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Mechanism of tachykinin NK_3 receptor-mediated colonic ion transport in the guinea pig

Jon Goldhill *, Iztchak Angel

Department of Internal Medicine, Synthelabo Recherche, 10 Rue des Carrières, BP 248, 92504 Rueil Malmaison, France

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Abstract

The guinea pig colon was used to elucidate the mechanism of tachykinin-induced secretion. Increased short-circuit current was observed in response to natural and synthetic tachykinins with rank orders of potency of substance P > neurokinin A = neuropeptide K ≫ neuropeptide γ; and senktide (tachykinin NK_3 receptor agonist) > Sar-substance P (tachykinin NK_1 receptor agonist) > βAla-neurokinin A (tachykinin NK_2 receptor agonist)). A functional role of tachykinin NK_1 receptors was confirmed as substance P and neurokinin A responsiveness was blocked by the tachykinin NK_1 receptor antagonist GR82334. The tachykinin NK_3 receptor antagonist SB222200 had no effect, leaving in doubt the identity of the natural tachykinin NK_3 receptor ligand in the colon. The response to tachykinin NK_3 receptor activation was abolished by tetrodotoxin and predominantly due to atropine sensitive cholinergic activation. The non-cholinergic component resulted from stimulation of tachykinin NK_1 and 5-HT receptors as the response to senktide was blocked by GR82334 and tropisetron. In conclusion, tachykinin NK_3 receptor activation stimulates cholinergic and non-cholinergic (tachykinin NK_1-receptor and serotonin-mediated) secretory pathways.

Keywords: Mucosa; Tachykinin NK_1 receptor; Tachykinin NK_3 receptor; Secretion; Tachykinin

1. Introduction

The tachykinin family is comprised of five peptides including the products of two genes, preprotachykinin I and preprotachykinin II. The preprotachykinin II gene produces neurokinin B (Kotani et al., 1986), a tachykinin which is present in very small quantities in peripheral organs such as the intestinal tract (Tateishi et al., 1990). Differential processing of preprotachykinin I RNA yields three different tachykinin precursor molecules: α preprotachykinin I which is converted into substance P; β preprotachykinin I which produces substance P, neurokinin A, neurokinin A (3–10) and neuropeptide K; and γ preprotachykinin I which is the precursor for substance P, neurokinin A, neurokinin A (3–10) and neuropeptide γ (Macdonald et al., 1989). Rat intestine expresses predominantly β preprotachykinin I and γ preprotachykinin I suggesting that substance P, neurokinin A, neuropeptide K and neuropeptide γ should be present in the intestine (Carter and Krause, 1990). Substance P, neurokinin A and neurokinin B bind to tachykinin NK_1, NK_2 and NK_3 receptors, respectively, although considerable overlap occurs. Both neuropeptide K and neuropeptide γ, which are N-terminally extended forms of neurokinin A, act via tachykinin NK_2 receptors (Van Giersbergen et al., 1992). In addition, Neuropeptide K appears to activate central tachykinin NK_1 receptors (Prat et al., 1994).

The tachykinins evoke a number of physiological responses which can be studied pharmacologically using the large number of specific agonists and antagonists now becoming available. The most frequently used tachykinin receptor agonists are [Sar^9,$Met(O_2)^{11}$]substance P (tachykinin NK_1), βAla-neurokinin A (tachykinin NK_2) and senktide (tachykinin NK_3) (Drapeau et al., 1987; Laufer et al., 1988), while commonly used receptor antagonists include GR82334 (tachykinin NK_1; Maggi et al., 1994a) and SR48,968 (tachykinin NK_2; Maggi et al., 1993). Although tachykinin NK_3 receptor antagonists are less abundant, SB222200 and SB223412 have recently been described as selective and potent receptor antagonists (Giardina et al., 1996).

Each of the tachykinin receptors has been localized to circular muscle cells in the small intestine (Hellstrom et

* Corresponding author. Tel.: +33-1-41-39-18-38; Fax: +33-1-41-39-13-09

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al., 1994), although it is of note that biological effects of tachykinin NK_3 receptor activation have been predominantly attributed to a neural site of action (see Croci et al., 1995). The response of intestinal smooth muscle to tachykinins has been characterised in a variety of species, and perhaps most importantly, human colonic and ileal circular muscle responses are both tachykinin NK_2 receptor preferring (Giuliani et al., 1991; Maggi et al., 1992), and furthermore, this receptor subtype plays a role in enteric neurotransmission. Moreover, the guinea pig, which is a particularly good model for tachykinergic control of human intestinal smooth muscle (see for example Barr and Watson, 1993; Maggi et al., 1993), has been used to demonstrate the importance of the tachykinins in the peristaltic reflex (Maggi et al., 1994b) and sensory afferent pathways (Bartho et al., 1994).

In addition to their contractile activity, tachykinins are potent secretagogues; however, in contrast to smooth muscle, few studies have been performed to characterize the effect of tachykinins on epithelial secretion. Of the reports published, the response to the tachykinins has been examined in the small and large intestinal mucosa. Substance P was reported to have neuronally-mediated and direct secretory effects with the former mediated by cholinergic nerves (Keast et al., 1985; Perdue et al., 1987). The guinea pig ileum possesses tachykinin NK_1 but not NK_2 or NK_3 receptors (Reddix and Cooke, 1992), while the guinea pig, rat and dog colons respond to tachykinin NK_1 and NK_3 receptor activation (Kuwahara and Cooke, 1990; Crowther et al., 1994; Cooke et al., 1997). Although cholinergic and non-neurally mediated responses to tachykinergic stimulation have been investigated, non-cholinergic pathways are less well understood. Thus, the aim of the present study was to characterize the guinea pig colonic secretory response to the tachykinins with an emphasis placed on elucidating the balance between the different receptor subtypes involved and the enteric neural circuitry mediating the response to their activation. Furthermore, the involvement of neuropeptide K and neuropeptide γ was also assessed for the first time.

2. Methods

2.1. Animals

Male Hartley guinea pigs (Charles River, France; 450–800 g), allowed free access to standard laboratory chow and water were used throughout this study. Animals were killed by desanguination following a stunning blow to the head, whereafter tissue was immediately removed and mounted in Ussing chambers.

2.2. Colonic epithelial preparation

The colonic epithelial preparation has previously been well documented (Kuwahara and Cooke, 1990). Briefly, the distal 5–10 cm of colon was removed, rinsed in cold Krebs' buffer and placed over a glass rod (6 mm diameter). The outer muscle layer was scored along its anti-mesenteric surface using a dull scalpel blade and peeled away from the mucosa using a piece of gauze previously dampened with Krebs' buffer. The preparation was opened along its anti-mesenteric surface, and the resultant epithelial preparation mounted as a flat sheet between two halves of an Ussing chamber (exposed area 1.24 cm^2). In all studies except those using senktide, two preparations were mounted from each animal, with one preparation always acting as a control. This protocol was chosen, rather than constructing successive response curves in individual preparations, to prevent desensitization. In contrast, when separated by an equilibration period of 30 min, senktide was found to generate two similar consecutive response curves and thus, the first curve was treated as a control while the second was used to test the activity of a given receptor antagonist. In both protocols receptor antagonist was incubated with the tissue for at least 30 min. Both the mucosal and serosal surfaces were perfused with 4 ml Krebs' buffer using a gas-lift ($95\%O_2/5\%CO_2$; pre-humidified by bubbling through distilled water), and maintained at 37 ± 1°C. Short-circuit current generated by the epithelium was continuously monitored using an EVC4000 voltage clamp (WPI, USA). In order to do this, one voltage sensing electrode and one current passing electrode was inserted into each half chamber, and the electrodes connected to the EVC4000 via a pre-amplifier. The voltage generated by the epithelium was continuously short-circuited by passing current across the tissue using the current passing electrodes.

Following a 30 min equilibration period in the presence of mucosal and serosal receptor antagonist or vehicle, various receptor agonists were added in a cumulative fashion to the serosal solution. Concentrations were administered at 2 min intervals to minimize desensitization. In studies performed using substance P, the endopeptidase inhibitor phosphoramidon (10 μM) was added to both halves of the Ussing chamber to prevent peptide breakdown. This treatment was not necessary for any of the other studies. Thirty minutes after the final washout, tissues were challenged with either acetylcholine or in the case of atropine pre-treatment, prostaglandin E_2 to determine tissue viability. Tissues responding to these receptor agonists with an increase in short-circuit current of less than 50 and 25 μA, respectively, were not used.

2.3. Data handling

Data were continuously collected using an acquisition package which automatically determines receptor agonist responses in microamperes. Data were then expressed as absolute values with the E_{max} being calculated for individual response curves. Data were then normalised to these E_{max} values, plotted and the pD_2 calculated. To determine

rank order of receptor agonist potencies, mean pD_2 values were compared using Student's *t*-test. In receptor antagonist studies, E_{max} values were expressed as percent of control E_{max} values. Variations in E_{max} were assessed statistically using a one sample *t*-test. If the E_{max} was not significantly reduced, the pD_2 was compared in the presence and absence of receptor antagonist, and when statistically different, as determined using Student's *t*-test, the dose ratio (DR), and subsequently the pK_b was calculated.

2.4. Drugs and solutions

The following receptor agonists, purchased from Sigma unless stated, were used: acetylcholine chloride; prostaglandin E_2; 5-hydroxytryptamine (creatine sulphate complex); substance P acetate; neurokinin A triflouric acid salt (RBI); neuropeptide K; neuropeptide γ (Bachem); [Sar^9,$Met(O_2)^{11}$]substance P; βAla^8-neurokinin A 4–10 triflouric acid salt (RBI); senktide (succinyl-[Asp^6, N–Me–Phe^8]substance P fragment 6–11). The following receptor antagonists were used: the mixed 5-HT_3/5-HT_4 receptor antagonist, tropisetron (synthesised by the chemistry department of Synthelabo Recherche); the muscarinic receptor antagonist, atropine sulfate; the neural toxin, tetrodotoxin; the tachykinin NK_1 receptor antagonist, GR82334 ({D-Pro^9(spiro-γ-lactam)Leu^{10},Trp^{11}]physalaemin-(1–11)}) triflouric acid salt; the tachykinin NK_2 receptor antagonist, SR48,968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]-benzamide) (synthesised by the chemistry department of Synthelabo Recherche); the tachykinin NK_3 receptor antagonist, (*RS*)SB222200 ((*RS*)-*N*(α-Ethyl-benzyl)-3-methyl-2-phenylquinoline-4-carboxamide) (synthesised by the chemistry department of Synthelabo Recherche); phosphoramidon (*N*-(α-rhamnopyranosyloxy-hydroxyphosphoinyl)Leu-Trp sodium salt). The Krebs' solution used was of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.64; KH_2PO_4, 1.18; glucose, 11.5; $NaHCO_3$, 24.88; $CaCl_2 \cdot 2H_2O$, 2.52. All peptides were dissolved in 0.1 N acetic acid. All other solutions and dilutions were made using distilled water on a daily basis, with the exception of SB222200 which was dissolved in dimethylsulphoxide (DMSO) and diluted in water to give a final vehicle concentration of 0.01%.

3. Results

3.1. Agonist responsiveness

The guinea pig colon responded to substance P, neurokinin A and neuropeptide K with a concentration-dependent increase in short-circuit current (Fig. 1). Maximal values obtained were 246 ± 29, 266 ± 51 and 212 ± 43 μA, respectively. In contrast this preparation was unresponsive to neuropeptide γ up to the highest concentration tested (12 ± 4 μA at 0.1 μM). This preparation was more sensitive ($P < 0.05$) to the tachykinin NK_1 receptor preferring substance P ($pD_2 = 8.40 \pm 0.21$; $n = 4$) than neuropeptide K ($pD_2 = 7.23 \pm 0.12$; $n = 4$) or the tachykinin NK_2 receptor preferring neurokinin A (7.18 ± 0.20; $n = 6$).

3.2. Receptor mechanisms involved in mediating tachykinin responses

Selective receptor agonists had a rank order of potency of senktide (tachykinin NK_3 receptor agonist) > Sar-substance P(tachykinin NK_1 receptor agonist) > βAla-neurokinin A(tachykinin NK_2 receptor agonist) (Fig. 1). Respective pD_2 values were 9.44 ± 0.12, 8.38 ± 0.19 and

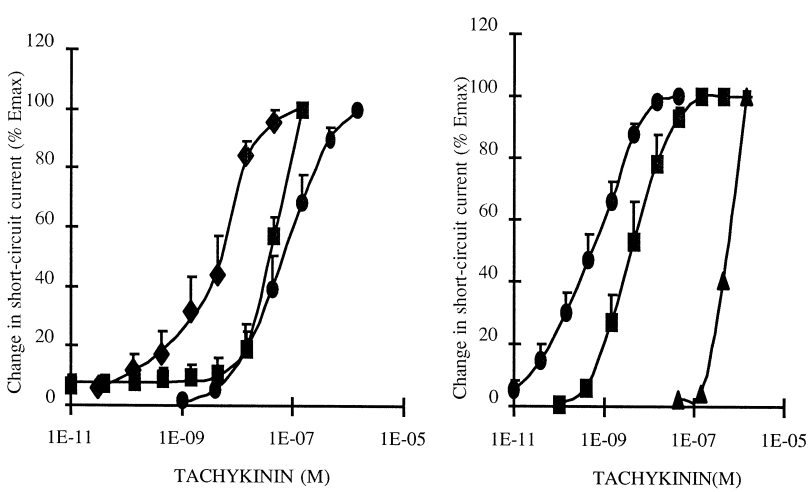

Fig. 1. Short-circuit current response of guinea pig distal colon to the natural tachykinins substance P (diamonds; $n = 4$), neurokinin A (circles; $n = 6$) and neuropeptide K (squares; $n = 4$) (left panel); and the selective synthetic tachykinin receptor agonists Sar-substance P (NK_1; squares; $n = 4$), βAla neurokinin A (NK_2; triangles; $n = 4$) and senktide (NK_3; circles; $n = 5$) (right panel). Each curve was cumulative. The response to substance P was performed in the presence of phosphoramidon (10 μM). Data points are normalized to individual E_{max} values.

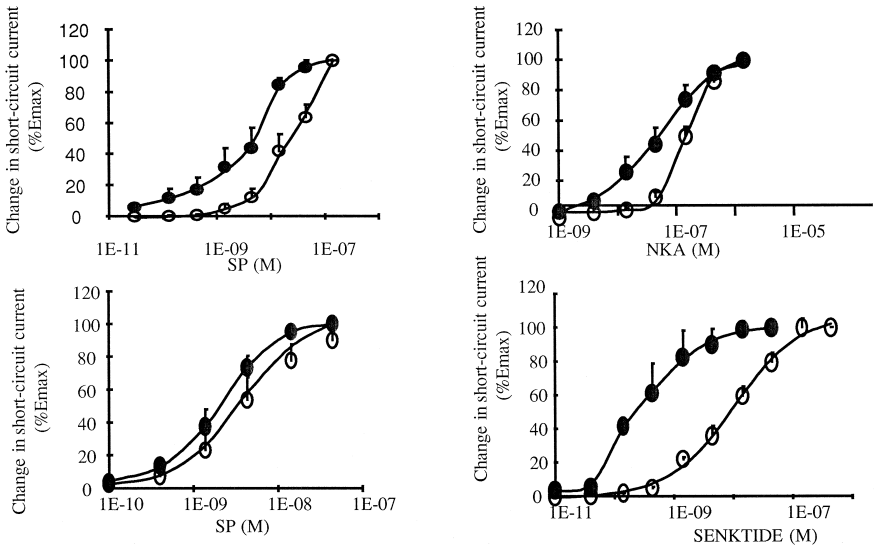

Fig. 2. Short-circuit current response of guinea pig distal colon to substance P (Left panel), neurokinin A (Upper right panel), or senktide (Lower right panel) in the presence (open circles) and absence (filled circles) of the tachykinin NK_1 receptor antagonist GR82334 (1 μM; $n = 4$; upper panel) or the tachykinin NK_3 receptor antagonist SB222200 (0.1 μM; $n = 3$–4; lower panel). Control vehicles for GR82334 and SB222200 were acetic acid (0.001 N) and DMSO (1%). Data points are normalized to individual E_{max} values.

6.26 ± 0.06. The E_{max} for the response to Sar-substance P was significantly ($P < 0.05$) larger than that for senktide (371 ± 50 and 199 ± 34 μA, respectively). The E_{max} for βAla-neurokinin A was not reached and could not be calculated. This confirms that tachykinin NK_2 receptors play a minor role in mediating colonic secretion in the guinea pig. In contrast tachykinin NK_1 and NK_3 receptors play a major role in epithelial ion transport.

A range of specific receptor antagonists were used to further characterize the involvement of the different receptor subtypes. The response to substance P was shifted to the right by GR82334 (1 μM) yielding a pK_b of 6.87 ± 0.29 (Fig. 2, upper left panel), but not by SB222200 (DR = 2.69 ± 1.25; $E_{max} = 209 \pm 97\%$ of control; $n = 6$; Fig. 2, lower left panel) at concentrations (0.1 μM) that inhibited the response to senktide (Fig. 2, lower right panel). This suggests that the response to substance P is mediated by tachykinin NK_1 but not NK_3 receptors. The response to neurokinin A was also antagonised by GR82334 (Fig. 2, upper right panel), in a non-competitive fashion as the E_{max} was reduced to $61 \pm 5\%$ ($P < 0.05$) of controls. Furthermore, the response to neurokinin A was shifted to the right and an apparent pK_b value of 6.27 ± 0.33 was calculated. The tachykinin NK_2 receptor antagonist SR48,968, at concentrations (0.1 μM) shown to be effective in preparations containing tachykinin NK_2 receptors (Maggi et al., 1992), did not effect the response to neurokinin A (DR = 1.11 ± 0.20; $E_{max} = 132 \pm 30\%$ of control). Similarly, the response to neurokinin A was not significantly altered by SB222200 (DR = 3.69 ± 1.64;

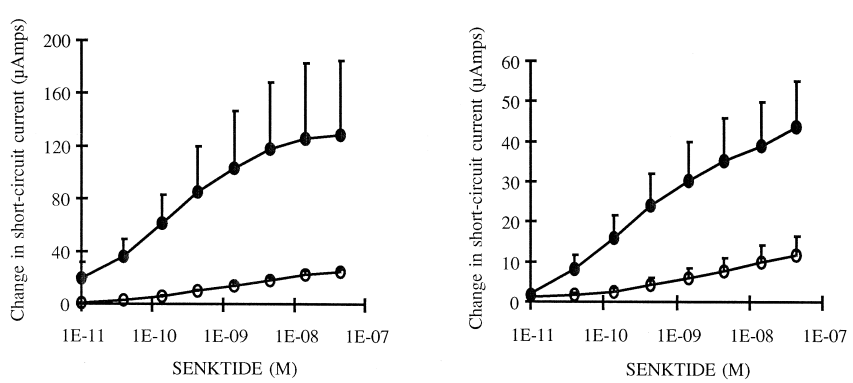

Fig. 3. Short-circuit current response of guinea pig distal colon to senktide in (Left Panel) the presence (open circles) or absence (filled circles) of the muscarinic receptor antagonist, atropine (10 μM; $n = 3$) and (Right Panel) the presence of atropine (10 μM; filled circles) or atropine and the neurotoxin tetrodotoxin (10 μM; $n = 3$) (right panel). The y axes represent absolute change in short-circuit current to demonstrate the reduction in the maximal response to senktide.

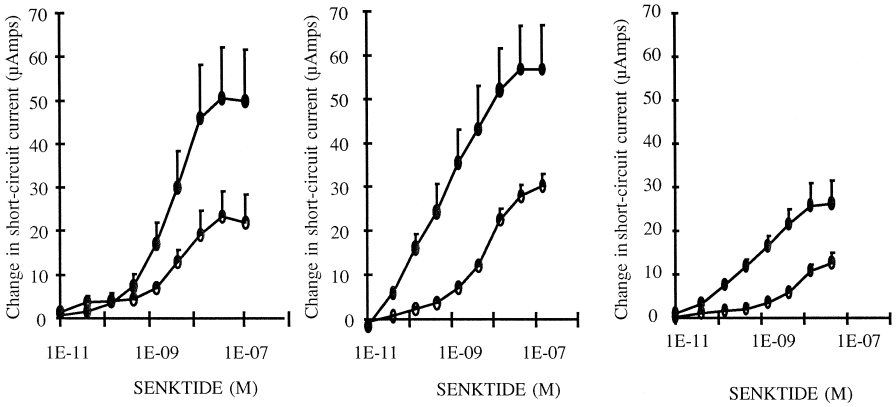

Fig. 4. Short-circuit current response of atropine-treated (10 μM) guinea pig distal colon to senktide in the presence (open circles) and absence (filled circles) of (Left panel) the tachykinin NK_1 receptor antagonist, GR82334 (1 μM; $n = 3$); (Middle panel) the mixed 5-HT_3/5-HT_4 receptor antagonist tropisetron (10 μM; $n = 4$); and (Right panel) a combination of these two receptor antagonists ($n = 4$). Note that the y axis represents absolute change in short-circuit current to demonstrate the reduction in the maximal response to senktide.

$E_{max} = 102 \pm 20\%$ of control). Thus, like substance P, the response to neurokinin A appears to be mediated by tachykinin NK_1 rather than NK_2 or NK_3 receptors.

3.3. Enteric neural pathways mediating tachykinin NK_3-induced secretion

The site of action of tachykinin NK_3 receptor agonists is generally neural (Croci et al., 1995). The response to senktide was reduced to $25 \pm 6\%$ of control values by atropine (10 μM; $P < 0.05$; Fig. 3) suggesting the stimulation of cholinergic nerves within the intestinal tract. Further addition of tetrodotoxin at a concentration that completely blocks the response of the present preparation to nerve stimulation (10 μM) reduced the residual response to $33 \pm 15\%$ of that in the presence of atropine alone ($P < 0.05$; Fig. 3). In the combined presence of tetrodotoxin and atropine the response to senktide was not significantly different to zero, suggesting that tachykinin NK_3 receptors are entirely neuronal, and are present predominantly on cholinergic nerves but also on non-cholinergic nerves supplying the mucosa. The non-cholinergic response to senktide appeared to be mediated by tachykinin NK_1 receptors, as in the presence of atropine, the response to senktide was reduced to $46 \pm 6\%$ of controls ($P < 0.05$; Fig. 4) by GR82334 (1 μM).

In addition to being blocked by tachykinin NK_1 receptor antagonists, we showed that the non-cholinergic response to senktide was reduced to $53 \pm 5\%$ ($P < 0.05$; Fig. 4) of controls by tropisetron (10 μM). This concentration has previously been shown to block both 5-HT_3 and 5-HT_4 receptors (Eglen et al., 1993), and in preliminary studies we showed that it was sufficient to almost abolish the response of the present preparation to 5-HT (data not shown). This suggests that both serotonin and tachykinin NK_1 receptor-mediated pathways are involved in the response to senktide. To determine interactions between these two pathways, the response to senktide was mea-

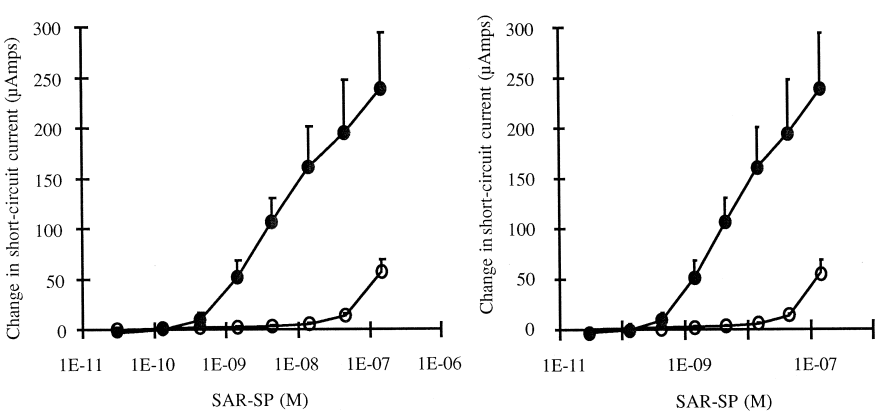

Fig. 5. Short-circuit current response of guinea pig distal colon to Sar-substance P in the presence (open circles) and absence (filled circles) of (Left panel) the mixed 5-HT_3/5-HT_4 receptor antagonist tropisetron (10 μM; $n = 4$); and (Right panel) the neurotoxin tetrodotoxin (10 μM; $n = 4$). Note that the y axis represents absolute change in short-circuit current to demonstrate the reduction in the maximal response to [Sar]substance P.

sured in the presence of both tropisetron and GR82334. Under these conditions the maximal response to senktide was reduced to 52 ± 11% of controls (Fig. 4), a value not significantly different to when either receptor antagonist was added on its own.

3.4. Pathways mediating tachykinin NK_1-induced secretion

The above findings suggest that the response to senktide is mediated by 5-HT which is in turn mediated by a tachykinin NK_1 receptor agonist, or visa versa. Thus, we determined the effect of tropisetron on the response to Sar-substance P, and found the E_{max} to be significantly ($P < 0.05$) reduced to 41 ± 14% of control values (Fig. 5), confirming that the response to tachykinin NK_1 activation is mediated by 5-HT. Since the response to 5-HT has previously been shown to be abolished by tetrodotoxin (Cooke et al., 1991), it follows that the response to Sar-substance P should also be reduced by tetrodotoxin. This was found to be the case with the E_{max} significantly ($P < 0.05$) reduced to 27 ± 7% of control value (Fig. 5). To determine whether cholinergic as well as serotonergic pathways mediate the response to tachykinin NK_1 activation, studies were performed using atropine, which was found to be without significant effect on the maximal response to Sar-substance P ($E_{max} = 95 \pm 22\%$ of controls).

4. Discussion

The present data confirm the findings of an earlier publication (Kuwahara and Cooke, 1990) showing that the guinea pig colonic epithelium is sensitive to the natural tachykinins substance P and neurokinin A. We extend these findings, demonstrating for the first time a secretory action of a third natural tachykinin, neuropeptide K, but not of neuropeptide γ. Neuropeptide K has been reported to have tachykinin NK_1 and NK_2 receptor-mediated effects, while tachykinin NK_2 receptor-mediated effects alone have been suggested for neuropeptide γ (Van Giersbergen et al., 1992; Prat et al., 1994). The lack of effect, in the present study, of neuropeptide γ suggests the absence of functional tachykinin NK_2 receptors, and moreover that the secretory action of neuropeptide K in the colon is likely to be mediated by tachykinin NK_1 receptors. A predominant role for tachykinin NK_1 over NK_2 receptors is supported by the greater potency of substance P compared to neurokinin A. The present study provides further evidence for this conclusion through the use of selective receptor agonists. The tachykinin NK_1 receptor agonist, Sar-substance P was found to be a potent receptor agonist in the colon, displaying similar potency to that described in a tachykinin NK_1 receptor assay system, the dog carotid artery (Drapeau et al., 1987). The tachykinin NK_3 receptor agonist, senktide was an even more potent secretagogue implicating these receptors, in addition to tachykinin NK_1 receptors, in the control of epithelial ion transport. In contrast the tachykinin NK_2 receptor agonist, βAla-neurokinin A was only weakly active, displaying an activity nearly 300-fold less potent than in the human intestinal smooth muscle, a relatively pure tachykinin NK_2 receptor preparation (Giuliani et al., 1991). Thus, there appears to be regional variation with tachykinin NK_3 receptors present in the guinea pig colon but not ileum in agreement with previous reports (Reddix and Cooke, 1992; Cooke et al., 1997). Moreover, there appears to be inter-species variation, with the guinea pig colon having a similar receptor profile to that of the dog (Crowther et al., 1994) but different to that of the rat which has additional functional tachykinin NK_2 receptors (Cox et al., 1993). GR82334 antagonised the response to substance P demonstrating a clear functional role for the tachykinin NK_1 receptor subtype. In fact, tachykinin NK_1 receptors were the only subtype identified to mediate the secretory action of substance P, as tachykinin NK_3 receptor antagonists were without effect, and as discussed earlier, tachykinin NK_2 receptors appear to have little functional role in the colonic epithelium. In addition to substance P, we examined the mechanism of action of the second major natural tachykinin neurokinin A. Although neurokinin A is tachykinin NK_2 receptor-preferring, the lack of effect of SR48,968 in the present study suggests that its secretory action was not mediated via this subtype. Furthermore, a tachykinin NK_3 receptor antagonist was without effect. In contrast, the response to neurokinin A was blocked by GR82334, and therefore, tachykinin NK_1 receptors play a major functional role in mediating the colonic epithelial response to neurokinin A as well as substance P. This is in line with the current hypothesis that the tachykinin NK_1 receptor exists in two states one which specifically binds substance P and a second state that binds, with a lower affinity, a range of additional tachykinins such as neurokinin A (Maggi and Schwartz, 1997).

While we have demonstrated a functional role for tachykinin NK_1 receptors, the role that tachykinin NK_3 receptors play in the control of epithelial ion transport requires further study. The tachykinin NK_3 receptor antagonist SB222200 is relatively specific for the tachykinin NK_3 receptor although it does have some affinity (277 nM) for the tachykinin NK_2 receptor (Giardina et al., 1996). This receptor antagonist when tested at concentrations similar to its affinity for the tachykinin NK_3 receptor and sufficient to block the response to senktide did not alter the response to substance P or neurokinin A. This leaves some doubt regarding the natural ligand for this receptor especially as neurokinin B is present in very small quantities in peripheral tissues such as the intestinal tract (Tateishi et al., 1990). This receptor may be an orphan receptor or alternatively its affinity for neurokinin B may be sufficiently high for low concentration to elicit an effect. An alternative explanation is that substance binds to

tachykinin NK_3 receptors. If substance P is the natural receptor agonist active concentrations must be higher than those used in the present study. If this is the case, the tachykinin NK_3 receptor may take on pathological significance in the intestinal tract.

Having demonstrated a potential role for tachykinin NK_1 and NK_3 receptors in the control of colonic ion transport, we attempted to further elucidate the neural circuitry that mediates the response to their activation. As previously shown in the dog colonic mucosa (Crowther et al., 1994), the response to senktide was abolished in the presence of tetrodotoxin, suggesting that tachykinin NK_3 receptor agonists activate nerves supplying the colonic mucosa. This differs from the rat, where a residual response to senktide remained in the presence of tetrodotoxin (Cox et al., 1993). It should perhaps be noted that tetrodotoxin blocks the Na^+ channel of certain immune cells (Munson et al., 1979) and this toxin therefore cannot be used to unequivocally demonstrate neural involvement. It has previously been shown that senktide stimulates acetylcholine release from the guinea pig ileal myenteric plexus (Guard and Watson, 1987). Likewise, here we show that a large proportion of the senktide secretory response is mediated, directly or indirectly, by enteric cholinergic nerves. In addition to cholinergic nerves, serotonergic and tachykinin NK_1 receptors also appear to mediate the response to tachykinin NK_3 receptor activation, as the response to senktide was antagonised by tropisetron and GR82334. From the present study it is not possible to identify the neurotransmitter acting at the tachykinin NK_1 receptor; however, the most likely candidates are substance P, neuropeptide K or neurokinin A. Serotonin- and tachykinin NK_1 receptor-mediated pathways appear to be arranged in series as the effect of tropisetron and GR82334 are not additive. To elucidate the order of events the effect of tropisetron on the response to Sar-substance P was investigated, and found to dramatically inhibit the E_{max}, suggesting that tachykinin NK_1 receptor activation liberates 5-HT. Thus, an enteric network appears to exist on the one hand involving tachykinin NK_3 receptor activation of cholinergic nerves, and on the other, tachykinergic nerves which in turn activate a 5-HT-mediated pathway. Tachykinin NK_3 receptor activation of the tachykinin NK_1 receptor- or cholinergic-mediated pathways as shown in the present study has previously been shown in the guinea pig ileal smooth muscle (Guard and Watson, 1987). However, the present study is one of the first to suggest that tachykinergic responsiveness is mediated by serotonin in the periphery. Serotonin does not appear to be the final neurotransmitter as the secretory response to 5-HT is abolished by tetrodotoxin (Cooke et al., 1991), and therefore, a secretory response evoked by serotonin may involve subsequent stimulation of nerve fibres. This is supported indirectly by the observation that the response to Sar-substance P (up to 30 nM) was abolished by tetrodotoxin. At higher concentrations a response to Sar-substance P was observed; however, the specificity of this effect is questionable. In contrast, the response to Sar-substance P was unaffected by atropine. Thus, in agreement with earlier data (Kuwahara and Cooke, 1990), the response to Sar-substance P was predominantly non-cholinergic in nature.

In summary, we have shown that the guinea pig colonic epithelium is tachykinin NK_1 and NK_3 receptor preferring, with little function implicated for tachykinin NK_2 receptors. The tachykinin NK_3 receptor appears to play a key neuromodulatory role, activating a cholinergic pathway and a non-cholinergic pathway involving tachykinin NK_1 and 5-HT receptors. This working model can now form the basis of further studies to investigate the role of tachykinins in intestinal secretion.

References

Barr, A.J., Watson, S.P., 1993. Non-peptide antagonists, CP-96,345 and RP 67580, distinguish species variants in tachykinin NK1 receptors. Br. J. Pharmacol. 108, 223–227.

Bartho, L., Maggi, C.A., Wilhelm, M., Patacchini, R., 1994. Tachykinin NK1 and NK2 receptors mediate atropine-resistant ileal circular muscle contractions evoked by capsaicin. Eur. J. Pharmacol. 259, 187–193.

Carter, M.S., Krause, J.E., 1990. Structure, expression, and some regulatory mechanisms of the rat preprotachykinin gene encoding substance P, neurokinin A, neurokinin K and neuropeptide γ. J. Neurosci. 10, 2203–2214.

Cooke, H.J., Wang, Y.Z., Frieling, W.T., Wood, J.D., 1991. Neural 5-hydroxytryptamine receptors regulate chloride secretion in guinea-pig distal colon. Am. J. Physiol. 261, G833–G840.

Cooke, H.J., Sidhu, M., Fow, P., Wang, Y.Z., Zimmerman, E.M., 1997. Substance P as a mediator of colonic secretory reflexes. Am. J. Physiol. 272, G238–G245.

Cox, H.M., Tough, I.R., Grayson, K., Yarrow, S., 1993. Pharmacological characterisation of neurokinin receptors mediating anion secretion in rat descending colon mucosa. Naunyn-Schmeideberg's Arch. Pharmacol. 348, 172–177.

Croci, T., Landi, M., Edmonds-Alt, X., Le Fur, G., Manara, L., 1995. Neuronal NK3-receptors in guinea-pig ileum and taenia caeci: in vitro characterization by their first non-peptide antagonist, SR142801. Life Sci. 57, 361–366.

Crowther, R., Jukic, D., Regoli, D., Rangachari, P.K., 1994. Functional subtyping of neurokinin receptors on canine proximal colonic mucosa. J. Pharmacol. Exp. Ther. 268, 1374–1380.

Drapeau, G., D'Orleans-Juste, P., Dion, S., Rhaleb, N.E., Rouissi, N.E., Regoli, D., 1987. Selective agonists for substance P and neurokinin receptors. Neuropeptides 10, 43–54.

Eglen, R.M., Alvarez, R., Johnson, L.G., Leung, E., Wong, E.H.F., 1993. The action of SDZ205,557 at 5-hydroxytryptamine (5-HT3 and 5-HT4) receptors. Br. J. Pharmacol. 108, 376–382.

Giardina, G.A.M., Sarau, H.M., Farina, C., Medhurst, A.D., Grugni, M., Foley, J.J., Raveglia, L.F., Schmidt, D.B., Rigolio, R., Vassallo, M., Vecchietti, V., Hay, D.W.P., 1996. 2-phenyl-4-quinolinecarboxamides: a novel class of potent and selective non-peptide competitive antagonists for the human neurokinin-3 receptor. J. Med. Chem. 39, 2281–2284.

Giuliani, S., Barbanti, G., Turini, D., Quartara, L., Rovero, P., Giachetti, A., Maggi, C.A., 1991. NK2 tachykinin receptors and contraction or circular muscle of the human colon: characterization of the NK2 receptor subtype. Eur. J. Pharmacol. 203, 365–370.

Guard, S., Watson, S.P., 1987. Evidence for neurokinin-3 receptor-mediated tachykinin release in the guinea-pig ileum. Eur. J. Pharmacol. 144, 409–412.

Hellstrom, P.M., Murthy, K.S., Grider, J.R., Makhlouf, G.M., 1994. Coexistence of three tachykinin receptors coupled to Ca++ signalling pathways in intestinal smooth muscle cells. J. Pharmacol. Exp. Ther. 270, 236–243.

Keast, J.R., Furness, J.B., Costa, M., 1985. Different substance P receptors are found on mucosal epithelial cells and submucous neurons of the guinea-pig small intestine. Arch. Pharmacol. 392, 382–387.

Kotani, H., Hoshimaro, M., Nawa, H., Nakanishi, S., 1986. Structure and gene organisation of bovine neuromedin K precursor. Proc. Natl. Acad. Sci. USA 83, 7074–7078.

Kuwahara, A., Cooke, H.J., 1990. Tachykinin-induced anion secretion in guinea pig distal colon: role of neural and inflammatory mediators. J. Pharmacol. Exp. Ther. 252, 1–7.

Laufer, R., Gilon, C., Chorev, M., Selinger, Z., 1988. Desensitization with a selective agonist discriminates between multiple tachykinin receptors. J. Pharmacol. Exp. Ther. 245, 639–643.

Macdonald, M.R., Tacite, J., Rice, C.M., Krause, J.E., 1989. Multiple tachykinins are produced and secreted upon post-translational processing of the three substance P precursor proteins α-, β- and γ-preprotachykinin. J. Biol. Chem. 264, 15578–15592.

Maggi, C.A., Schwartz, T.W., 1997. The dual nature of the tachykinin NK_1 receptor. Trends Pharmacol. Sci. 18, 351–354.

Maggi, C.A., Giuliani, S., Patacchini, R., Santicioli, P., Theodorsson, E., Barbanti, G., Turini, D., Giachetti, A., 1992. Tachykinin antagonists inhibit nerve-mediated contractions in the circular muscle of the human ileum. Gastroenterology 102, 88–96.

Maggi, C.A., Patacchini, R., Giuliani, S., Giachetti, A., 1993. In vivo and in vitro pharmacology of SR48,968, a non-peptide tachykinin NK_2 receptor antagonist. Eur. J. Pharmacol. 234, 83–90.

Maggi, C.A., Patacchini, R., Meini, S., Quartara, L., Sisto, A., Potier, E., Giuliani, S., Giachetti, A., 1994a. Comparison of tackykinin NK1 and NK2 receptors in the circular muscle of the guinea pig ileum and proximal colon. Br. J. Pharmacol. 112, 150–160.

Maggi, C.A., Patacchini, R., Bartho, L., Holzer, P., Santicioli, P., 1994b. Tachykinin NK_1 and NK_2 receptor antagonists and atropine-resistant ascending excitatory reflex to the circular muscle of the guinea-pig ileum. Br. J. Pharmacol. 112, 161–168.

Munson, R., Westermark, B., Glaser, L., 1979. Tetrodotoxin-sensitive sodium channels in normal human fibroblasts and normal human glia-like cells. Proc. Natl. Acad. Sci. USA 76, 3429–6425.

Perdue, M.H., Galbraith, R., Davison, J.S., 1987. Evidence for substance P as a functional neurotransmitter in guinea pig small intestinal mucosa. Regul. Pept. 18, 63–74.

Prat, A., Picard, P., Couture, R., 1994. Cardiovascular and behavioural effects of centrally administered neuropeptide K in the rat: receptors characterization. Br. J. Pharmacol. 112, 250–256.

Reddix, R.A., Cooke, H.J., 1992. Neurokinin 1 receptors mediate substance P-induced changes in ion transport in guinea pig ileum. Regul. Pept. 39, 215–225.

Tateishi, K., Kishimoto, S., Kobayashi, H., Koburo, K., Matsuora, Y., 1990. Distribution and colocalization of neurokinin A-like immunoreactivity and neurokinin B-like immunoreactivity in rat peripheral tissue. Regul. Pept. 30, 193–200.

Van Giersbergen, P.L.M., Shatzer, S.A., Burcher, E., Buck, S.H., 1992. Comparison of the effects of neuropeptide K and neuropeptide γ with neurokinin A at NK2 receptors in the hamster urinary bladder. Arch. Pharmacol. 345, 51–56.