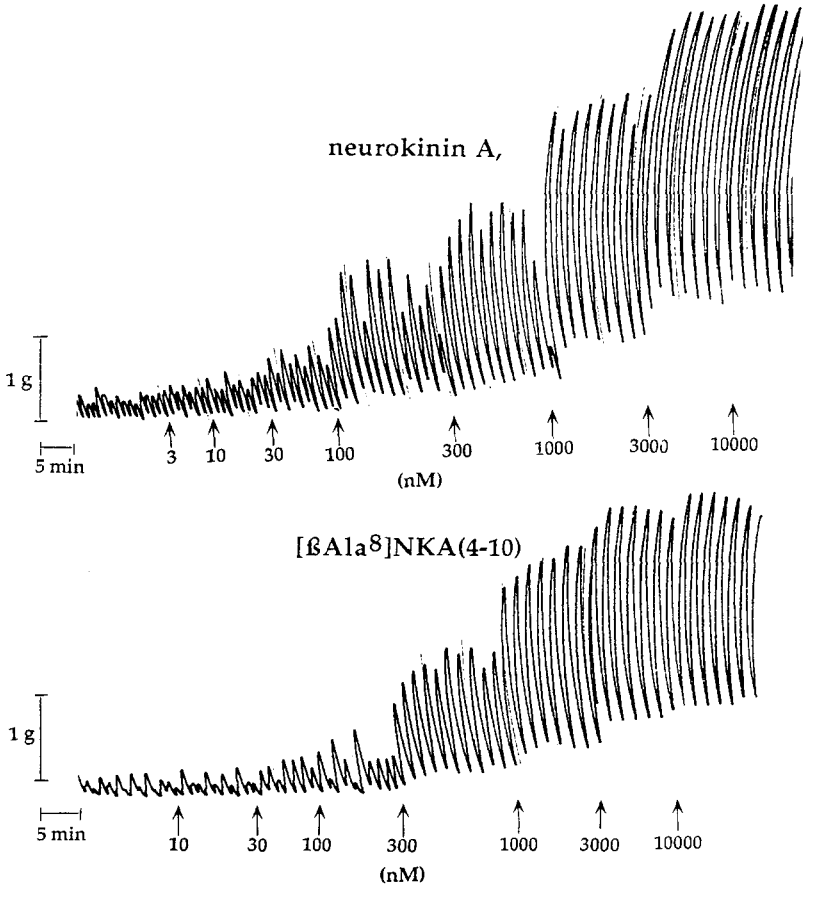

Fig. 1. Typical tracings showing the contractile effect of increasing concentrations of neurokinin A (upper panel) and [β-Ala^8]neurokinin A-(4–10) (lower panel) on the circular muscle of the dog proximal colon. All experiments were carried out in the presence of a cocktail of peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each, added to the bath 60 min before).

tachykinin NK_2-selective receptor agonist [β-Ala^8]neurokinin A-(4–10) (Rovero et al., 1989; Parlani et al., 1995) was slightly less potent than neurokinin A ($pD_2 = 6.8 \pm 0.1$) and had a lower efficacy ($103 \pm 8\%$ of the carbachol response, $n = 6$). The selective tachykinin NK_3 receptor agonist [MePhe^7]neurokinin B (Drapeau et al., 1987) produced a concentration-dependent response only in the μM range, and at the highest concentration tested (30 μM) its maximal effect did not exceed $37 \pm 4\%$ ($n = 6$) of the carbachol response and its pD_2 was 5.6 ± 0.05. The maximal contractile response induced by [Sar^9,Met(O_2)^{11}]substance P (0.3 μM) was completely inhibited by the tachykinin NK_1 receptor antagonist (±)-CP 96,345 at 1 μM ($n = 4$; data not shown). The maximal effect of [MePhe^7]neurokinin B was blocked by (±)-CP 96,345 (1 μM) plus SR 48,968 (0.1 μM) ($n = 3$; data not shown).

3.2. Effect of tachykinin NK_2 receptor antagonists

To study the effect of tachykinin NK_2 receptor antagonists, further experiments were carried out by using neurokinin A as agonist and in the presence of (±)-CP 96,345 (1 μM) to avoid any possible interference by tachykinin NK_1 receptors. Neurokinin A-induced contractions were only marginally affected by (±)-CP 96,345 (1 μM) producing a shift of its pD_2 from 6.8 ± 0.1 to 6.2 ± 0.1. The linear peptide MEN 10,376 (1–10 μM) and the cyclic one L 659,877 (0.03–1 μM) exhibited weak competitive antagonism ($pA_2 = 6.2 \pm 0.2$, $n = 9$, and 6.8 ± 0.1, $n = 7$, respectively). MDL 28,564 (1–10 μM) showed instead a partial agonistic activity and a weak competitive antagonism ($pA_2 = 5.6 \pm 0.2$, $n = 9$). A marked jump in potency was observed with the two newly developed antagonists MEN 10,627 (3–100 nM) and SR 48,968 (3–100 nM), which exerted a potent and concentration-dependent antagonism already in the nanomolar range (Fig. 3). Schild plot analysis indicated a slope not statistically different from unity, suggesting for both drugs a competitive type of antagonism (Fig. 3). MEN 10,627 (0.1 μM) (Fig. 4) and SR 48,968 (0.1 μM) ($n = 3$, data not shown) were ineffective in inhibiting the maximal contractile response induced by the tachykinin NK_1 receptor agonist [Sar^9,Met(O_2)^{11}]substance P (0.3 μM).

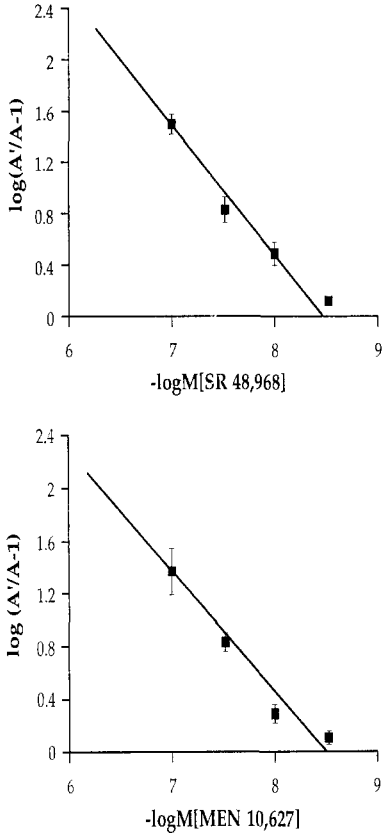

Fig. 3. Schild plots for the antagonism of the contractile responses to neurokinin A in the dog isolated proximal colon by SR 48,968 ($pA_2 = 8.40 \pm 0.09$; slope $= 0.98 \pm 0.13$; upper panel) or MEN 10,627 ($pA_2 = 8.50 \pm 0.1$; slope $= 0.90 \pm 0.11$; lower panel). All experiments were carried out in the presence of (±)-CP 96,345 (1 μM, 1 h incubation time) and a cocktail of peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each, added to the bath 60 min before). Each value is the mean ± S.E.M. of at least three experiments.

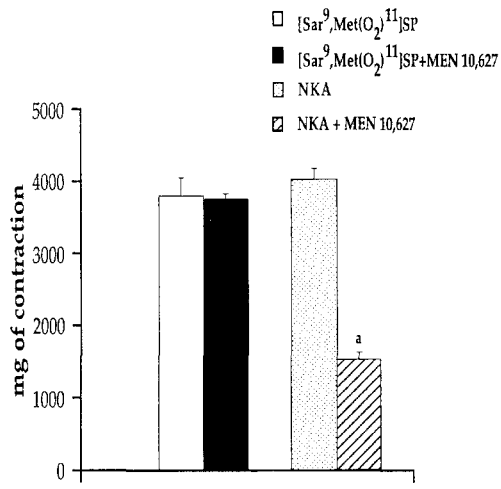

Fig. 4. Lack of inhibition by MEN 10,627 (0.1 μM) of the maximal contractile response induced by the tachykinin NK_1 receptor agonist [Sar^9,Met(O_2)^{11}]substance P (0.3 μM) and by an equieffective concentration of neurokinin A (0.1 μM). The contractile response of neurokinin A was obtained in the presence of (±)-CP 96,345 (1 μM, 1 h incubation time). Each value represents the mean ± S.E.M. of at least six experiments. [a] $P < 0.01$ vs. neurokinin A (0.1 μM).

4. Discussion

The present data show that a prominent spasmogenic action can be elicited by neurokinin A in the circular muscle of isolated dog proximal colon, with an efficacy greater than that of a cholinergic agonist. A potent motor effect could be observed with selective NK_1 [Sar^9,Met(O_2)^{11}]substance P or NK_2 [β-Ala^8]neurokinin A-(4–10) receptor agonists, but not with [MePhe^7]neuro-

kinin B, a selective ligand for the tachykinin NK_3 receptor. These data indicate the presence of functional tachykinin NK_1 and NK_2 receptors mediating the contractile response to tachykinins in the circular muscle of the dog colon. This conclusion is in keeping with autoradiographic data indicating that both tachykinin NK_1 and NK_2 receptors are expressed on smooth muscle layers of the canine colon (Mantyh et al., 1988). Indeed, tachykinin NK_2 receptors have been identified on the circular but not on longitudinal smooth muscle layer (Mantyh et al., 1988) and this localization could be in line with a greater involvement of tachykinin NK_2 receptors in the propulsive peristaltic function of the canine colonic musculature. In fact, a greater efficacy of tachykinin NK_2 receptor agonists as compared to a tachykinin NK_1 receptor agonist in inducing colonic spasm in circular muscle preparations has been demonstrated in dogs (this study) and in humans (Giuliani et al., 1991).

This finding could be linked to a greater transduction efficiency of NK_2-mediated responses and/or to a greater density of this kind of receptor on smooth muscle cells. Autoradiographic studies indicate the presence of tachykinin NK_1 receptors also on circular muscle, but some of them could be expressed on vascular smooth muscle and endothelial cells (Mantyh et al., 1988) subserving an inflammatory role rather than a motor role.

In other mammalian visceral muscles a mixed population of tachykinin NK_1 and NK_2 receptors mediating contraction has been described, for example the guinea pig bronchial musculature (Maggi et al., 1991b). In this tissue, the NK_1-mediated response can be largely unmasked in the presence of a cocktail of peptidase inhibitors, and it plays a subsidiary role as compared to the predominant NK_2 spasmogenic drive (Maggi et al., 1993 for review). In humans, it has been demonstrated that the spasmogenic action of tachykinins on colon (circular muscle), ileum (circular muscle), prostatic urethra (circular muscle) and bronchus relies almost entirely on tachykinin NK_2 receptors (Parlani et al., 1990; Giuliani et al., 1991; Maggi et al., 1992a; Astolfi et al., 1994).

The study with tachykinin NK_2 receptor antagonists demonstrated the following rank order of potency MEN 10,627 = SR 48,968 $\gg$ L 659,877 > MEN 10,376 > MDL 28,564. Species-dependent heterogeneity in the potency of tachykinin NK_2 receptor antagonists has been described, suggesting the possible existence of tachykinin receptor subtypes (Maggi et al., 1992b), although the existence of a new tachykinin receptor has been recently proposed (Daniel et al., 1995).

The observed pattern of efficacy resembles that obtained in rat and hamster preparations (Lee et al., 1982; Buck et al., 1986; Maggi et al., 1990b), while in a guinea pig colonic preparation the affinity of SR 48968 was almost one order of magnitude higher (Maggi et al., 1994b). However, it has to be underlined that the newly developed potent and selective tachykinin NK_2 receptor antagonists MEN 10,627 and SR 48,968 exhibit strong competitive antagonism against tachykinin NK_2 receptor-mediated contractions of dog proximal colon, with pA_2 values that are quite similar to the ones measured in human tissues (Emonds-Alt et al., 1992; Giuliani et al., 1993, 1994). This suggests that their effects on canine colonic motility could be representative of their potential efficacy on human colon. The observation that MEN 10,627 was virtually ineffective against [Sar^9,$Met(O_2)^{11}$]substance P-induced contraction further strengthens the selectivity of its pharmacological action (Giuliani et al., 1994; Maggi et al., 1994a).

In conclusion, we have gathered findings indicating that the stimulation of tachykinin NK_2 receptors elicits a marked remarkable contraction in dog proximal colon. This response is strongly antagonized by MEN 10,627 and SR 48,968, with an affinity similar to that observed in human tissues. The effect of these drugs on canine colonic motility could be predictive of their pharmacological action in humans.

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