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Short communication

Response heterogeneity of 5-HT_3 receptor antagonists in a rat visceral hypersensitivity model

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Abstract

Subcutaneous administration of granisetron (BRL 43694, *endo*-1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1.]non-3-yl-1*H*-indazole-3-carboxamide) and zacopride (4-amino-*N*-(1-azabicyclo[2.2.2.]oct-3-yl)-5-chloro-2-methoxybenzamide), two 5-HT_3 receptor antagonists, at doses ranging from 3 to 1000 μg/kg, inhibited abdominal contractions induced by distension (30 mmHg, 10 min) of irritated colon (0.6% acetic acid) in conscious rats with a bell-shaped dose-response curve. The ED_{50} of granisetron and zacopride were 17.6 and 8.2 μg/kg, respectively. In contrast, both tropisetron (ICS 205-930, (3-a-tropanyl)l-indole-3-carboxylic ester) and ondansetron (GR38032F, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one hydrocloride dihydrate) were inactive in this model. These data further support the concept of a heterogeneity in the potency of 5-HT_3 receptor antagonists in modulating visceral hypersensitivity in conscious rats. This finding is in agreement with a reported efficacy of granisetron but not of ondansetron in patients with irritable bowel syndrome.

Keywords: 5-HT_3 receptor antagonist; Visceral hypersensitivity; Colonic distension; (Rat)

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) plays an important role in the regulation of gastrointestinal functions as well as in pain modulation and inflammatory processing (Kilpatrick et al., 1990). Among the 5-HT receptor subtypes, 5-HT_3 receptors have been implicated in visceral nociception pathways both in animals and humans (Talley, 1992), and have received increased attention related to pathologies involving abnormal visceral perception.

However, 5-HT_3 receptor antagonists demonstrate a wide heterogeneity of potency and efficacy against visceral pain. Granisetron (Prior and Read, 1993) but not ondansetron (Hammer et al., 1993) is able to reduce rectal sensitivity in patients with irritable bowel syndrome. In conscious rats granisetron, zatosetron, bemesetron, renzapride and metoclopramide increase the threshold values to colo-rectal distension after 5-hydroxytryptophan (5-HTP)-induced colonic hypersensitivity, while tropisetron and ondansetron display no significant such effect (Banner and Sanger, 1995). Similarly, granisetron and tropisetron but not ondansetron reduce depressor response to duodenal distension in anaesthetized rats (Moss and Sanger, 1990).

We have developed a model of colonic hypersensitivity to ballon distension in conscious rats involving a colonic irritation procedure (0.6% acetic acid intracolonically) which turn a low painful colonic distension into a high painful stimulus without significant tissue injury. In this experimental model, μ- and κ-opioid receptor agonists were able to restore normal colonic sensitivity to balloon distension, and these effects were blocked with selective antagonists at μ- and κ-sites, respectively (Langlois et al., 1994).

In the present study we have compared the effect of various 5-HT_3 receptor antagonists, granisetron, zacopride, ondansetron and tropisetron, and provided further evidence

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of the heterogeneity of this class of compounds, regarding their ability to modulate visceral hypersensitivity.

2. Materials and methods

Male Sprague-Dawley rats (Iffa Credo, Les Oncins, France) weighing 300–350 g were used. After an overnight fast, each animal was placed in a transparent plastic cage lined up with sawdust and allowed 45 min to get used to its surrondings. Procedures for the maintenance and use of the experimental animals were carried out in accordance with the guidelines of the International Association for the Study of Pain.

The visceral stimulus employed in all experiments was a distension of the descending colon by inflation of a 5-cm-long latex balloon inserted via the anal route and kept in place by taping the polyethylene tube holding the ballon to the base of the tail, in order to ensure that the tip of the ballon remained 10 cm from the anal verge. In all experiments, pressure within the balloon was continuously monitored by a pressure transducer (Bioblock, Illkirch, France) and care was taken to apply a constant pressure distension.

1.5 ml of 0.6% acetic acid was injected intracolonically through a small catheter mounted along the ballon assembly and 1 h later, a first period of 30 mmHg distension was applied for 10 min (control period). This distension period was followed by subcutaneous injection of vehicle or drugs, and 20 min later a second period of distension (30 mmHg for 10 min) was again applied (treatment period). Pain was scored by visual counting of abdominal contractions over the two 10-min distension periods. Drugs were given as follows: granisetron (BRL 43694, endo-1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1.]non-3-yl-1*H*-indazole-3-carboxamide) and zacopride (4-amino-*N*-(1-azabicyclo[2.2.2.]oct-3-yl)-5-chloro-2-methobenzamide) at doses ranging from 3 to 1000 μg/kg s.c.; tropisetron (ICS 205-930, (3-α-tropanyl)l-indole-3-carboxylic ester) and ondansetron (GR38032F, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one hydrochloride dihydrate) at doses ranging from 10 to 100 μg/kg s.c. Granisetron, ondansetron and zacopride were dissolved in distilled water. Tropisetron was dissolved in distilled water with 0.2% HCl, 1 M. All drugs were administered in a volume of 1 ml/kg body weigth. Granisetron, ondansetron and zacopride were synthetized at Jouveinal's Department of Medicinal Chemistry. Tropisetron was obtained from RBI (Bioblock, Illkirch, France).

Results are expressed as means ± S.E.M. Statistical significance between control and treatment periods was assessed using the Wilcoxon test. Differences were considered statistically significant at $P < 0.05$. The antinociceptive effect of 5-HT_3 receptor antagonists was expressed by the following equation: % antinociception = $100 \times [1 -$ (AC after treatment/AC before treatment)] (AC = cumulative abdominal contraction). The ED_{50} was calculated using the method of Litchfield and Wilcoxon (1949).

3. Results

After intracolonic administration of saline, the applied distension procedure (30 mmHg, 10 min) induced a small number of abdominal contractions, which remained less than five (cumulative response during the distension period: 4.8 ± 1.4 abdominal contractions). In contrast, the same stimulus resulted in a dramatic increase in the pain score (cumulative response during the distension period: 23.4 ± 4.1 abdominal contractions, $P < 0.001$ vs. saline) 1 h after intracolonic administration of 0.6% acetic acid. This response was stable and reproducible as shown by the number of abdominal contractions obtained during the second period of distension applied 20 min later (22.9 ± 3.4 abdominal contractions). In both groups, abdominal contractions totally disappeared at the end of distension period.

Subcutaneous injection of granisetron significantly inhibited abdominal contractions induced by colonic distension, with a bell-shaped dose-response curve (Fig. 1) as also reported from several other studies with 5-HT_3 receptor antagonists (Moss and Sanger, 1990; Banner and Sanger, 1995). As shown in Fig. 1, 81.5 ± 4.6% of antinociception was obtained at 100 μg/kg and further increase in the dose of granisetron produced a lower degree of antinociception. The ED_{50} of granisetron was 17.6 μg/kg s.c. (95% confidence intervals: 6.5–47.4). Zacopride also significantly inhibited abdominal contractions with a bell-shaped dose-response curve (Fig. 1). A maximal antinociceptive effect (84.3 ± 5.6% of antinociception) was achieved with 30 μg/kg and the ED_{50} of zacopride was 8.2 μg/kg s.c. (95% confidence intervals: 3.3–20.1).

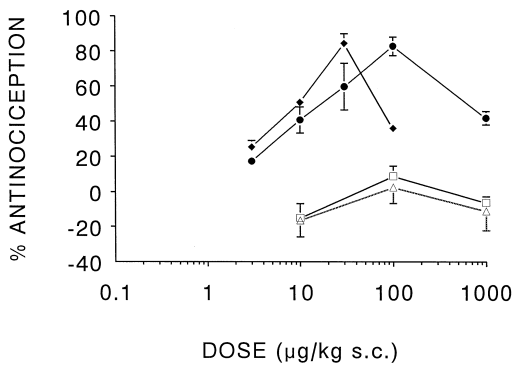

Fig. 1. Dose-response relationships of antinociceptive actions produced by s.c. administration of granisetron (●), tropisetron (△), ondansetron (□) and zacopride (♦) on abdominal contractions during colonic distension (30 mmHg, 10 min) in acetic acid-treated rats. Results are expressed as means ± S.E.M of 5–8 animals per dose.

Tropisetron and ondansetron, on the other hand, did not produce any significant inhibition of the abdominal contractions at 10, 100 or 1000 μg/kg s.c. (Fig. 1).

None of the drugs tested in this study significantly modified the volume of air required to obtain a constant distension pressure (data not shown), indicating that the compliance of the colon was not altered and thus did not contribute to the antinociceptive effect of the drug tested.

4. Discussion

It has previously been demonstrated that abdominal electromyographic recordings and visual evaluation of abdominal muscle contractions produce similar pain scores (Ness and Gebhart, 1988). In the present model of visceral hypersensitivity induced by intracolonic administration of 0.6% acetic acid, granisetron and zacopride were able to reduce colonic hypersensitivity while ondansetron and tropisetron were ineffective. These results are in good agreement with previously reported animal and human data. Banner and Sanger (1995) reported that granisetron reverses 5-HTP-induced colonic hypersensitivity to distension while both tropisetron and ondansetron were inactive. Similarly, granisetron (Prior and Read, 1993) but not ondansetron (Hammer et al., 1993) reduced rectal sensitivity in patients with irritable bowel syndrome.

The present data, which are in agreement with previous observations in pain models, cannot be explained by differential affinities or potencies of the tested compounds at 5-HT_3 receptors. It was interesting to note that granisetron and zacopride were active in this model, while tropisetron and ondansetron were not. The four compounds had similar affinities to 5-HT_3 sites in rat endothelial cortex, and similar potencies in a test of antiemetic properties in the ferret (for review, see Kilpatrick et al., 1990). Furthermore, neither a possible interaction with 5-HT_4 receptors may explain the heterogeneity in their antinociceptive response. Indeed, in mouse colliculi neurons, zacopride had 5-HT_4 receptor agonist activity while tropisetron had 5-HT_4 receptor antagonist activity and granisetron and ondansetron were inactive (Costall and Naylor, 1990). It is possible that the observed differences in activity of the 5-HT_3 receptor antagonists might have been partly due to different rates of absorption from the injection site and diffusion to the site of action. However, tropisetron and ondansetron, which were inactive in our study, had been demonstrated to be active 20 min after subcutaneous injection in a somatic pain model (Eschalier et al., 1989).

The existence of 5-HT_3 receptor subtypes has been proposed from pharmacological studies but has not yet been confirmed by molecular biology experiments. There is pharmacological evidence for species variations in the ligand affinity for 5-HT_3 receptors (for review see Hoyer et al., 1994), and differences in the response to 5-HT_3 receptor antagonists within the same species have been reported. Nanomolar concentrations of granisetron, tropisetron and MDL72222 decreased 5-HT release in enterochromaffin cells from guinea-pig isolated small intestine while ondansetron did not modify 5-HT release (Gebauer et al., 1993). Bonhaus et al. (1993) reported that there was a difference between the affinities of ligands for 5-HT_3 recognition sites in the ileum and cerebral cortex of CD-1 mice. Furthermore, it has been demonstrated that granisetron (Rowat et al., 1991) but not tropisetron (Ferrari et al., 1991) had an effect in the treatment of migraine attacks. As an alternative to an hypothesis based on different isoforms of 5-HT_3 receptors, one may not exclude an action outside the serotoninergic system. Whatever the specific mechanism, the present finding, together with several previous studies, indicate a putative therapeutic interest of some but not all 5-HT_3 receptor antagonists in pathologies involving abnormal visceral pain perception.

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