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Role of tachykinin receptors in the modulation of colonic peristaltic activity in mice

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ABSTRACT

Tachykinins are important mediators of neuroneuronal and neuromuscular transmission in the gastrointestinal tract, however their contribution to colonic peristalsis in mice remains unclear. Therefore, our aim was to characterise the functional role of tachykinins in mediating peristalsis by evaluating the effect of selective tachykinin NK_1, NK_2 and NK_3 receptor agonists and antagonists on *in vitro* colonic peristaltic activity in mice. Using a modified Trendelenburg set-up, gradual distension of proximal and distal colonic segments evoked rhythmic, aborally migrating contractions. Peristaltic activity was assessed by quantifying the amplitude and interval of the corresponding pressure waves. Stimulation of NK_1 receptors showed regional differences as both the pressure amplitude and interval were enhanced in the distal colon without affecting peristalsis proximally. Blockade of NK_1 receptors reduced the peristaltic pressure amplitude in the proximal and distal colon while the interval was not significantly altered. NK_2 receptor stimulation resulted in a modest enhancement of the amplitude in proximal and distal segments and a slightly prolonged interval distally. Blockade of NK_2 receptors reduced the peristaltic pressure amplitude and interval in the distal colon. NK_3 receptor stimulation significantly augmented the amplitude in both segments and prolonged the interval distally. However, NK_3 receptor blockade had no effect on peristaltic activity. In conclusion, tachykinins contribute to colonic peristalsis in mice by acting mainly on NK_1 and NK_2 receptors and their effects show a proximal-to-distal gradient. NK_3 receptors might play a role in conditions of excess tachykinin release but appear not to be involved under the conditions of the present study.

1. Introduction

Propulsion of intraluminal content through the gastrointestinal tract is achieved by peristalsis, a distinct motor pattern that is constituted by ascending excitatory and descending inhibitory reflexes (Bayliss and Starling, 1899). The ascending excitatory reflex releases acetylcholine and tachykinins, initiating simultaneous contraction of circular muscle and relaxation of longitudinal muscle orad to the site of stimulation. Aborally, the descending inhibitory reflex elicits relaxation of circular muscle and contraction of longitudinal muscle, mainly through the release of nitric oxide, vaso-active intestinal polypeptide and adenosine triphosphate (ATP) (Burnstock, 2008; Grider, 2003a, 2003b; Holzer et al., 1993; Kottegoda, 1969).

Substance P, neurokinin (NK) A and neurokinin B pertain to a group of neuropeptides, termed tachykinins. They are involved in the regulation of various gastrointestinal functions, including peristalsis and motility (Holzer and Holzer-Petsche, 1997a). Tachykinins act through binding on specific receptors, designated as tachykinin NK_1, NK_2 and NK_3 receptors, each exhibiting a characteristic rank-order of affinity (Maggi, 2000). In the gastrointestinal tract, tachykinin receptor distribution is subject to regional and species-related differences (Holzer and Holzer-Petsche, 1997a). Nevertheless, it is generally believed that NK_1 receptors are localised on smooth muscle cells of the circular and longitudinal muscle layer, as well as on enteric neurons, modulating the activity of motor neurons, sensory neurons and interneurons (Holzer and Holzer-Petsche, 1997a). In addition, NK_1 receptors have been identified on interstitial cells of Cajal, endothelial cells, enterocytes and inflammatory cells within the lamina propria and submucosa (Lecci et al., 2006). Tachykinin NK_2 receptors are largely confined to smooth muscle cells. However, recent evidence points towards additional neuronal expression as NK_2 receptors were demonstrated on descending nitrergic neurons in the myenteric plexus of guinea-pig gastrointestinal tract and on nerve varicosities within the rat myenteric and submucosal plexus (Lecci et al., 2004). NK_3 receptors are present mainly on neuronal cells as intrinsic primary afferent neurons, ascending and descending interneurons, excitatory and inhibitory motor neurons and secretomotor neurons (Holzer and Holzer-Petsche, 1997a). Nevertheless, there is some evidence of NK_3 receptor expression on smooth muscle cells as well (Lecci et al., 2006).

In the gastrointestinal tract, tachykinins can either facilitate or inhibit motility (Holzer, 1998; Holzer and Holzer-Petsche, 1997a). It appears that their net effect on motility is dependent on the receptor subtype involved, the gastrointestinal region under investigation and the animal model studied (Holzer and Holzer-Petsche, 2001). While

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most of the research into the field of tachykinins was conducted in guinea-pig and rat gastrointestinal tissue, few studies are described in mice. Considering the substantial differences in tachykinin receptor distribution between species and the continuously growing importance of studies in knock-out mice, it is important to fully understand the role of tachykinins in peristaltic activity in mice.

Therefore our aim was to investigate the functional role of tachykinins and the tachykinin NK_1, NK_2 and NK_3 receptors in mediating peristaltic activity by studying the effect of selective NK_1, NK_2 and NK_3 receptor agonists and antagonists on *in vitro* distension-induced peristaltic activity in the mouse colon.

2. Material and methods

2.1. In vitro measurement of colonic peristaltic activity

All animal procedures and experimental protocols were approved by the Medical Ethical Committee of the University of Antwerp, Belgium. A modified Trendelenburg set-up was used to induce peristaltic activity in isolated colonic segments (Holzer et al., 1998; Holzer and Maggi, 1994; Seerden et al., 2007; Trendelenburg, 2006). Briefly, Swiss OF1 mice were anaesthetized with diethyl ether and were exsanguinated. After laparotomy, the colon was rapidly excised, transferred to ice-cold Krebs-Ringer solution (118.3 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaCHO_3$, 0.026 mM CaEDTA and 11.1 mM glucose) and gently flushed to clear the lumen of intestinal contents. The ceacum was removed and the remaining colon was divided in a proximal and distal segment (approximately 3.5 cm each), discarding the redundant mid-colonic tissue if the total length exceeded 7 cm. Segments were mounted horizontally in a 4 ml organ bath, filled with warm, oxygenated Krebs-Ringer solution (37 °C, oxygenated with 5% CO_2/95% O_2). The oral side of the segment was connected to a pressure transducer for the recording of intraluminal pressure differences and to a perfusion pump allowing continuous intraluminal infusion of warm, oxygenated Krebs-Ringer solution (37 °C, oxygenated with 5% CO_2/95% O_2), at a rate of 0.3 ml/min. The aboral end was secured to an open, adjustable outlet that could be raised in height. The tissue was allowed to equilibrate for approximately 15 min and then the outlet was gradually raised to a height of 7.5 cm by increments of 2.5 cm per 5 min. This gradual distension of the colonic tissue resulted in rhythmic and repetitive peristaltic contractions propagating aborally. These contractions were recorded by the pressure transducer at the oral side of the segment as cyclic pressure waves.

2.2. Experimental protocol

All experiments were conducted after at least 15 min of equilibration at an outlet-height of 7.5 cm. This outlet-height resulted in highly reproducible pressure waves that persisted for several hours. In a first series of experiments, the effect of tetrodotoxin (inhibitor of neuronal conductance; 1 μM), hexamethonium (inhibitor of nicotinic receptors; 100 μM) and atropine (inhibitor of muscarinic receptors; 1 μM) on peristalsis was evaluated. The incubation time for each agent was 10 min. Secondly, tachykinin receptor stimulation or inhibition by selective agonists and antagonists respectively allowed assessment of the relative contribution of each receptor subtype to peristaltic activity. In detail, the effect of tachykinin NK_1, NK_2 and NK_3 receptor agonists was evaluated by exposing colonic segments to septide (10–100 nM) (De Schepper et al., 2005), β-A-NKA (10–100 nM) (De Schepper et al., 2005) and senktide (1–10 nM) (Wormser et al., 1986). Tissues were exposed to two cumulative doses of a single agonist with an incubation time of 8 min per dose. The following selective antagonists were applied for receptor inhibition: RP 67580 (2 μM) (Beaujouan et al., 1993; De Schepper et al., 2005; Nsa Allogho et al., 1997), nepadutant (0.1–1 μM) (De Schepper et al., 2005; Nsa Allogho et al., 1997) and SR 142801 (100–300 nM) (De Man et al., 2008; Emonds-Alt et al., 1995; Laufer et al., 1986) for the NK_1, NK_2 and NK_3 receptor respectively. In a final series of experiments, the overall effect of tachykinins on peristalsis was evaluated by subsequently blocking the NK_3, NK_2 *plus* NK_1 receptors. The incubation time for all antagonists was 15 min. The concentration of tachykinin receptor agonists and antagonists was chosen according to the literature. Receptor selectivity was established by testing each tachykinin NK_1, NK_2 and NK_3 receptor agonist in the presence of the respective antagonist, in accordance with our previous studies in the mouse ileum (De Schepper et al., 2005).

2.3. Data analysis

Colonic peristaltic activity was registered by a pressure transducer as differences in intraluminal pressure. The signal was amplified (Octal Bridge Amplifier, ADInstruments) and recorded by a data-acquisition system (ADInstruments, Powerlab 8/30). Analysis of the pressure tracings was performed using Chart™ 5 (ADInstruments). Peristaltic activity was assessed for each colonic segment by quantifying the mean amplitude (cm H_2O) and interval (s) of 3 consecutive pressure waves. If exposure to a specific drug abolished peristaltic activity, the amplitude was considered to be zero when calculating the mean amplitude. In those cases however, the interval could not be measured and thus the segment was not included in the analysis of the mean interval. Results are shown as mean ± S.E.M. Each experiment was conducted in 6 proximal and 6 distal segments unless otherwise mentioned. Whenever possible, one proximal and one distal segment from the same animal were used. The unpaired Student's *t*-test was used to compare proximal and distal colonic segments in baseline conditions. The paired Student's *t*-test and repeated measures ANOVA were used for statistical analysis of drug effects, followed by Dunnett's multiple comparison test in the single agonist or antagonist experiments and by Bonferroni's multiple comparison test after combined NK_3, NK_2 *plus* NK_1 receptor blockade. *P*-values ≤ 0.05 were considered significant.

2.4. Drugs and solutions

The following drugs were used: tetrodotoxin, atropine, hexamethonium (Sigma-Aldrich Ind., St-Louis, USA), septide, RP 67580, [beta-Ala8]-neurokinin A (4–10) (β-A-NKA) (Calbiochem, San Diego, CA, USA), nepadutant (kindly gifted by Menarini, Florence, Italy), senktide (Tocris Bioscience, Bristol, UK) and SR 142801 (kindly gifted by Sanofi-Synthelabo Recherche, Chilly-Mazarin, France). Stock solutions of RP 67580 and SR 142801 were dissolved in 100% DMSO and further diluted in 50% DMSO. All other drugs were dissolved in water. Solutions were injected in the 4 ml organ bath in volumes of 4–8 μl. The final volume of DMSO in the organ bath did not exceed 0.5% and had no effect on peristaltic contractions (data not shown).

3. Results

3.1. Characteristics of murine colonic peristaltic activity

Distension of the proximal and distal colonic segments resulted in repetitive and reproducible pressure waves. In the proximal colon, peristaltic pressure waves had a mean pressure amplitude of 6.6 ± 0.3 cm H_2O and a mean interval of 66 ± 2 s (n = 63). A representative tracing of peristaltic activity in the proximal colon is shown in Fig. 1A. In distal colonic segments, the mean peristaltic pressure amplitude was 7.9 ± 0.5 cm H_2O and the mean interval was 65 ± 3 s (n = 63) (Fig. 1B). The amplitude, but not the interval of the peristaltic pressures waves, was significantly different between proximal and distal segments ($p \leq 0.05$).