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Tachykinin NK_2 receptor mediates contraction and ion transport in rat colon by different mechanisms

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Abstract

We have characterized the tachykinin NK_2 receptor-mediated contraction and vectorial ion transport responses in the muscularis mucosae and mucosa of the rat isolated distal colon, respectively. The tachykinin NK_2 receptor-selective antagonist nepadutant (c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β-5β)}) produced competitive antagonism of [βAla^8]neurokinin A-(4-10)-induced contraction ($pK_B = 9.3$) in the muscularis mucosae, and insurmountable blockade of increases in short-circuit current (I_{sc}) responses ($pK_B = 8.6$) in the mucosa. However, this latter effect was completely reversed by washout of the antagonist. [βAla^8]Neurokinin A-(4-10)-induced contractions were unaffected by indomethacin (3 μM). In sharp contrast, I_{sc} responses induced by [βAla^8]neurokinin A-(4-10) (100 nM) were inhibited (> 70%) by indomethacin (3 μM), while I_{sc} responses to substance P (3 μM) were unchanged. Our study provides the first evidence that in the same organ stimulation of tachykinin NK_2 receptors leads to two independent responses mediated by different effector mechanisms both of which are blocked (albeit with different kinetics) by the potent and selective tachykinin NK_2 receptor antagonist, nepadutant.

Keywords: Nepadutant; Tachykinin NK_2 receptor; Ion transport, vectorial; Colon mucosa (rat)

1. Introduction

Tachykinins are a family of neuropeptides distributed in the mammalian central and peripheral nervous system which produce a wide range of biological effects through the stimulation of at least three distinct receptor types, termed NK_1, NK_2 and NK_3 (Regoli et al., 1989; Guard and Watson, 1991; Maggi et al., 1993). In the gastrointestinal tract both anatomical and biochemical evidence indicates the existence of tachykinin-like immunoreactivity: at this level the bulk of extractable tachykinin-like immunoreactivity originates from enteric (intrinsic) neurons, while the remainder is contributed by the peripheral endings of capsaicin-sensitive afferents (Maggi, 1995). The tachykinin NK_2 receptor has been shown to mediate a variety of effects produced by tachykinins in the gastrointestinal tract, including: (1) direct smooth muscle contraction (e.g., Maggi et al., 1992; Giuliani et al., 1991), (2) secretion of fluid and electrolytes from intestinal mucosa (Cox et al., 1993; Eutamene et al., 1995), and (3) visceral hypersensitivity and pain (Julia et al., 1994; Julia and Buéno, 1997). Accordingly, tachykinin NK_2 receptor antagonists are viewed as possible drug candidates for the treatment of various intestinal diseases involving motility alterations, abnormal visceral sensation, diarrhoea and gut inflammation (Holzer and Holzer-Petsche, 1997a,b; Evangelista, 2000).

Among the intestinal preparations used to study the effects of tachykinins in vitro, the rat distal colon is a useful model in which both epithelial ion transport and muscle contractile responses have been reported. Cox et al. (1993) showed that in the rat descending colon mucosa under voltage-clamp conditions, tachykinins evoked increases in short-circuit current (I_{sc}) responses by activating a combination of tachykinin NK_1, NK_2 and NK_3 receptor types. Furthermore, in the rat distal colon muscularis mucosae tachykinins produce contractile responses that are

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apparently selectively mediated by tachykinin NK_2 receptors alone (Astolfi et al., 1993).

In the present work, we have studied the effects of a tachykinin NK_2 receptor antagonist, nepadutant (or MEN 11420; Catalioto et al., 1998a), upon both ion transport and smooth muscle contraction stimulated by an tachykinin NK_2 receptor-preferring agonist in the rat isolated colon mucosa and muscularis mucosae, respectively. From these experiments, we observed that nepadutant has divergent inhibitory mechanisms in the two assays, being competitive in the muscularis mucosae and insurmountable in the mucosa. Thus, we have undertaken further studies to address whether different effector mechanisms may underlie the two responses, especially in relation to the generation of prostanoids. These are known to be produced following activation of tachykinin NK_2 receptors both in transfected cells (Eistetter et al., 1991, 1993) and in intact tissues (Tramontana et al., 2000), and may therefore contribute to both the contractile and secretory responses previously recorded for tachykinins in rat colonic tissue.

2. Materials and methods

2.1. Functional studies on the colon muscularis mucosae

Male albino rats (Wistar strain, 300–350 g) were stunned and bled. A 10-cm long segment of distal colon was quickly excised about 1 cm from the rectum, and placed in oxygenated (96% O_2 and 4% CO_2) Krebs–Henseleit solution (with the following composition: NaCl, 119 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.5 mM; $CaCl_2$, 2.5 mM; KCl, 4.7 mM and glucose 11 mM). Both circular and longitudinal muscle layers were peeled off with the use of a wisp of cotton wool, as described previously (Bailey and Jordan, 1984; Astolfi et al., 1993). The remaining tissue, consisting of epithelium and muscularis mucosae, was cut into four longitudinal segments and placed in 5-ml organ baths filled with oxygenated Krebs–Henseleit solution at 37°C containing atropine (1 μM) and indomethacin (3 μM). Each preparation was connected to an isometric transducer under a resting tension of 5 mN, for recording mechanical activity. Cumulative concentration–response curves to the tachykinin NK_2 receptor-selective agonist [βAla^8]neurokinin A-(4-10) (Rovero et al., 1989) were constructed, each concentration being added when the effect of the preceding addition had reached a steady state. Nepadutant (incubation period of 60 min) was assayed for its ability to block the effects produced by [βAla^8]neurokinin A-(4-10). In order to ascertain whether inhibition of prostaglandin production could alter the activity of both [βAla^8]neurokinin A-(4-10) and nepadutant, a series of experiments were performed in preparations taken from a same animal, which were incubated either in the presence or in the absence of indomethacin (3 μM).

2.2. Short circuit current (I_{sc}) measurement in the colon mucosa

Sprague–Dawley rats (male, 225–275 g) were stunned and killed by cervical dislocation and the descending colon removed and placed immediately in fresh Krebs–Henseleit solution (composition as above). Mucosal preparations of descending colon were prepared, removing overlying smooth muscle by dissection and the resulting stripped mucosa was placed in between two halves of modified Ussing chambers with a window size of 0.64 cm^2. The transmural short-circuit current (I_{sc}) was recorded continuously under voltage clamp (0 mV) conditions as described in detail previously (Cox et al., 1993). Colonic mucosae were allowed to equilibrate for 30 min during which time the basal I_{sc} stabilised, before addition of peptides or drugs to the basolateral compartment only. Under these conditions, we performed preliminary experiments to assess the reproducibility of the response to [βAla^8]neurokinin A-(4-10). In these experiments, we observed that a single concentration (1 μM) of [βAla^8]neurokinin A-(4-10), administered 30 min after washout of [βAla^8]neurokinin A-(4-10) given cumulatively to preparations (1.4 μM was the highest concentration added at the end of the curve), produced only $2.2 \pm 4\%$ of the maximal effect obtained previously ($n = 12$; $P < 0.01$), so showing a significant tachyphylaxis had established. Thus, to circumvent this problem all studies with the antagonist were performed following a modified protocol: four adjacent pieces of mucosa were used, one of which served to construct a control curve to [βAla^8]neurokinin A-(4-10), while the other three preparations were exposed to single increasing concentrations of nepadutant. Nepadutant was added for different time periods (30, 60 or 90 min) before addition of the agonist, and a curve to [βAla^8]neurokinin A-(4-10) was constructed thereafter. All the curves obtained in the presence of nepadutant were compared to the control one obtained in the absence. In a series of experiments the reversibility of nepadutant was evaluated as follows: the preparations received initial additions of [βAla^8]neurokinin A-(4-10) and these responses (at time $= -30$ min) were denoted as 100%. Following washout 15 min prior (time $= -15$ min) to the second [βAla^8]neurokinin A-(4-10) addition, nepadutant was added, or vehicle to controls. All subsequent agonist responses were calculated as a percentage of the internal [βAla^8]neurokinin A-(4-10) control. Washouts were performed at 30-min intervals thereafter and increases in I_{sc} were pooled from responses within each respective group. Results were expressed as the mean peak increase in I_{sc} (μA/0.64 cm^2). Increases in I_{sc} were analysed using Graphpad Prism (San Diego, USA; version 2.01).

2.3. Data analysis

The "Schild plot" method (Arunlakshana and Schild, 1959) was used to check the competition of nepadutant in

the muscularis mucosae. A plot with linear regression line and slope not significantly different from unity was considered as proof of competition. The antagonist affinity was expressed as a pK_B value (negative logarithm of the antagonist dissociation constant) and, assuming a slope of −1.0, was estimated as the mean of the individual values obtained with the equation: $pK_B = \log[\text{dose ratio} - 1] - \log[\text{antagonist concentration}]$ (Kenakin, 1997; Jenkinson, 1991). In the colonic mucosa, nepadutant caused nonparallel rigthward shifts of the concentration–response curves to [βAla^8]neurokinin A-(4-10), and decreased the E_{max}. Thus, the affinity of nepadutant for tachykinin NK_2 receptors in this tissue was estimated by the "double reciprocal plot method" described by Kenakin (1997) for noncompetitive and/or pseudo-irreversible antagonists. In practice, a double-reciprocal plot of equieffective concentrations of agonist (A) in the absence (1/A) and in the presence (1/A′) of the antagonist (B) was constructed, and K_B derived from the equation: $K_B = [B]/(\text{slope} - 1)$.

2.4. Statistical analysis

The values in the text, tables or figures are expressed as means ±95% confidence limits (CL), or ±S.E.M. Statistical analysis was performed using Student's *t*-test for paired or unpaired data where applicable. $P < 0.05$ was considered the level of statistical significance. Maximal agonist induced increases in I_{sc} (quoted throughout as μA/0.64 cm^2) were pooled within each group and statistical comparisons of the resulting means ± S.E.M. were performed using unpaired Student's *t*-test ($P \leq 0.05$ was significantly different).

2.5. Drugs and solutions

c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β-5β)} (Nepadutant) and [βAla^8]neurokinin A-(4-10) were synthesized at Menarini Ricerche (Florence, Italy) by conventional solid-phase methods. Other drugs used were: indomethacin (Sigma, St. Louis, MO, USA), atropine (Serva, Heidelberg, FRG), substance P (Peninsula Laboratories, Merseyside, UK). Peptide stocks in aqueous solution were stored at −25°C and never underwent more than one freeze–thaw cycle.

3. Results

3.1. Tachykinin NK_2 receptor-mediated contractions in the rat isolated distal colon muscularis mucosae

In the presence of indomethacin (3 μM), the tachykinin NK_2 receptor agonist [βAla^8]neurokinin A-(4-10) produced concentration-dependent and reproducible contractile responses ($pD_2 = 7.6$; 95% CL 7.3; 7.8). Nepadutant potently inhibited [βAla^8]neurokinin A-(4-10)-induced contractions, producing parallel rightward shifts in the agonist concentration–response curve without depressing E_{max} ($pK_B = 9.3 \pm 0.08$; $n = 14$; Fig. 1). The competitive nature of antagonism exerted by nepadutant was confirmed by the Schild plot slope: −1.2 (95% CL: −1.5; −1.0). To investigate the influence of prostanoid production on the contractile responses mediated by tachykinin NK_2 receptor stimulation, the effects of both [βAla^8]neurokinin A-(4-10) and nepadutant were compared in further experiments performed in the absence of indomethacin vs. experiments in the presence of indomethacin. [βAla^8]neurokinin A-(4-10) produced comparable effects in the absence ($pD_2 = 7.4 \pm 0.06$; $n = 4$) and in the presence ($pD_2 = 7.5 \pm 0.09$; $n = 4$) of indomethacin (3 μM). Nepadutant (10 nM) was as potent in the absence ($pK_B = 9.4 \pm 0.03$; $n = 4$) as in the presence ($pK_B = 9.6 \pm 0.11$; $n = 4$) of cyclo-oxygenase

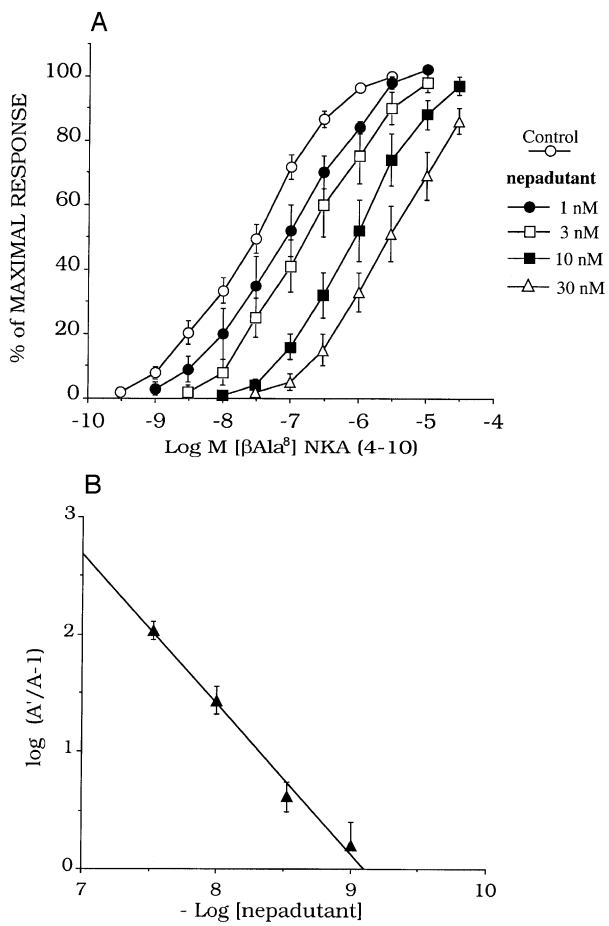

Fig. 1. Antagonism by nepadutant of tachykinin NK_2 receptor-mediated contractions in the rat isolated distal colon muscularis mucosae. (A) Log concentration–response curves for [βAla^8]neurokinin A-(4-10) in the absence and presence of nepadutant. Each value is mean ± S.E.M. of 3–4 experiments. (B) Corresponding Schild plot of agonist dose ratios vs. nepadutant concentrations (slope −1.2; 95% CL: −1.5; −1.0). Each value is the mean ± S.E.M. of 3–4 experiments.

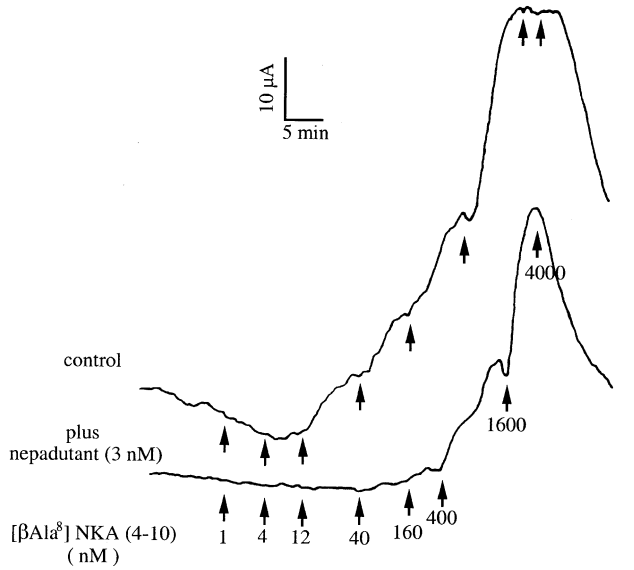

Fig. 2. Representative traces of the changes in short-circuit current (I_{sc}) induced by cumulative additions of [βAla^8]neurokinin A-(4-10) to rat descending colon mucosa, in the absence (upper trace) or presence (lower trace) of nepadutant (3 nM) added 60 min prior to the first addition of agonist. Basal I_{sc} values were 32 and 19 μA for the upper and lower traces, respectively. Basolateral additions of [βAla^8]neurokinin A-(4-10) are shown by arrows.

inhibitor, showing apparent competitive kinetics in both cases.

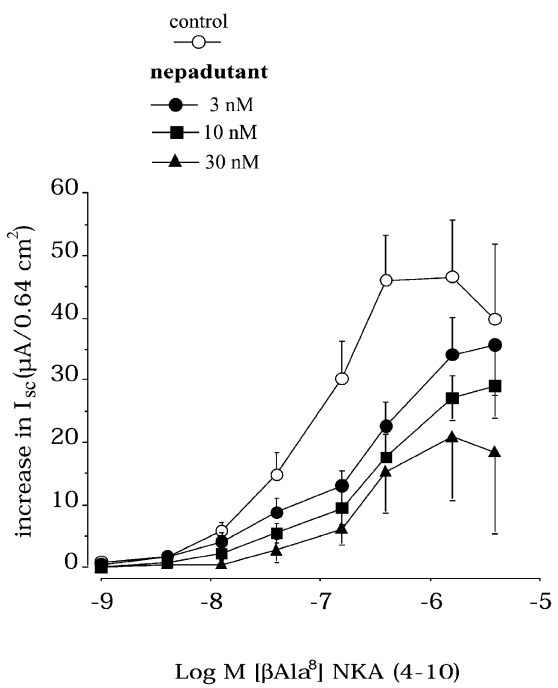

Fig. 3. Antagonism by nepadutant of tachykinin NK_2 receptor-mediated elevation of short-circuit current (I_{sc}) in the rat descending colon mucosa. Log concentration–response curves for [βAla^8]neurokinin A-(4-10) are shown in the absence and in the presence of increasing concentrations of nepadutant (preincubation period was 60 min throughout). Each value is the mean ± S.E.M. of 4–10 experiments.

3.2. Tachykinin NK_2 receptor-mediated changes in ion secretion in the mucosa of the rat distal colon

The basal I_{sc} value recorded from the mucosa at the end of the equilibration period averaged 14.1 ± 3 μA/0.64 cm^2 ($n = 10$). Addition of [βAla^8]neurokinin A-(4-10) produced a concentration-dependent increase of I_{sc} ($pD_2 = 7.2$; 95% CL: 6.9; 7.4); $E_{max} = 46.5 \pm 9$ μA/0.64 cm^2 ($n = 10$) (Figs. 2 and 3). Nepadutant (3–30 nM; 60-min incubation period) produced a nonparallel rightward shift of the agonist curve, accompanied by a depression of E_{max} (77 ± 7%; 62 ± 9%; 45 ± 9% were the maximal responses obtained in the presence of nepadutant 3, 10 and 30 nM compared to the control maximum, respectively; Fig. 3). In order to investigate the role of prostanoid production upon

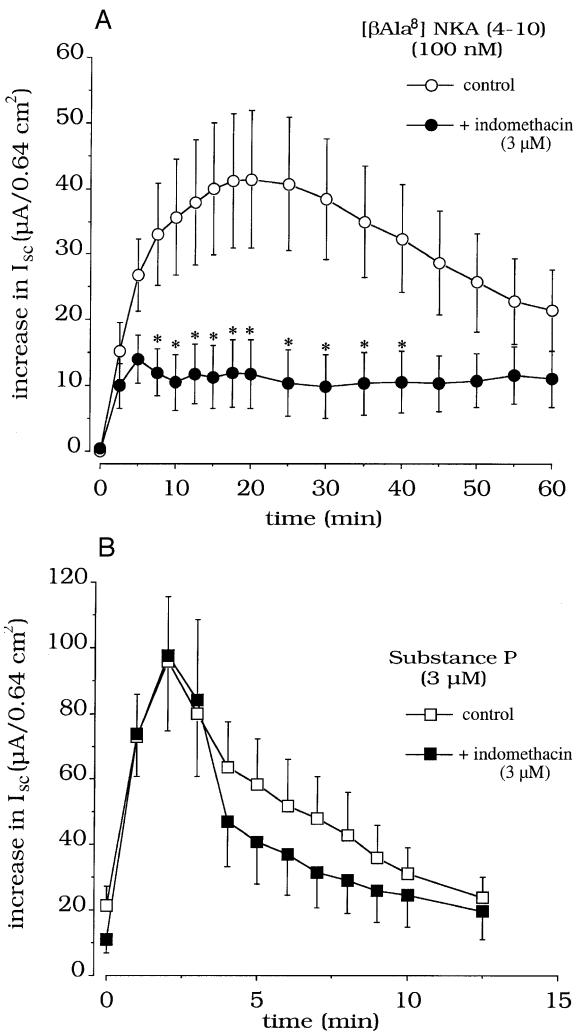

Fig. 4. Effect of indomethacin on elevation of short-circuit current (I_{sc}) produced by [βAla^8]neurokinin A-(4-10) and substance P in the rat descending colon mucosa. The time-course of the response to [βAla^8]neurokinin A-(4-10) (100 nM) and substance P (3 μM) is shown ± indomethacin (3 μM) in panels (A) and (B), respectively. Each value is the mean ± S.E.M. of seven experiments. * Significantly different from time-matched control response; $P < 0.05$.

I_{sc}, we compared the effects produced by [βAla^8]neurokinin A-(4-10) in preparations pretreated with indomethacin vs. vehicle-pretreated preparations. Indomethacin (3 μM; for 30 min) significantly decreased basal I_{sc} per se ($-11.6 \pm 4\%$, $n = 7$; $P < 0.05$ as compared to vehicle). In the presence of indomethacin (3 μM) the response to [βAla^8]neurokinin A-(4-10) (100 nM) was greatly inhibited (> 70% reduction; Fig. 4A) as compared to control. In sharp contrast, responses to the tachykinin NK_1 receptor-preferring agonist substance P (3 μM; administered 45–60 min after indomethacin) to tissues previously exposed to [βAla^8]neurokinin A-(4-10), was totally unaffected by indomethacin (Fig. 4B). In control experiments, the maximal response to [βAla^8]neurokinin A-(4-10) (100 nM, 41.4 ± 10.5 μA. μA/0.64 cm^2, $n = 7$) was reached between 15 and 20 min after agonist addition, whereas in the presence of indomethacin the maximum (14.0 ± 3.6 μA/0.64 cm^2, $n = 7$) was reached within 5 min (Fig. 4A). The response to substance P developed quickly (maximum reached within 2–3 min) and was not significantly altered by the presence of indomethacin (Fig. 4B).

3.3. Reversibility and time-dependency of the effect of nepadutant in the mucosa of the rat distal colon

To investigate whether the insurmountable antagonism exhibited by nepadutant on I_{sc} responses could result from an irreversible interaction with tachykinin NK_2 receptors, both the reversibility and the time-dependency of nepadutant inhibition were studied. In the first set of experiments the inhibition exerted by nepadutant (10 nM) on [βAla^8]neurokinin A-(4-10) responses (100 nM) was slowly reversed over a period of 30–90 min following washout of the antagonist (Fig. 5). In a subsequent series of experiments nepadutant (10 nM) was kept in contact with the colonic mucosa for 30, 60 or 90 min before constructing a concentration–response curve to the agonist. The prolongation of the incubation period from 30 to 60 min resulted in a greater rightward shift of the agonist curve and a further decrease of the E_{max} ($77 \pm 10\%$ vs. $63 \pm 9\%$ of the corresponding control, respectively; $n =$ 6–10), whereas no difference was noted between the curves obtained after 90 and 60 min of antagonist ($E_{max} = 63 \pm$ 9% vs. $61 \pm 9\%$ of control, respectively). The latter findings indicate that a 60-min incubation period is sufficient for nepadutant to attain equilibrium with tachykinin NK_2 receptors present in the colonic mucosa. Since the insurmountable antagonism exerted by nepadutant against [βAla^8]neurokinin A-(4-10) is not apparently a consequence of an irreversible interaction with the tachykinin NK_2 receptors, the apparent pK_B value was estimated by the "double reciprocal plot method" (see Materials and methods) for noncompetitive and/or pseudo-irreversible antagonists and was calculated as 8.6 ± 0.14 ($n = 3$).

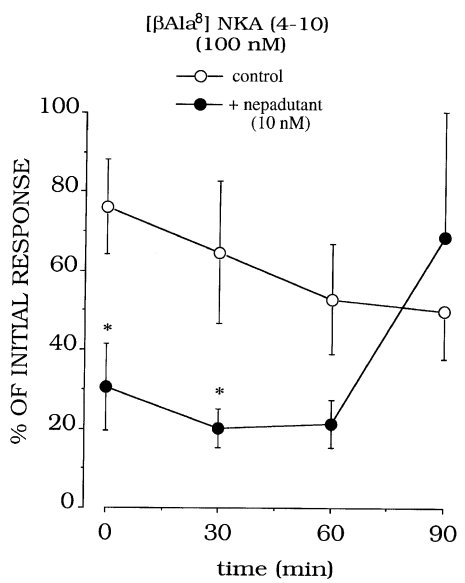

Fig. 5. Reversibility of the blockade produced by nepadutant upon [βAla^8]neurokinin A-(4-10)-induced elevation of short-circuit current (I_{sc}) in the rat descending colon mucosa. The responses to single concentrations of [βAla^8]neurokinin A-(4-10) (100 nM) are shown in the absence or in the presence (time = 0) of nepadutant (10 nM; 15 min incubation), and after 30, 60 and 90 min from washout of the antagonist. * Significantly different from the corresponding value obtained in controls. Each point is the mean ± S.E.M. of 8–10 experiments.

4. Discussion

Previously published studies investigating tachykinin NK_2 receptors stably transfected into different host cells, have indicated that stimulation of these receptors leads to activation of several intracellular pathways, including phosphatidylinositol hydrolysis, cAMP formation and increased arachidonic acid metabolism (Henderson et al., 1990; Nakajima et al., 1992; Eistetter et al., 1991, 1993; Arkinstall et al., 1994). Recently we have provided evidence for independent coupling of the human tachykinin NK_2 receptor with two distinct intracellular pathways namely activation of: (i) phospholipase C leading to formation of inositol trisphosphate (IP_3), and (ii) phospholipase A_2 leading to the release of arachidonic acid and prostaglandin E_2 formation (Catalioto et al., 1998b). In the present study we have explored the possibility that tachykinin NK_2 receptor-stimulated changes in epithelial ion secretion and smooth muscle contraction are dependent upon the formation of endogenous prostanoids. Our data clearly show that blockade of prostanoid synthesis by pretreatment with indomethacin, greatly reduced mucosal ion transport induced by [βAla^8]neurokinin A-(4-10). In contrast, the contractile response of the muscularis mucosae to [βAla^8]neurokinin A-(4-10) was totally independent of prostanoid formation. These observations are in agreement with the results obtained by Tramontana et al. (2000) who, using the hamster isolated urinary blad-

der found [βAla^8]neurokinin A-(4-10) capable of stimulating both contraction of smooth muscle and release of prostaglandin E_2. However, the contractile response to [βAla^8]neurokinin A-(4-10) was unaffected by blockade of cycloxygenase (Tramontana et al., 2000).

Nepadutant (MEN 11420) is a bicyclic peptide compound with high potency and selectivity for tachykinin NK_2 receptors (Catalioto et al., 1998a). In the rat urinary bladder it competitively blocks with nanomolar affinity ($pK_B = 9.0$) tachykinin NK_2 receptor-mediated contractions produced by [βAla^8]neurokinin A-(4-10) (Catalioto et al., 1998a). The present data obtained in the muscularis mucosae ($pK_B = 9.3$) and mucosa ($pK_B = 8.6$) of the rat colon provide further evidence that nepadutant is a potent antagonist of the tachykinin NK_2 receptor in this species. However, we found a difference in the type of antagonism exerted by nepadutant in the two preparations; insurmountable antagonism in the colon mucosa and simple competitive antagonism in the muscularis mucosae. Our consequent assessment of the reversibility and time-dependency of this receptor blockade by nepadutant in the colon mucosa showed that the antagonist did not act as a pure irreversible ligand. In fact, the insurmountable blockade of tachykinin NK_2-mediated changes in ion transport induced by nepadutant was slowly but completely reversed by washout. A 60-min incubation period was sufficient for nepadutant to attain equilibrium with the tachykinin NK_2 receptors. In principle it may still be possible that the insurmountable blockade produced by nepadutant depends on the slow kinetics observed (i.e., it is a question of pseudo-irreversible antagonism, as defined by Kenakin, 1997). An alternative explanation such as different rates of nepadutant degradation in the two tissues, probably does not account for the observed differences. This is because, irrespective of its peptidic structure, nepadutant has been shown to be extremely resistant to degradation by peptidases and other degrading enzymes (Catalioto et al., 1998a; Lippi et al., 1998). In particular, less than 10% of nepadutant undergoes degradation after a 6-h incubation period with liver or rat intestine homogenates (Catalioto et al., 1998a).

Rather, we think that the different types of antagonism produced by nepadutant in the two bioassays may be dependent upon the different effector mechanisms underlying either contraction or increased I_{sc}. Thus, while the contractile response evoked in the muscularis mucosa is likely to be obtained through direct stimulation of tachykinin NK_2 receptors present on smooth muscle cells (Holzer and Holzer-Petsche, 1997a), mucosal responses are most likely a combination of direct epithelial (tetrodotoxin-resistant; Cox et al., 1993) and indirect, neurogenic responses (tetrodotoxin-sensitive, Cox et al., 1993), together with another tetrodotoxin-resistant mechanism, possibly mast cell-mediated and resulting in the generation of prostanoids which elevate I_{sc} over a more prolonged timescale.

Both the capacity of the effector system(s) involved, and the kinetics of the effects produced by the chemical mediators participating to the tachykinin NK_2 receptor-induced changes in ion secretion might be important steps in determining the apparent noncompetitive behaviour of nepadutant. To this respect it is worth noting that although [βAla^8]neurokinin A-(4-10) produced contraction and elevated I_{sc} with comparable potencies ($pD_2 = 7.6$ and 7.2, respectively), the contractile response could be obtained without significant desensitization, whereas the secretory response was subject to marked desensitization. The reasons for the latter behaviour of [βAla^8]neurokinin A-(4-10) are currently unknown.

Another possible explanation for the different antagonism produced by nepadutant in the two preparations is that nepadutant is capable of distinguishing between two conformers of the tachykinin NK_2 receptor in the rat colon. The first could be coupled to a G-protein leading to prostanoid formation and increased ion transport, and the second one coupled to a G-protein leading to activation of a nonprostanoid-mediated mechanism producing contraction. Thus, nepadutant might bind the two putative conformers of the tachykinin NK_2 receptor with competitive vs. noncompetitive kinetics, and quite different affinities ($pK_B = 9.3$ vs. 8.6), respectively.

In the present study ion transport evoked by the tachykinin NK_1 receptor-preferring agonist substance P was not altered by indomethacin. This strongly suggests that stimulation of tachykinin NK_1 receptors in the rat colon mucosa leads to increased I_{sc} via a different mechanism(s) to that activated by tachykinin NK_2 receptor stimulation (prostanoid-independent vs. prostanoid-dependent). Another difference between the responses produced by substance P and [βAla^8]neurokinin A-(4-10) in the colonic mucosa is that the former develops more rapidly (maximal within 5 min from agonist addition) than the latter (maximal at 15-20 min). It is worth noting that the maximum of the residual anion secretion induced by [βAla^8]neurokinin A-(4-10) in the presence of indomethacin was reached much earlier than that obtained in the absence of indomethacin. This latter observation suggests that this residual effect may be due to stimulation of tachykinin NK_1 receptors by [βAla^8]neurokinin A-(4-10), whose ability to activate tachykinin NK_1 receptors at high concentrations has been reported previously (Patacchini et al., 1994).

In conclusion, our study provides evidence that in the rat colon stimulation of tachykinin NK_2 receptors leads to two independent responses; contraction (obtained in the muscularis mucosae preparation) and increased vectorial ion transport responses (obtained in the mucosa). The latter are mediated by a combination of prostanoid-dependent and independent mechanisms. The selective tachykinin NK_2 receptor antagonist, nepadutant, blocked agonist responses in these two colonic target tissues with different kinetics, competitive vs. noncompetitive, respectively. The underlying reasons for the differences in observed antago-

nism remain unclear but are likely to be dependent upon the effector mechanisms predominantly responsible for the responses stimulated by tachykinin NK_2 receptor activation in the rat colon.

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