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SPECIAL REPORT

The influence of neuronal 5-hydroxytryptamine receptor antagonists on non-cholinergic ganglionic transmission in the guinea-pig enteric excitatory reflex

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A partitioned bath made it possible to separate the site of recording of the ascending excitatory reflex of the ileal circular muscle (oral compartment) from the site of reflex induction (caudal compartment), evoked by inflating an intraluminal balloon. In the caudal compartment, blockade of cholinergic ganglionic transmission by hexamethonium (100 μM) and hyoscine (0.3 μM) caused an approximately 65% reduction in the amplitude of reflex contractions, suggesting that the remaining response was mediated by non-cholinergic transmission near the distension site. This non-cholinergic component of ganglionic transmission was insensitive to the action of methiothepin (1 μM), ondansetron (1 μM), tropisetron (1.5 μM), DAU 6285 (1 μM) and renzapride (1 μM), agents that antagonize the action of 5-hydroxytryptamine (5-HT) at neural 5-HT_1-like, 5-HT_3, 5-HT_4 and putative 5-HT_{1P} receptors. These findings suggest that the neural pathways subserving non-cholinergic ganglionic transmission in the ascending excitatory reflex in the guinea-pig ileum do not involve 5-HT as neurotransmitter.

Keywords: Isolated small intestine; ascending excitatory reflex; non-cholinergic ganglionic transmission; 5-hydroxytryptamine (5-HT); 5-HT receptor antagonists

Introduction The ascending excitatory reflex is a neurogenic orally-directed motor response of the circular musculature which is triggered by gut wall distension. In the guinea-pig ileum, both cholinergic (nicotinic and muscarinic) and non-cholinergic transmission are involved in this reflex (Holzer, 1989; Tonini & Costa, 1990). Evidence that 5-hydroxytryptamine (5-HT) or substance P might be the transmitter mediating non-cholinergic transmission between myenteric neurones has previously raised considerable debate (see Furness & Costa, 1987). Although substance P is currently considered to be the most likely transmitter candidate (Furness & Costa, 1987), an equivalent role for 5-HT has not yet been ruled out. In the guinea-pig ileum, in fact, 5-HT causes fast and slow synaptic excitation through activation of 5-HT_3 (Surprenant & Crist, 1988; Mawe *et al.*, 1989) and putative 5-HT_{1P} receptors (Mawe *et al.*, 1989), respectively. Recently, other 5-HT receptor subtypes have been characterized in myenteric neurones. These include excitatory 5-HT_4 receptors which cause prejunctional and presynaptic acetylcholine release (for review see Tonini *et al.*, 1991), and inhibitory 5-HT_1-like receptors (Galligan *et al.*, 1988).

This study was designed to assess whether the 5-HT receptor antagonists methiothepin (5-HT_1-like), ondansetron (5-HT_3), tropisetron (5-HT_3/5-HT_4), DAU 6285 (5-HT_4) (Peroutka, 1990; Dumuis *et al.*, 1992) and the prokinetic agent renzapride, which was shown to antagonize putative 5-HT_{1P} receptors (Mawe *et al.*, 1989), affect the non-cholinergic component of ganglionic transmission in the ascending excitatory reflex in the guinea-pig ileum.

Methods The experimental model was similar to that described in detail previously (Tonini & Costa, 1990). Guinea-pigs of either sex (300–350 g) were stunned and bled. Seven cm long segments of ileum were excised about 20 cm from the ileocaecal junction and set up horizontally in a three-compartment bath containing standard Tyrode solution maintained at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. In this bath, a small intermediate compartment made it possible to separate the site of recording of the ascending excitatory reflex (oral compartment) from the site of reflex induction (caudal compartment). Reflex contractions of the circular coat were recorded isometrically (tension: 5 mN). Inflation of an intraluminal rubber balloon with 0.3 ml of water was used as a gut distending stimulus to evoke reflex responses at 2 min intervals. Drugs were applied in the caudal compartment only. The non-cholinergic component of ganglionic transmission in this compartment was revealed by concomitant administration of 100 μM hexamethonium and 0.3 μM hyoscine (Tonini & Costa, 1990). 5-HT receptor antagonists were added to the compartment in the presence of cholinergic transmission blockade, and reflex responses were followed for 20 min. Reflex contractions elicited in the presence of hexamethonium and hyoscine with and without 5-HT receptor antagonists were expressed as percentages of the response obtained in the absence of any drug treatment.

All data are expressed as mean ± s.e.mean. The significance of difference between mean values was estimated by one-way analysis of variance with Scheffé F-test for multiple comparisons. P values of 0.05 or less were considered significant.

The following drugs were used: hyoscine hydrochloride (Sigma), hexamethonium bromide (Sigma), tetrodotoxin (Sankyo), dimethyl-phenyl-piperazinium iodide (DMPP) (Fluka), methiothepin maleate (Roche), tropisetron (ICS 205-930) (RBI), ondansetron (GR 38032F) (Glaxo), DAU 6285 (endo-6-methoxy-8-methyl-8-azabicyclo [3.2.1.] oct-3-yl-2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylate hydrochloride) (Boehringer Ingelheim, Italy) and renzapride (BRL 24924) (Beecham Pharmaceuticals).

Results Distension of the gut wall in the caudal compartment by inflation of an intraluminal balloon with 0.3 ml of water, elicited a reflex monophasic contraction of the circular

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muscle in the oral compartment. As reported previously (Tonini & Costa, 1990), the concomitant addition of hexamethonium (100 μM) and hyoscine (0.3 μM) to the caudal compartment reduced the amplitude of reflex contractions to 37.7 ± 5.5% ($n = 8$, $P < 0.01$) and tetrodotoxin (0.6 μM, $n = 6$) caused their disappearance (Figure 1a). In untreated preparations, 20 μM DMPP applied to the caudal compartment caused a contractile response, the amplitude of which was 84.3 ± 6.5% ($n = 8$) of the reflex responses (Figure 1b). These DMPP-induced contractions were abolished by 100 μM hexamethonium ($n = 8$) (Figure 1c), indicating that the latter drug was able to suppress completely responses to nicotinic receptor activation comparable in amplitude to those evoked by gut distension. In the presence of hexamethonium and hyoscine, blockade of 5-HT_1-like receptors by 1 μM methiothepin ($n = 8$), 5-HT_3 receptors by 1 μM ondansetron ($n = 7$), 5-HT_3 and 5-HT_4 receptors by micromolar concentrations of tropisetron (1.5 μM, $n = 7$), 5-HT_4 by 1 μM DAU 6285 ($n = 8$) and putative 5-HT_{1P} receptors by 1 μM renzapride ($n = 10$), did not affect the amplitude of reflex responses (Figure 1d).

Discussion The ascending excitatory reflex response of the circular muscle in the guinea-pig ileum involves both cholinergic and substance P-mediated non-cholinergic transmission at both the neuro-neuronal and neuromuscular level (Holzer, 1989; Tonini & Costa, 1990). Nevertheless, the possibility that 5-HT might possess a transmitter role at the ganglionic level (Mawe *et al.*, 1989) had not yet been adequately explored in functional motility experiments.

Based on present findings, the failure of a variety of antagonists of neural 5-HT receptors to antagonize the non-cholinergic component of ganglionic transmission in the ascending excitatory reflex, argues against 5-HT subserving this type of transmission. In fact, if 5-HT is released by balloon distension into the caudal compartment (i.e. the site where, under our experimental conditions, non-cholinergic ganglionic transmission occurs), it could act at both excitatory (5-HT_3, 5-HT_4 and putative 5-HT_{1P}) or inhibitory (5-HT_1-like) receptors (Tonini *et al.*, 1991; Mawe *et al.*, 1989; Galligan *et al.*, 1988). The inefficiency of ondansetron, tropisetron and DAU 6285 in modifying reflex contractions, excludes an involvement of 5-HT_3 or 5-HT_4 receptors on distension-induced non-cholinergic transmission in the caudal compartment. In agreement with our results, tropisetron at micromolar concentrations was previously found not to affect the amplitude of ascending contractions of the circular muscle to transmural electrical stimulation in the guinea-pig ileum (Jin *et al.*, 1989). In our experiments, even renzapride at 1 μM concentration did not affect the amplitude of reflex contractions. In myenteric neurones this compound at the same concentration was found to abolish 5-HT-mediated slow depolarizations and slow e.p.s.ps. through antagonism

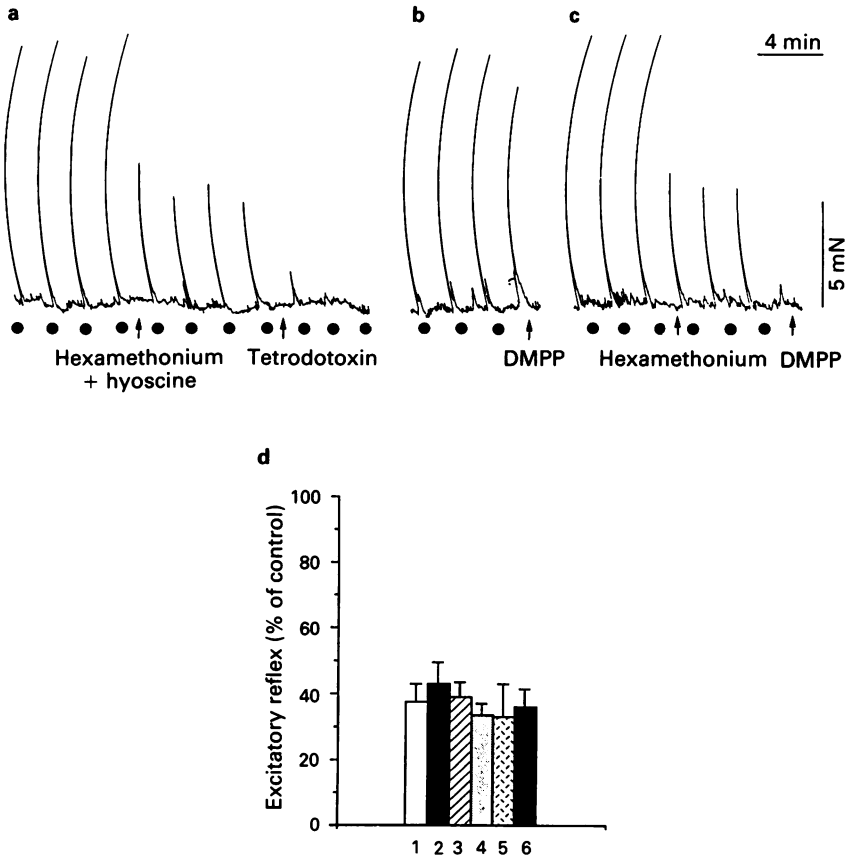

Figure 1 Ascending reflex contractions recorded by a technique which made it possible to separate the site of reflex recording (oral compartment) from the site of reflex induction and drug administration (caudal compartment). In (a) a combination of hexamethonium (100 μM) and hyoscine (0.3 μM) reduced to approximately 35% the amplitude of reflex contractions (dots), which were subsequently abolished by tetrodotoxin (0.6 μM). In (b) dimethylphenylpiperazinium iodide (DMPP, 20 μM) caused the appearance in the oral compartment of contractile responses the amplitude of which was 85% of reflex responses (dots). In (c) administration of hexamethonium (100 μM) depressed by 60% the amplitude of reflex responses (dots), and abolished DMPP-induced contractions. In (d) reflex contractions in the presence of hexamethonium (100 μM) and hyoscine (0.3 μM) before (1) ($n = 8$) and after treatment with (2) methiothepin (1 μM, $n = 8$); (3) ondansetron (1 μM, $n = 7$); (4) tropisetron (1.5 μM, $n = 7$); (5) DAU 6285 (1 μM, $n = 8$); (6) renzapride (1 μM, $n = 10$). Each group of reflex contractions was significantly lower ($P < 0.01$) than those observed in controls. The amplitude of reflex contractions in the presence of hexamethonium and hyoscine plus each of the five 5-HT receptor antagonists was not different from that recorded with hexamethonium and hyoscine alone.

at putative 5-HT_{1P} receptors (Mawe *et al.*, 1989). The latter finding suggests that 5-HT does not mediate non-cholinergic orally-directed slow excitatory ganglionic transmission in the guinea-pig ileum. Inhibitory 5-HT_1-like receptors (probably the 5-HT_{1A} subtype) are widely distributed in the myenteric plexus, in which they reduce neuronal excitability leading to depression of cholinergic and non-cholinergic synaptic transmission (Galligan *et al.*, 1988). The fact that in the presence of methiothepin the amplitude of reflex activity was unaffected further suggests that the nerve pathways subserving the ascending excitatory reflex do not release 5-HT following a physiological stimulus like radial distension of the gut wall.

References

DUMUIS, A., GOZLAN, H., SEBBEN, M., ANSANAY, H., RIZZI, C.A., TURCONI, M., MONFERINI, E., GIRALDO, E., SCHIANTARELLI, P., LADINSKY, H. & BOCKAERT, J. (1992). Characterization of a novel 5-HT_4 receptors antagonist of the azabicycloalkyl benzimidazolone class: DAU 6285. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **345**, 264–269.

FURNESS, J.B. & COSTA, M. (1987). *The Enteric Nervous System.* Edinburgh: Churchill Livingstone.

GALLIGAN, J.J., SURPRENANT, A., TONINI, M. & NORTH, R.A. (1988). Differential localizatioin of 5-HT_1 receptors in myenteric and submucosal neurons. *Am. J. Physiol.*, **255**, G603–G611.

HOLZER, P. (1989). Ascending enteric reflex: multiple neurotransmitter systems and interactions. *Am. J. Physiol.*, **19**, G540–G545.

JIN, J.-G., NEYA, T. & NAKAYAMA, S. (1989). Myenteric 5-HT-containing neurones activate the descending cholinergic excitatory pathway to the circular muscle of guinea-pig ileum. *Br. J. Pharmacol.*, **98**, 982–988.

MAWE, G.M., BRANCHEK, T.A. & GERSHON, M.D. (1989). Blockade of 5-HT-mediated enteric slow EPSPs by BRL 24924: gastrokinetic effects. *Am. J. Physiol.*, **257**, G386–G396.

PEROUTKA, S.J. (1990). 5-Hydroxytryptamine receptor subtypes. *Pharmacol. Toxicol.*, **67**, 373–383.

SURPRENANT, A. & CRIST, J. (1988). Electrophysiological characterization of functionally distinct 5-hydroxytryptamine receptors on guinea-pig submucous plexus. *Neuroscience*, **24**, 283–295.

TONINI, M. & COSTA, M. (1990). A pharmacological analysis of the neuronal circuitry involved in distension-evoked enteric excitatory reflex. *Neuroscience*, **38**, 787–795.

TONINI, M., RIZZI, C.A., MANZO, L. & ONORI, L. (1991). Novel enteric 5-HT_4 receptors and gastrointestinal prokinetic action. *Pharmacol. Res.*, **24**, 5–14.

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