J. Pharm. Pharmacol. 1993, 45: 521–524
Received May 14, 1992

Granisetron and Ondansetron: Effects on the Ileal Brake Mechanism in the Rat

N. J. BROWN*, A. HORTON, R. D. E. RUMSEY AND N. W. READ

Department of Biomedical Science, The University, Sheffield S10 2TN, UK

Abstract—Studies were carried out on 20 male adult rats to investigate how the action of the selective 5-HT_3-receptor antagonists, granisetron and ondansetron, influence gastrointestinal transit under control conditions and when stomach-to-caecum transit was delayed by ileal infusion of lipid. Stomach-to-caecum transit time (SCTT) was measured using environmental hydrogen analysis. Subcutaneous administration of granisetron (BRL 43694, 40, 80 or 150 $\mu g\ kg^{-1}$) significantly delayed the passage of the head of the baked bean meal through the stomach and the small intestine under control conditions ($P < 0.05$). Similarly, subcutaneous administration of ondansetron (GR 38032F, 80 or 150 $\mu g\ kg^{-1}$) delayed control SCTT of the head of the meal but this did not reach statistical significance. In contrast, granisetron significantly reversed the delay in SCTT induced by ileal infusion of lipid at 40 ($P < 0.001$), 80 ($P < 0.01$) and 150 $\mu g\ kg^{-1}$ ($P < 0.05$). Ondansetron also reversed the lipid-induced delay at 40 ($P < 0.01$), 80 ($P < 0.001$) and 150 $\mu g\ kg^{-1}$ ($P < 0.001$). These apparently conflicting results may be rationalized by postulating the presence of 5-HT_3 receptors on afferent nerves which, when inhibited by the specific antagonists, initiate reflexes that both accelerate and delay transit.

Many experimental and clinical observations have suggested that 5-hydroxytryptamine (5-HT) plays an important role in mediating intestinal responses to chemical and mechanical stimuli. Over 90% of the total 5-HT in the body is contained in enterochromaffin cells of the gastrointestinal mucosa which are closely associated with afferent nerve terminals (Andrews & Hawthorn 1988). Stimulation of 5-HT_3 receptors with the agonist 2-methyl-5-HT leads to the release of substance P, acetylcholine and noradrenaline from enteric neurones (Richardson et al 1985). In-vitro pharmacological preparations have shown 5-HT_3 receptors in the peripheral nervous system located on cholinergic, sympathetic and vagal fibres (Watling 1989) and binding studies have demonstrated 5-HT_3 receptors in both the peripheral and central nervous sytems (Watling 1989).

Blockade of 5-HT_3 receptors using both of the specific antagonists granisetron and ondansetron, has shown a reduction in the emetic responses to cisplatin and radiation (Hawthorn et al 1988; Falkson et al 1990; Khojasteh et al 1990). Granisetron decreases the sensitivity of the rectum to distension in patients with irritable bowel syndrome (Prior & Read 1990) and both antagonists reverse the delay in gastric emptying induced by the presence of lipid in the proximal small intestine (Gamse 1989; Buchheit et al 1989). These observations have led to the suggestion that 5-HT_3 receptors may be present on afferent neurones.

We have already demonstrated that inhibition of 5-HT_3 receptors with granisetron (40 $\mu g\ kg^{-1}$) delays stomach-to-caecum transit of the head of a meal under control conditions but reverses the lipid-induced delay in stomach-to-caecum transit (Brown et al 1991). The aim of the present series of studies was to investigate whether granisetron has a dose-dependent effect on gastrointestinal transit and to determine whether the effects on gastrointestinal transit are drug-specific effects or 5-HT_3-specific effects, by investigating the intestinal response to another 5-HT_3-receptor antagonist ondansetron, under both control and lipid-delayed stomach-to-caecum transit.

* Present address and correspondence: N. J. Brown, University Surgical Unit, Floor K, Royal Hallamshire Hospital, The University, Sheffield S10 2JF, UK.

Materials and Methods

Animals

Experiments were carried out on a total of 20 adult male albino rats, 250–300 g, obtained from Sheffield Field Laboratories. The rats were equipped with a chronic indwelling cannula and housed singly in cages. The animals were deprived of food (Diet 86, Oxoid, London, UK) 18 h before the experiment but water was freely available. Animals were allowed at least one week post-operative recovery before any experiments were carried out.

Drugs

The drugs used in this study were granisetron (BRL 43694) obtained from Beechams Pharmaceuticals, ondansetron (GR 38032F) obtained from Glaxo Pharmaceuticals and 20% Intralipid (KabiVitrum, Uxbridge, UK), a triglyceride emulsion. The dose range used was similar to that used in-vivo in the rat by subcutaneous injection to inhibit the Bezold-Jarisch reflex, suggesting blockade of 5-HT_3 receptors (Butler et al 1988).

Preparation of the test meal

'Californian' white beans (H. J. Heinz Co. Ltd, Hayes, Middlesex, UK) were washed to remove the tomato sauce and homogenized with a little water. Lactose (May & Baker Ltd, Dagenham, UK) was added to produce a concentration of 10% w/v.

Surgical procedure

A plastic cannula (Silastic i.d. 0·02 in, o.d. 0·037 in, Dow Corning Corp., Medical Products, Midland, MI, USA), 25 cm

in length was implanted in the ileum of animals under sodium pentobarbitone anaesthesia (Sagatal 60 mg kg^{-1}; May & Baker Ltd, Dagenham, UK). The abdomen was opened via a midline incision and the cannula placed in the ileal lumen approximately 20 cm proximal to the ileo–caecal junction. The intestinal wound was closed using a purse-string suture around the cannula making sure the lumen of the cannula was not occluded. Sufficient cannula was left free in the abdominal cavity to allow the gastrointestinal tract full mobility. The intestinal incision was closed in two separate layers, the muscle and then the skin using a sterile braided silk suture 5-0 (Mersilk, Ethicon, Edinburgh, UK). Two to three centimetres from the end of the cannula a small square of nylon mesh was secured using silicone glue (Medical Adhesive Type A, Dow Corning Co., Medical Products, Midland, MI, USA). The cannula was tunnelled subcutaneously from an abdominal stab wound to the midscapular region where it was exteriorized via a cutaneous puncture wound, the piece of nylon mesh lying under the skin forming an anchorage point. The cannula was secured in position as the damaged tissues under the skin regenerated, forming a platform over the mesh. In the meantime, three stitches were used to secure the cannula and the exposed end was covered with a blunt-ended pin. Each rat was allowed a post-operative recovery period of one week before any experimental procedures were performed. Every day a small volume of saline (0·3 mL) was infused into the ileum of the rat to ensure the cannula remained patent.

Experimental protocol

The 20 animals were divided into two groups, one group used to test granisetron and the other to test ondansetron. Two control procedures were performed on all animals; a subcutaneous saline injection with ileal saline infusion, and subcutaneous saline injection with ileal Intralipid (20%) infusion. Each rat was then injected with each dose of the drug (20–150 $\mu g\ kg^{-1}$) on separate days, each dose requiring two further experiments: a subcutaneous drug injection with ileal saline infusion and subcutaneous drug injection with ileal Intralipid infusion. Control and test procedures were randomized. The two 5-HT_3 antagonists or 0·9% NaCl (saline) were injected subcutaneously in a volume of 0·25 mL saline, 30 min before the ileal infusion of either saline or Intralipid.

The effect of each of these combinations on the passage of the head of a bean meal through the stomach and the small intestine was investigated using the environmental hydrogen technique (Brown et al 1987).

After starvation for 18 h, rats were placed in Bollman restraining cages and injected subcutaneously with either placebo or active drug. Thirty minutes later, rats were connected to the infusion pump (Braun, Germany) by a metal connector and plastic tubing. Solutions were placed directly into the ileum via the cannula at a rate of 0·3 mL h^{-1} for 30 min. Then the rats were gavaged with 5 mL of the bean/lactose test meal and placed in the perspex chambers. The infusion tube was attached to a pulley system allowing the animal free movement within the chamber. The infusion continued for another 165 min after gavage. The perspex chambers provided a controlled environment from which the hydrogen concentrations could be monitored easily and solutions infused into the animals without causing them any disturbance. An explanation of how the monitoring of the breath hydrogen is controlled and recorded is described in a previous publication (Brown et al 1987).

Stomach-to-caecum transit time (SCTT) of the head of the bean/lactose test meal was defined as the time taken from gavage, to an increase in the hydrogen concentration in the rats environment of 2 ppm sustained for at least three consecutive readings (Brown et al 1987) which was assumed to occur when the unabsorbable carbohydrate component of the meal reached the caecum and was fermented by the caecal bacteria in the rats (Brown et al 1987). This is analogous to the human situation, where the unabsorbable carbohydrate is fermented by the colonic bacteria (Bond & Levitt 1975; Read et al 1980), since the caecum is redundant in terms of fermentation in man.

Statistical analysis

The differences in transit times produced during the different experimental conditions was assessed using Student's *t*-test, a Wilcoxon test for nonparametric data and a one-way analysis of variance.

Results

Control SCTT

The 5-HT_3-receptor antagonist granisetron significantly slowed the SCTT of the head of the baked bean meal during ileal infusion of saline at 40 ($P < 0.05$), 80 ($P < 0.05$) and 150 ($P < 0.001$) $\mu g\ kg^{-1}$ when compared with the control injection of saline (Fig. 1). The optimum dose for the greatest delay in SCTT was 80 $\mu g\ kg^{-1}$, the higher dose being no more effective in delaying SCTT. The ID50 was 40 $\mu g\ kg^{-1}$. The 5-HT_3-receptor antagonist ondansetron had no significant effect on control SCTT although at the higher doses (80 and 150 $\mu g\ kg^{-1}$) there was a trend towards a delay in SCTT of the head of the meal.

Lipid-delayed SCTT

Ileal infusion of Intralipid significantly delayed SCTT of the head of the meal when compared with control saline infusion (Fig. 2). Granisetron administered at 40, 80 or 150 $\mu g\ kg^{-1}$ significantly reversed the Intralipid-induced delay in SCTT

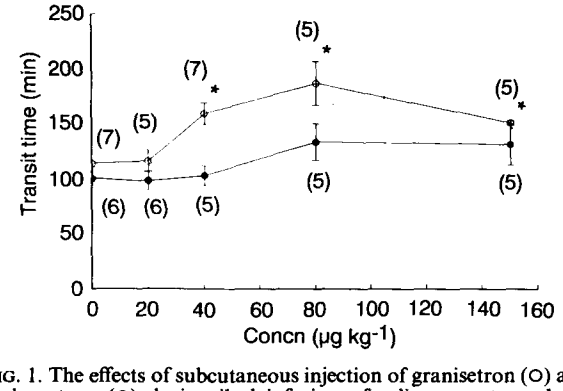

FIG. 1. The effects of subcutaneous injection of granisetron (○) and ondansetron (●) during ileal infusion of saline, on stomach-to-caecum transit time of the head of a baked bean meal. Values are mean ± s.e.m., n values are shown in parenthesis. *$P < 0.05$ compared with control.

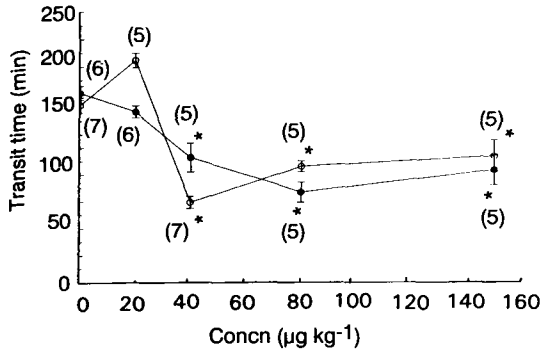

FIG. 2. The effects of subcutaneous injection of granisetron (○) and ondansetron (●) during ileal infusion of lipid, on stomach-to-caecum transit time of the head of a baked bean meal. Values are mean ± s.e.m., n values are shown in parenthesis. $*P<0.05$ compared with control.

of the head of the meal. The greatest inhibition of lipid-delayed transit was achieved at a dose of 40 μg kg^{-1}, no greater effects were seen at higher doses. At the lowest dose of 20 μg kg^{-1}, granisetron significantly prolonged the lipid-delayed transit ($P<0.05$).

Similarly, ondansetron when administered at 40, 80 and 150 μg kg^{-1} significantly reversed the Intralipid-induced delay in SCTT of the head of the meal. The greatest inhibition of lipid-delayed transit was at the higher dose of 80 μg kg^{-1} with a similar inhibition of lipid-delayed transit at 150 μg kg^{-1}. The ID50 was 40 μg kg^{-1}.

Discussion

The results of this study demonstrate that blockade of 5-HT_3 receptors with the specific receptor antagonists granisetron and ondansetron slowed gastrointestinal transit of a baked bean/lactose test meal under control conditions, but reversed the delay in gastrointestinal transit observed when lipid was infused into the ileum. The data therefore suggest a specific 5-HT_3 receptor-mediated effect rather than a single drug effect which could have been postulated from our initial experiment (Brown et al 1991). The conflicting results, however, may be reconciled by proposing that luminal factors may either accelerate or delay transit by stimulating mechanisms that are mediated or modulated by the same pool of 5-HT or mediated by separate and distinct pools of 5-HT.

The physical presence of food within the intestine may stimulate mucosal mechanoreceptors or tension receptors to stimulate propulsion and digestive secretions which would accelerate transit (Beubler & Juan 1978; Caren et al 1974; Read 1986), whereas the infusion of lipid into the ileum could act via specific mucosal chemoreceptors to delay transit (Read et al 1984; Melone 1986). If we make the assumption that the 5-HT_3 antagonists are acting at a single site on the afferent neurone to suppress responses to intestinal stimuli, then the effects may depend on the predominant intestinal stimuli, delaying transit that is accelerated and accelerating transit that is delayed. However if both stimuli act on the same receptors then stomach-to-caecum transit time should return to the same neutral value in the presence of the antagonists.

An alternative explanation is to propose an additional 5-HT_3 receptor-mediated mechanism by ileal lipid, which might involve the activation of sympathetic or cholinergic neurones. 5-HT_3 receptors have been shown to activate the release of noradrenaline from these neurones (Richardson & Engel 1986) and we have previously shown that the ileal brake phenomenon can be inhibited by blockade of α_1- and β_1-adrenoceptors with prazosin and atenolol, respectively, and enhanced by the blockade of α_2-receptors with idazoxan (Brown et al 1992). However, granisetron has been shown to have negligible agonist or antagonist activity on either α- or β-adrenoceptors, histamine or muscarinic receptors in-vivo (Butler et al 1988). There is also evidence suggesting the presence of 5-HT_3- and 5-HT_1-like receptors situated on cholinergic nerve terminals (Schuurkes 1990) and stimulation by 5-HT released by distal small intestinal infusion of lipid could produce delayed transit time. Blockade of the receptors would reverse the delay in transit time produced by ileal infusion of lipid.

The breath hydrogen technique used in this study as an index of stomach-to-caecum transit of the meal is a combined measurement of gastric emptying and small intestinal transit and only gives information on the transit of the head of a meal introduced into the stomach. The delay in control stomach-to-caeum transit time may therefore be a consequence of either delayed gastric emptying or small intestinal transit. Similarly the reversed lipid-induced delay of the head of the meal may be due to increased gastric emptying or intestinal transit. It is not possible from these studies to determine whether the 5-HT_3 antagonists are effecting either gastric emptying or small intestinal transit.

It has been suggested that different 5-HT_3 antagonists have different affinities within the gastrointestinal tract (Richardson et al 1985) and have different actions on the rate of gastric emptying (Smith et al 1984; Costall et al 1987; Buchheit et al 1989; Forster & Dockray 1990). The different test meals used in these studies makes direct comparison difficult, especially since it has been shown that 5-HT_3-antagonists reverse gastric emptying delayed by lipid or protein solutions but not when delayed by glucose or hypertonic saline solutions (Gamse 1989; Forster & Dockray 1990). However, our results indicate that the two 5-HT_3-receptor antagonists ondansetron and granisetron used in this study have similar effects, at least on the head of the baked bean test meal, slowing control gastrointestinal transit and reversing lipid-delayed transit, but that granisetron is more potent than ondansetron. The ID50 values obtained for granisetron in these studies are consistent with data derived from studies in the rat on the Bezold-Jarisch reflex (Butler et al 1988) demonstrating the involvement of 5-HT_3 receptors.

The results from this present study confirm our previous observations demonstrating the involvement of 5-HT_3 receptors in the control of gastrointestinal transit under control conditions and when gastrointestinal transit is delayed by distal intestinal infusion of lipid. In conclusion, our data support the concept that 5-HT is an important mediator of sensory stimuli in the gastrointestinal tract.

Acknowledgements

The financial support of Kabi Novum Huddinge Sweden is gratefully acknowledged.

References

Andrews, P. L. R., Hawthorn, J. (1988) The neurophysiology of vomiting. Clin. Gastroenterol. 2: 141–168

Beubler, E., Juan, H. (1978) PGE-released blood flow and transmucosal water movement after mechanical stimulation of the rat jejunal mucosa. Naunyn Schmiedebergs Arch. Pharmacol. 305: 91–95

Bond, J. H., Levitt, M. D. (1975) Investigation of small bowel transit time in man utilising pulmonary hydrogen (H_2) measurements. J. Clin. Invest. 85: 546–555

Brown, N. J., French, S. J., Rumsey, R. D. E., Read, N. W. (1991) The effect of a 5-HT_3 antagonist on the ileal brake mechanism in the rat. J. Pharm. Pharmacol. 43: 517–519

Brown, N. J., Rumsey, R. D. E., Read, N. W. (1987) Adaptation of hydrogen analysis to measure stomach to caecum transit time in the rat. Gut 28: 849–854

Brown, N. J., Rumsey, R. D. E., Read, N. W. (1992) The effects of adrenoceptor antagonists on the ileal brake in the rat. Br. J. Pharmacol. 105: 751–755

Buchheit, K. H., Gamse, R., Berholet, A., Buscher, H. H. (1989) Antagonists at serotonergic-5-HT_3-receptors increase gastric emptying of solids and liquids in the rat. Gastroenterology 96: A64

Butler, A., Hill, J. M., Ireland, S. J., Jordan, C. C., Tyers, M. B. (1988) Pharmacological properties of GR38032F, a novel antagonist at 5-HT_3 receptors. Br. J. Pharmacol. 94: 397–412

Caren, J. F., Meyer, J. H., Grossman, M. (1974) Canine intestinal secretion during and after rapid distension of the small bowel. Am. J. Physiol. 227: 183–188

Costall, B., Gunning, S. J., Naylor, R. J., Tyers, M. B. (1987) The effect of GR38932F, a novel 5-HT_3-receptor antagonist on gastric emptying in the guinea pig. Br. J. Pharmacol. 91: 263

Falkson, H. C., Falkson, C. I., Falkson, G. (1990) High versus low dose granisetron, a selective 5-HT_3 antagonist, for the prevention of chemotherapy induced nausea. Invest. New Drugs 8: 407–409

Forster, E. R., Dockray, G. J. (1990) The effect of ondanestron on gastric emptying in the conscious rat. Eur. J. Pharmacol. 191: 235–238

Gamse, R. (1989) 5-HT_3 antagonists accelerate fat-delayed, but not hyperosmolar glucose delayed gastric emptying in mice. J. Gastro. Motil. 1: 54

Hawthorn, J., Ostler, K. H., Andrews, P. L. R. (1988) The role of abdominal visceral innervation and 5-hydroxytryptamine M-receptors in vomiting induced by the cytotoxic drugs, cyclophosphamide and cisplatin in the ferret. J. Exp. Physiol. 73: 7–21

Khojasteh, A., Sartiano, G., Tapazoglou, E., Lester, E., Gandara, D., Bernard, S., Finn, A. (1990) Ondansetron for the prevention of emesis induced by high-dose cisplatin. A multi-centre dose-response study. Cancer 66: 1101–1105

Melone, J. (1986) Vagal receptors sensitive to lipids in the small intestine of the cat. J. Aut. Nerv. Syst. 17: 231–241

Prior, A., Read, N. W. (1990) Reduction of rectal sensitivity and postprandial motility by granisetron, a 5-HT_3 receptor antagonist, in patients with irritable bowel syndrome (IBS). Gut 31: A1174

Read, N. W. (1986) Diarrhoea motrice. Clin. Gastroenterol. 15: 657–686

Read, N. W., Miles, C. A., Fisher, D., Holgate, A. M., Kime, N. D., Mitchell, M. A., Reeve, A. M., Rocke, T. B., Walker, M. (1980) Transit of a meal through the stomach, small intestine and colon in normal subject and its role in the pathogenesis of diarrhoea. Gastroenterology 79: 1276–1282

Read, N. W., McFarlane, A., Kinsman, R. I., Bates, T. E., Blackhill, N. W., Farrar, G. B. J., Hall, J. C., Moss, G., Morris, A. P., O'Neill, B., Welch, I., Lee, Y., Bloom, S. R. (1984) The effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon. Gastroenterology 86: 274–280

Richardson, B. P., Engel, G. (1986) The pharmacology and function of 5-HT_3 receptors. Trends Neurol. Sci. 9: 424–428

Richardson, B. P., Engel, G., Donatsch, P., Stadler, P. A. (1985) Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. Nature 316: 126–131

Schuurkes, J. (1990) Effect of cisapride on gastric motility. Z. Gastroenterologie 28 (Suppl. 1): 27–30

Smith, W. L., Droppleman, D. A., Gregory, R. L., Alphin, R. S. (1984) Dazopride (AH-5531): a novel gastrokinetic agent. Gastroenterology 86: 1257

Watling, K. J. (1989) 5-HT_3 receptor agonists and antagonists. Neurotransmissions V: 1–8