J. Pharm. Pharmacol. 1993, 45: 1033–1036
Received February 2, 1993

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The Effect of the Cholecystokinin Antagonist Devazepide (L364718) on the Ileal Brake Mechanism in the Rat

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Abstract—Studies were carried out on 28 male adult rats to investigate whether the selective cholecystokinin-receptor antagonist devazepide influences gastrointestinal transit under control conditions and when it is delayed by ileal infusion of lipid. Stomach-to-caecum transit time of the head of the test meal was measured using environmental hydrogen analysis and the distribution of the meal was assessed using the radiolabelled meal technique. Oral administration of devazepide (4 mg kg^{-1}) had no significant effect on transit time of the head of the baked bean test meal under control conditions, but significantly reversed the delay in transit time induced by ileal infusion of lipid ($P < 0.01$). Studying the distribution of the meal showed that Intralipid delayed transit time by delaying both gastric emptying ($P < 0.01$) and small bowel transit ($P < 0.05$). Devazepide did not alter the control distribution of the meal during ileal saline infusion, but during ileal infusion of lipid, devazepide further delayed gastric emptying ($P < 0.01$); the geometric centre of the meal was situated more proximally in the gastrointestinal tract ($P < 0.05$), but there was more of the meal in the colon ($P < 0.01$). The latter is compatible with the early rise in environmental hydrogen during devazepide administration and ileal lipid infusion and suggests that peripheral cholecystokinin receptors may modulate or mediate the delay in small bowel transit induced by ileal lipid. However, the data also suggest that mechanisms other than those involving cholecystokinin play a dominant role in the regulation of postprandial and lipid-delayed gastric emptying of a meal.

Although there is evidence that the delay in gastric emptying induced by the presence of nutrients in the upper small intestine may be mediated or modulated by cholecystokinin (CCK) (Dockray 1989), little is known about the role of CCK on small intestinal transit. We have previously shown that both gastric emptying and small bowel transit time of a meal is delayed by infusion of lipid into the ileum in both man and animals (Read et al 1984; Brown et al 1987; Welch et al 1987; Brown 1988). However, it seems unlikely that CCK is directly involved in this effect by liberation from the distal small intestine, since CCK-containing cells are distributed mainly in the epithelium of the proximal small intestine (Bishop et al 1982), with a limited number present in the distal small intestine (Solcia et al 1987). If CCK is involved in lipid-delayed gastrointestinal transit of a meal it may be released from other sites in the reflex pathway, such as the peripheral ganglia or the afferent nerve pathway.

Recently, a non-peptide orally active CCK-receptor antagonist, devazepide (L364718), specific for peripheral CCK_A receptors has been developed (Chang & Lotti 1986). In-vitro studies have shown this compound to inhibit binding of CCK to pancreatic and gall bladder tissues effectively with little affinity to other receptor types such as adrenergic, dopaminergic, benzodiazepine and gastrin (Chang & Lotti 1986). Devazepide has also been shown to inhibit CCK-stimulated pancreatic exocrine secretions, gall bladder contractility, colonic contractions, and antagonize CCK-stimulated delayed gastric emptying in rats, mice and dogs in-vivo (Lotti et al 1987). However, delayed gastric emptying produced by a mixed meal in man (Liddle et al 1989), or by amino acid solution (Lotti et al 1987), hydrochloric acid and hypertonic saline (Green et al 1988) in rats, is not reversed by administration of devazepide.

The aim of the following series of studies was to investigate whether peripheral CCK receptors are involved in mediating or modulating the delay in gastric emptying and small intestinal transit produced by infusion of lipid into the distal small intestine, mimicking the effects of exogenously administered CCK on a meal. The effects of the specific peripheral CCK_A-receptor antagonist devazepide on stomach-to-caecum transit time of the head of the meal were assessed using environmental hydrogen analysis and the distribution of a radiolabelled meal during basal and lipid-delayed conditions.

Materials and Methods

Animals

Experiments were carried out on a total of 28 adult male albino rats, obtained from Sheffield Field Laboratories, weighing between 250 and 300 g. 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.

Preparation of the test meal

'Californian' white beans (H. J. Heinz Co. Ltd, Hayes, UK) were washed to remove the tomato sauce, homogenized with a little water, and lactose (May & Baker Ltd, Dagenham, UK) was added to produce a concentration of 10% w/v to increase the concentration of unabsorbable carbohydrate in the meal. The radioactive test meal was produced in the same way except 1 mBq technetium-sulphur colloid (Amersham

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International PLC, Amersham, UK) was added to the water used to homogenize the beans.

Preparation of the drug

The CCK antagonist devazepide was obtained as a gift from Merck Sharp & Dohme, Hoddesdon, UK. Devazepide was dissolved in 0·5% carmellose in saline to give a concentration allowing a dose of 4 mg kg^{-1} in 2 mL.

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 with 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). 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, as the regenerating tissues under the skin formed a platform over the mesh. 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 (0·9% NaCl, 0·3 mL) was infused into the ileum of the rat to ensure the cannula remained patent.

Experimental protocol

Environmental hydrogen analysis. Eight animals were used for this part of the study. Four experiments were carried out in a Latin square design on each of the group of eight animals: oral administration of the drug vehicle (carmellose) and ileal saline infusion; oral administration of drug vehicle and ileal Intralipid (20%) infusion; oral administration of the CCK antagonist devazepide and ileal saline infusion; and oral administration of CCK antagonist and ileal Intralipid infusion. The specific CCK antagonist was given orally by gavage at a dose of 4 mg kg^{-1} in a volume of 2 mL suspended in 0·5% carmellose in 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 dosed by gavage with either the vehicle or the CCK antagonist suspended in the vehicle and after 30 min were placed in Bollman restraining cages. Thirty minutes later, rats were connected to the infusion pump (Braun, Germany) by a metal connector and plastic tubing and either saline or Intralipid was infused directly into the ileum via the cannula at a rate of 0·3 mL h^{-1} for 30 min. Rats were then dosed by gavage 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 dosing. The perspex chambers provided a controlled environment from which the hydrogen concentrations could be readily monitored 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 of the head of the bean/lactose test meal was defined as the time taken from dosing, 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).

Radiolabelled meal technique. The remaining 20 animals were divided into four groups, those to be given placebo by gavage and either saline or lipid infusion into the ileum, and those to be given devazepide by gavage and either saline or lipid. All animals were killed at 200 min after gavage by exposure to cyclopropane gas (BOC Gases Ltd) in a closed chamber. Surgical anaesthesia was rapidly obtained and death occurred within 30 s without causing any obvious signs of movement of contents or trauma to the internal organs (Paton & Payne 1968; Atkinson & Lee 1973).

After death, the gastrointestinal tract was ligated at the lower oesophageal sphincter, the pylorus and the ileo-caecal valve completely removed from the animal and transferred to a longitudinal perspex trough filled with warm saline. Care was taken in handling the intestine to avoid stretching and displacement of luminal contents. The trough was pulled by an electric motor at a rate of 10 cm min^{-1} at a constant depth of 46 mm under a single crystal scintillation detector (type MS 310E, J & P Engineering Ltd, Reading, UK). The resultant radioactivity profile was displayed on a chart recorder also running at 10 cm min^{-1} and recorded on computer for further analysis. The remainder of the carcass was monitored using a scintillation meter to ensure all radiation used was contained within the excised gastrointestinal tract.

The radioactivity profiles from the chart recorder were divided into 12 sections. These consisted of the stomach, caecum and 10 equal sections of the small intestine from the pylorus to the ileo-caecal valve. The area under each section was obtained from the computer and expressed as a percentage of the total radioactivity present. The geometric centre of the gastrointestinal distribution of the technetium-sulphur colloid at each time interval for each meal was calculated as the G sum of the fraction of Tc per segment multiplied by the segment number (Miller et al 1981).

Statistical analysis

The differences in transit times using the breath hydrogen technique during the different experimental conditions was assessed using the Wilcoxon test for non-parametric data

and a one way analysis of variance. The radioactivity data were analysed using the Mann Whitney U-test for non-parametric data.

Results

Environmental hydrogen analysis

Ileal infusion of Intralipid significantly delayed the stomach-to-caecum transit of the head of the baked bean test meal when compared with ileal saline infusion (Intralipid vs saline; mean ± s.e.m., 192·5 ± 13·1 vs 107·5 ± 6·3 min; n = 8; $P < 0·001$). Devazepide had no effect on the stomach-to-caecum transit of the meal during ileal infusion of saline, but significantly reversed the delay in transit time of the head of the test meal produced by ileal infusion of Intralipid (devazepide/Intralipid vs saline/Intralipid; 80 ± 12·1 vs 192·5 ± 13·1 min; n = 8; $P < 0·01$).

Distribution of radioactivity

Analysis of the distribution of radioactivity at 200 min after dosing showed that during ileal lipid infusion, less of the meal had left the stomach ($P < 0·01$) and less had entered the colon ($P < 0·001$); also there was a proximal shift in the distribution of the meal in the small intestine ($P < 0·05$; Table 1) when compared with the distribution during ileal infusion of saline.

Devazepide had no effect on the distribution of the radiolabelled meal during ileal infusion of saline (Table 1). However, during ileal infusion of lipid, devazepide significantly increased the amount of the meal in the distal small intestine ($P < 0·05$) and the colon at 200 min ($P < 0·01$), although devazepide produced a further delay in gastric emptying of the meal ($P < 0·01$), with less of the meal present in the more proximal parts of the intestine ($P < 0·01$; Table 1).

Discussion

The results from these experiments using the specific CCK_A-receptor antagonist, devazepide, suggest that CCK may be involved in delaying stomach-to-caecum transit time of the head of the test meal induced by ileal infusion of lipid. This delay in transit time of the head of the meal is reversed by oral administration of devazepide, indicating that CCK receptors are involved in mediating or modulating this response to distal small intestinal lipid infusion. Devazepide has no effect on transit time of the head of the meal during control conditions when saline is infused into the ileum.

Results from the breath hydrogen analysis, which demonstrate a reverse in the lipid-delayed stomach-to-caecum transit time of the head of the meal during devazepide administration but no effect on control stomach-to-caecum transit time of the head of the meal, are confirmed by analysis of the distribution of the radiolabelled meal. Ileal lipid infusion delays both gastric emptying and small intestinal transit. Devazepide administration results in more of the meal present in the colon 200 min after ingestion. At the same time, however, more of the meal is present in the stomach. Devazepide has no effect on the control meal distribution during ileal infusion of saline. The data, therefore, suggest that the delay in small intestinal transit induced by ileal lipid infusion may be mediated or modulated by CCK, but that mechanisms other than those involving CCK play a dominant role in regulating the postprandial gastric emptying rate of a meal.

CCK may mediate the delay in small intestinal transit time, induced by ileal lipid, by a direct action on vagal afferent nerves. Recent reports have indicated that CCK-8 can increase vagal afferent discharge from the ileum of the rat (Richards & Grundy 1993) and that CCK-binding sites are transported distally in vagal afferent nerves (Forster & Dockray 1992). Radioimmunoassays of peptide hormones carried out on human tissue, however, suggest that CCK-containing cells are primarily located in the mucosa of the duodenum and upper jejunum (Bishop et al 1982), with some also located in the more distal regions of the small intestine (Solcia et al 1987). The distribution of this peptide is similar in the rat, in as much as there are fewer CCK-releasing cells in the distal small intestine when compared with the proximal small intestine (Solcia et al 1987). CCK is also present in the peripheral ganglia which relay signals from the ileum to the stomach and the rest of the small intestine, as well as being situated on enteric ganglia. Thus, an alternative possibility is that devazepide could inhibit the effect of CCK on small intestinal transit via an effect on neural ganglia.

Previous animal studies have shown that devazepide has no effect on control gastric emptying of physiological saline and does not reverse the delay in gastric emptying produced by hypertonic saline, 50 mM hydrochloric acid (Green et al 1988), or a liquid amino acid meal in rats (Lotti et al 1987) and dogs (Pendleton et al 1987). The lack of effect of devazepide on the control meal distribution and in reversing the lipid-induced delayed gastric emptying in our experiments and these previous studies may be explained by the composition of the meal. It has been shown in the rat that meals containing protein and protease inhibitors delay the rate of gastric emptying by liberating CCK, whereas other nutrients delay gastric emptying by different mechanisms (Green et al 1988). Similarly, studies in man have shown that devazepide depresses postprandial plasma levels of CCK and gall bladder contractility in response to a mixed meal, but has

Table 1. Effect of devazepide (D) on the percentage distribution of a radiolabelled meal in the gastrointestinal tract of the rat 200 min after dosing during ileal saline and lipid infusions.

	Saline		Lipid	
	Control	D	Control	D
Stomach	5·3 ± 1·1	4·1 ± 1·5	15·0** ± 2·8	46·3** ± 8·4
Duodenum	8·1 ± 1·1	7·4 ± 1·6	15·9** ± 0·4	0·3** ± 0·07
Small intestine				
proximal	4·4 ± 1·0	5·3 ± 1·4	8·2*** ± 0·5	0·4*** ± 0·08
mid	4·9 ± 0·6	5·5 ± 0·3	7·9*** ± 0·4	4·0*** ± 1·9
distal	15·2 ± 0·6	14·9 ± 0·5	6·7*** ± 0·4	9·7* ± 1·9
Colon	13·8 ± 2·1	12·8 ± 2·6	1·3*** ± 0·5	11·5** ± 2·6
Geometric centre of meal in small intestine	5·5 ± 0·4	5·7 ± 0·3	4·1*** ± 0·2	3·5 ± 0·8

Values are mean ± s.e.m., n = 5. * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ compared with the corresponding experiment with saline.

no effect on liquid or solid emptying (Liddle et al 1989) of the meal, or in reversing delayed gastric emptying induced by meals containing guar or glucose (Meyer et al 1989). Those observations have again been attributed to the composition of the meal and suggest that certain mixed meals and nutrient solutions do not delay gastric emptying by predominantly releasing endogenous CCK but do so by other mechanisms. Studies performed in mice, rats and dogs which demonstrated devazepide to reverse delayed gastric emptying of a meal, employed CCK-induced inhibition of gastric emptying of a meal (Lotti et al 1986, 1987) rather than the inherent ability of that meal to delay gastric empyting by releasing endogenous CCK. Those studies (Lotti et al 1987) therefore confirmed the ability of devazepide to block peripheral CCK receptors by which exogenous administration of CCK induces delayed gastric emptying. Thus, the most likely explanation for the discrepancies observed in the ability of devazepide to reverse delayed gastric emptying may be attributed to the mechanism by which gastric emptying of the meal is delayed.

Results from our studies and previous experiments (Green et al 1988) suggest that delayed gastric emptying may be mediated or controlled by mechanisms other than those involving the release of endogenous CCK. An alternative mediator of delayed gastric emptying by distal small intestinal infusion of lipid is peptide YY (PYY). PYY is present in specialized endocrine cells located in the epithelium of the small and large intestine particularly the ileum and rectum (Adrian et al 1985). Small intestinal release of PYY is relatively specific for lipid in the dog (Adrian et al 1985) and it has been shown to delay gastric emptying and small bowel transit in man (Savage et al 1987), possibly by the inhibition of propagative motor patterns (Al-Saffer et al 1985). Thus, lipid-induced release of PYY from the distal small intestine may be responsible for the delayed gastric emptying and small intestinal transit in our studies, with small intestinal transit also being regulated by endogenous CCK release.

In conclusion, our data suggest that peripheral CCK receptors may mediate or modulate the delayed small intestinal motor response induced by distal small intestinal infusion of lipid. Although both distal small intestinal lipid infusion and exogenous administration of CCK-8 induces delayed gastric emptying of a meal, it is unlikely that an increase in endogenous CCK release and elevated plasma levels of CCK are responsible for the delayed gastric emptying of a meal in these studies, since it is not reversed by the peripheral CCK_A-receptor antagonist devazepide. Alternative mechanisms involving mediators such as PYY may be involved in the regulation of gastric emptying of a meal in the rat.

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