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Short communication

5-HT_3 receptor involvement in descending reflex relaxation in the rabbit isolated distal colon

Eliana Messori [a], Stefano M. Candura [b], Teresa Coccini [a], Barbara Balestra [a], Marcello Tonini [b,*]

[a] *Department of Internal Medicine and Therapeutics, Division of Pharmacology and Toxicology, University of Pavia, Piazza Botta 10, I-27100 Pavia, Italy*

[b] *Clinica del Lavoro Foundation, Pavia Medical Centre, Toxicology Unit, Pavia, Italy*

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Abstract

In whole segments of rabbit distal colon with mucosa removed, descending reflex relaxations of the circular muscle (descending inhibition) elicited by inflating (0.1–1 ml) an intraluminal balloon, were partially antagonized by 100 μM hexamethonium and the 5-HT_3 receptor antagonist, ondansetron (3 μM), and abolished by 1 μM tetrodotoxin. The inhibitory effects of hexamethonium and ondansetron were additive. Conversely, hexamethonium (100 μM) and ondansetron (3 μM) failed to reduce electrically induced non-adrenergic non-cholinergic (NANC) relaxations of colonic circular muscle. It is concluded that interneuronally released acetylcholine and 5-hydroxytryptamine (5-HT) activate descending inhibitory pathways supplying the circular coat, via nicotinic and 5-HT_3 receptors, respectively. This evidence suggests a functional involvement of 5-hydroxytryptaminergic transmission in the descending inhibition of rabbit colon.

Keywords: Neuro-neuronal transmission; 5-HT (5-hydroxytryptamine, serotonin); 5-HT_3 receptor antagonism; Descending inhibition

1. Introduction

5-Hydroxytryptamine (5-HT) has been proposed as a transmitter in the enteric nervous system. In the guinea-pig small intestine and colon, 5-HT immunoreactive cells are mainly myenteric interneurons which send their axonal processes in an anal direction to synapse the cell body of other neurons in the myenteric and submucous ganglia (Furness and Costa, 1982; Wardell et al., 1994). Thus, the main physiological role of 5-HT as neuro-neuronal transmitter should be associated, at least in theory, with the function of descending (inhibitory) pathways (Wardell et al., 1994). Of the four neural receptors (5-HT_{1A}, 5-HT_{1P}, 5-HT_3 and 5-HT_4) identified so far in the intestine of mammals, 5-HT_3 receptors are those located in the cell body of S-type enteric neurons (including motor neurons), where they cause fast membrane depolarizations and transmitter release (Tonini et al., 1991). Recently, it has been demonstrated that the hexamethonium (100 μM)-resistant component of fast excitatory postsynaptic potentials in myenteric neurons is mediated by ATP and 5-HT acting at P_2 purinoceptors and 5-HT_3 receptors, respectively (Galligan, 1994). It is likely, therefore, that the latter receptors may represent a primary target for the action of neurally released 5-HT.

In the intestine, the activation of 5-HT_3 receptors is associated with excitatory (Tonini et al., 1991) and inhibitory responses. With regard to the latter responses, 5-HT_3 receptor-mediated neurogenic relaxations have been demonstrated in the isolated ileum of rat (Kanada et al., 1993), in the canine terminal ileum and ileocolonic junction (Boeckxstaens et al., 1990), and in the longitudinal muscle of guinea-pig distal colon (Woollard et al., 1994). In the rat ileum, 5-HT released from interneurons following radial distension of the gut wall participates, via 5-HT_3 receptors, in the descending inhibition since the latter response is sensitive to the 5-HT_3 receptor antagonists, tropisetron and

* Corresponding author. Tel.: 39-382-506363; fax: 39-382-22741.

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MDL 72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate methansulphonate) (Kanada et al., 1993).

This study was designed to investigate whether 5-HT_3 receptors partially mediate the distension-evoked reflex relaxation of the circular muscle in whole segments of rabbit distal colon with mucosa removed, to avoid any interference of non-neural 5-HT (i.e. mucosal 5-HT) possibly released by distension stimuli.

2. Materials and methods

New Zealand White rabbits (S. Morini, S. Polo d'Enza, Italy) of either sex, weighing 2000–2400 g were killed by stunning and bleeding. A 15-cm segment of distal colon was excised with the aboral end cut 1 cm above the pubis symphysis and transferred to a Petri dish containing prewarmed Tyrode solution (composition in mM: NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.04, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5; pH 7.4) to remove the intraluminal content. From this specimen two kinds of preparation were obtained, after the mucosa was peeled off with a wet cotton pad. All preparations were equilibrated for at least 90 min before the experiments were started.

2.1. Evaluation of descending reflex relaxation in the circular muscle

The descending reflex relaxation of the circular muscle, which occurs anally from the site of radial gut wall distension, was studied as described previously (Ciccocioppo et al., 1994). Briefly, a 6 cm-long segment was set up horizontally in a bath containing 100 ml Tyrode solution (bubbled with a mixture of 95% O_2 + 5% CO_2 and maintained at 37°C) with its mesenteric border attached to a rod placed immediately below the organ. Reflex relaxations were elicited by distending an intraluminal stationary rubber balloon with 0.1, 0.2, 0.6 and 1 ml of water. These responses were measured at 2.5 cm aborally from the site of distension and were recorded by connecting the serosa of the preparation's antimesenteric border to an isotonic transducer with a 0.5-g load. Reflex activity was elicited at 5-min intervals by maintaining the balloon distended until peak responses were obtained. All reflex relaxations in the absence and in the presence of drugs (100 μM hexamethonium and 3 μM ondansetron either alone or in combination) were expressed as percentages of the relaxation elicited by 1-ml balloon distension. The above antagonist concentrations were selected as being those generally used to isolate pharmacologically other receptor subtypes from nicotinic (Galligan, 1994) and 5-HT_3 receptors (Elswood et al., 1991; Wardle and Sanger, 1993) in isolated intestinal preparations.

2.2. Electrically induced NANC relaxation in the circular muscle

To study electrically induced non-adrenergic non-cholinergic (NANC) relaxations of the circular muscle in the presence of hyoscine (0.3 μM), propranolol and phentolamine (each at 1 μM), colon segments were set up in a manner similar to that described for recording reflex relaxations. Electrical stimulation was performed using a pair of platinum electrodes placed horizontally (10 mm apart) and parallel to a portion of gut wall, i.e. the site where neurogenic relaxations of the circular muscle were recorded by connecting the antimesenteric border of the preparation to a 0.5 g loaded isotonic transducer. The effects of 100 μM hexamethonium and 3 μM ondansetron, administered either alone or in combination, were evaluated on submaximal neurogenic relaxations evoked every 5 min by electrical stimulation (60 V, 0.5 ms, 1–10 pulses; multiple pulses being delivered at 1-s intervals) after 20-min drug treatment and were expressed as percent changes of the maximal response (10 pulses).

2.3. Drugs

The drugs used in this study were hyoscine hydrobromide, hexamethonium chloride, propranolol hydrochloride, phentolamine hydrochloride, tetrodotoxin (all from Sigma Chemicals, USA), and ondansetron ((±)-1,2,3,9-tetrahydro-3-[(methylimidazol-1-yl)methyl]-9-methyl-4H-carbazol-4-one hydrochloride) (Glaxo, UK). All drugs were dissolved in distilled water.

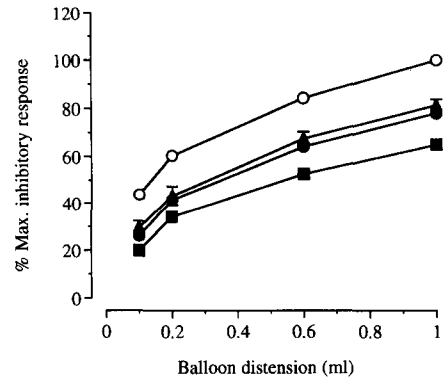

Fig. 1. Distension-evoked descending reflex relaxations of the circular muscle in mucosa-free segments of rabbit distal colon. Control responses (○); responses after treatment with 100 μM hexamethonium (●), 3 μM ondansetron (▲), and a combination of both drugs (■). Values are expressed as percentages of the maximal inhibitory response elicited by 1 ml of balloon distension. All values in the presence of drugs are different from the control values ($P < 0.05$) (analysis of variance). The inhibitory effect produced by hexamethonium and ondansetron in combination was significantly greater than that of hexamethonium or ondansetron alone ($P < 0.05$). Each point represents the mean ± S.E.M. of six experiments.

2.4. Data analysis

The significance of differences between groups was determined by means of Student's t-test or an analysis of variance followed by the Scheffé F-test for multiple comparisons.

3. Results

Inflation of an intraluminal balloon with 0.1–1 ml of water produced a descending reflex relaxation of the circular muscle anally from the site of gut wall distension. This response was unaffected by the concomitant exposure to hyoscine (0.3 μM), phentolamine and propranolol (each at 1 μM) ($n = 4$) and was abolished by 1 μM tetrodotoxin ($n = 4$). The amplitude of reflex relaxations was directly related to the intensity of the stimulus (Fig. 1). Reflex relaxations were partially inhibited by 100 μM hexamethonium and, to a similar extent, by 3 μM ondansetron. Co-incubation with hexamethonium and ondansetron caused a more pronounced inhibitory effect, which was the sum of the effects caused by each individual agent (Fig. 1).

Electrical field stimulation in the presence of muscarinic and α- and β-adrenoceptor blockade caused the circular muscle to relax by a tetrodotoxin-sensitive (1 μM; $n = 4$) mechanism. The amplitude of non-adrenergic non-cholinergic (NANC) relaxations was directly related to the number of pulses (range 1–10). Quantitation of these responses is shown in Fig. 2. Blockade of nicotinic and 5-HT_3 receptors (either alone or in combination) with hexamethonium (100 μM) and ondansetron (3 μM) failed to modify electrically induced NANC relaxations (Fig. 2).

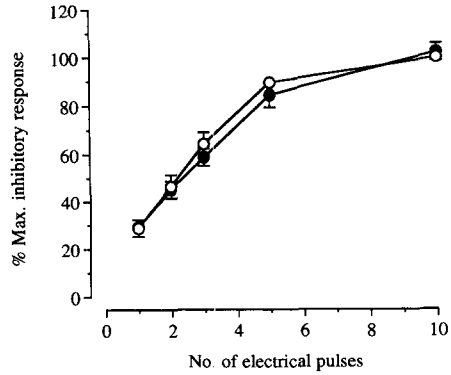

Fig. 2. Electrically induced non-adrenergic non-cholinergic (NANC) relaxations of the circular muscle in mucosa-free segments of rabbit distal colon. Control responses (○); responses in the presence of 100 μM hexamethonium and 3 μM ondansetron (●). For clarity, the curves in the presence of hexamethonium and ondansetron alone are not illustrated. Each point represents the mean ± S.E.M. of six experiments.

4. Discussion

The use of hexamethonium and the selective 5-HT_3 receptor antagonist, ondansetron, allowed us to establish that nicotinic and 5-HT_3 receptors partially mediate a distension-evoked descending inhibition in the rabbit isolated distal colon. This evidence supports the notion that 5-HT is a transmitter in the enteric nervous system of this species, where, as observed in the intestine of guinea-pig (Furness and Costa, 1982; Wardell et al., 1994) and rat (Kanada et al., 1993), it is likely to be contained in and released from descending interneurons, which synapse inhibitory pathways supplying the circular muscle. However, the finding that more than 50% descending inhibition still occurred after nicotinic (Smith et al., 1990) and 5-HT_3 receptor blockade suggests that transmitters other than acetylcholine and 5-HT mainly contribute to neuro-neuronal transmission in descending pathways. Like nicotinic receptors, 5-HT_3 receptors should be located at the ganglionic level, since NANC relaxations in response to electrical field stimulation were unaffected by both nicotinic and 5-HT_3 receptor blockade. In a previous study, descending inhibition in the rabbit colon was found to be mediated in part by nitric oxide and, mainly, by transmitter(s) sensitive to apamin (Ciccocioppo et al., 1994).

To date, there is little information about the role of 5-HT_3 receptors in the motor function of the gastrointestinal tract. Although there is evidence for the involvement of 5-HT_3 receptors in the initiation of migrating motor complexes in the canine and human (Wilmer et al., 1993) gastrointestinal tract, these receptors do not seem to play a major functional role in another coordinated motor event, such as intestinal peristalsis in vitro. In fact, tropisetron, ondansetron and granisetron were repeatedly found ineffective on peristaltic activity in the isolated guinea-pig ileum (Craig and Clarke, 1991; Buchheit and Buhl, 1993).

In the colon, however, the suppression of 5-HT_3 receptor function by tropisetron, granisetron and ondansetron is associated with a reduction of faecal pellet transit in rodents (Sanger et al., 1991). Peristalsis experiments using isolated colonic segments are required to establish whether the disruption of the descending inhibition caused by 5-HT_3 receptor antagonists may explain, at least in part, their slowing effect on pellet propulsion.

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