lipophilicity of HNE suggests that the effect of HNE might be limited to the membrane, and it is therefore likely that membrane-associated enzymes are mainly affected by HNE. Other membrane associated enzymes can be targets for sulphydryl-reactive agents. It is well established that Ca^{2+}-ATPases are vulnerable to oxidative stress, and which are shown to be inactivated by modification of sulphydryl groups (Thor et al 1985; Kaneko et al 1989). This will lead to a disturbance of the Ca^{2+} homeostasis, which affects a variety of cellular functions and might also cause a diminished smooth muscle contractility.

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The effect of a 5-HT_3-antagonist on the ileal brake mechanism in the rat

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Abstract—Studies have been carried out on 7 male adult rats to investigate how the action of the selective 5-HT_3 receptor antagonist, granisetron, influences gastrointestinal transit under control conditions and when it is 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 $\mu g\ kg^{-1}$) significantly delayed the passage of the head of the baked bean meal through the stomach and the small intestine under control conditions ($P < 0.05$). The same compound, however, significantly reversed the delay in SCTT induced by ileal infusion of lipid ($P < 0.001$). These apparently paradoxical results may be rationalized by postulating inhibition of receptors on afferent nerves initiating reflexes that both accelerate and delay transit.

A number of observations suggest that 5-hydroxytryptamine (5-HT) may play an important role in mediating the responses to chemical and mechanical stimulation of the intestinal wall. Over 90% of the total 5-HT in the body is contained in enterochromaffin cells of the gastrointestinal mucosa in close association with afferent nerve terminals (Andrews & Hawthorn 1988). Blockade of 5-HT_3 receptors has been shown to reduce the emetic responses to cisplatin and radiation (Andrews et al 1988), decrease the sensitivity of the rectum to distension in patients with the irritable bowel syndrome (Prior & Read 1990) and reverse the delay in gastric emptying induced by the presence of lipid in the proximal small intestine (Gamse 1989). These observations have led to the suggestion that 5-HT_3 receptors may be present on afferent neurones. Stimulation of 5-HT_3 receptors with 2-methyl-5-HT leads to the release of substance P, acetylcholine and noradrenaline from enteric neurones (Richardson et al 1985). The aim of our studies was to determine how inhibition of 5-HT_3 receptors with granisetron would influence gastrointestinal transit both under control conditions and when it is delayed by the presence of lipid in the ileum.

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Materials and methods

Animals. Experiments were carried out on a total of 7 adult male albino rats, 250–300 g, from Sheffield Field Laboratories. The

rats, equipped with the chronic indwelling cannulae and housed singly in cages, were deprived of food (Diet 86, Oxoid, London, UK) 18 h before the experiment but water was freely available. At least one week post-operative recovery was allowed before experiments were carried out.

Drugs. Drugs used were granisetron (BRL 43694) 40 $\mu g\ kg^{-1}$ from Beechams Pharmaceuticals and 20% Intralipid (Kabi-Vitrum, Uxbridge, UK), a triglyceride emulsion.

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 inches, o.d. 0·037 inches, 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, Scotland, UK). A small square of nylon mesh was secured 2–3 cm from the end of the cannula using silicone glue (Medical Adhesive Type A, Dow Corning Corp., 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. Each 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. Four experiments were carried out on each of the animals, (i) subcutaneous saline injection and ileal saline infusion, (ii) subcutaneous saline injection and ileal Intralipid (20%) infusion, (iii) subcutaneous drug injection and ileal saline infusion and (iv) subcutaneous drug injection and ileal Intralipid infusion, in a Latin square design. The specific 5-HT_3-antagonist granisetron (BRL 43694) or normal saline was injected subcutaneously at a dose of 40 $\mu g\ kg^{-1}$ 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 min 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. 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 detail 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 colon and was fermented by the colonic bacteria (Bond & Levitt 1975; Read et al 1980).

Statistical analysis. The differences in transit times produced during the different experimental conditions was assessed using Student's paired *t*-test and a one way analysis of variance (ANOVA).

Results

The specific 5-HT_3-antagonist granisetron (BRL 43694, 40 $\mu g\ kg^{-1}$) significantly slowed the SCTT of the head of the baked bean meal during ileal infusion of saline when compared with the control injection of saline (granisetron/saline vs saline/saline; means ± s.e.m. 160 ± 10 vs 115 ± 3 min, n = 7; $P < 0.05$; Fig. 1).

Ileal infusion of Intralipid significantly delayed SCTT of the head of the meal when compared with control saline infusion (saline/Intralipid vs saline/saline; means ± s.e.m. 181·7 ± 3·1 vs 115 ± 3 min; n = 7; $P < 0.001$; Fig. 1). This delay in transit was not seen in the presence of granisetron which reversed the Intralipid-induced delay in SCTT of the head of the meal (granisetron/Intralipid vs saline/Intralipid; means ± s.e.m.

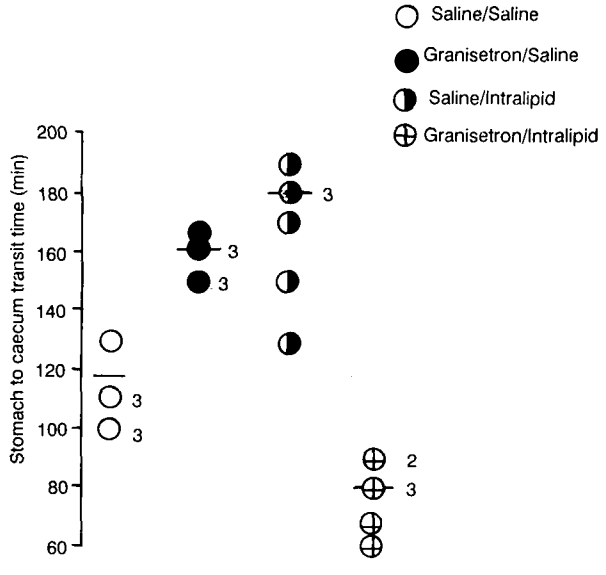

FIG. 1. Individual data representing stomach to caecum transit time (min) of the head of the baked bean test meal during either saline or granisetron injections and saline or Intralipid infusions into the ileum, n = 7 in all groups. The number next to some of the data points indicates more than one animal having this value. The horizontal bar represents the mean of the data. Statistical significances are indicated in the text.

80·2±6 vs 181·7±3·1 min; n=7; $P<0·001$; Fig. 1) to a value significantly shorter than that for the control SCTT (saline/saline).

Discussion

Our results show that blockade of 5-HT_3 receptors with the specific receptor antagonist granisetron 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. How is it possible to reconcile such paradoxical results?

Luminal factors may either accelerate or delay small bowel transit. The physical presence of food may stimulate mucosal mechanoreceptors or tension receptors to stimulate propulsion and digestive secretions that would accelerate transit (Caren et al 1974; Beubler & Juan 1978; 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). Thus if we assume that granisetron is acting at a single site on the afferent neurone to inhibit responses to intestinal stimuli, then its effects could depend on the predominant intestinal stimuli, delaying transit that is accelerated and accelerating transit that is delayed.

The major problem with this explanation is that if both types of stimulus act on the same receptors then stomach to caecum transit time should return to the same 'neutral' value in the presence of granisetron. However, in the presence of granisetron, stomach to caecum transit time during infusion of saline into the ileum is twice that observed when lipid is infused into the ileum. An alternative explanation is to postulate activation of an additional 5-HT_3 receptor-mediated mechanism by ileal lipid. Such a mechanism might involve the activation of sympathetic neurones, since 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 & French 1989; Brown et al 1990). Activation of sympathetic neurones by 5-HT could delay transit by inhibiting the release of acetylcholine from enteric neurones. It has also recently been shown that the prokinetic agent cisapride which has 5-HT_3-antagonistic properties reverses delayed gastric emptying by facilitating cholinergic transmission (Schuurkes 1990).

In conclusion our results have shown the involvement of 5-HT_3 receptors in the control of gastrointestinal transit under control conditions and when gastrointestinal transit is delayed by infusion of lipid into the ileum. Although the opposing effects suggest an action on afferent neurones, 5-HT_3 receptors are present at many sites. 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 systems (Watling 1989). It is therefore possible that the resulting effect may be due to a combination of actions which cannot be determined from this series of studies.

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