Gut hormones in acute diarrhoea

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SUMMARY The gut hormone response to a breakfast meal was studied in 12 subjects hospitalised for an episode of acute diarrhoea (presumed infective) who were otherwise well and in 13 healthy control subjects. Fasting blood glucose concentrations were low but basal insulin concentrations were raised. Basal concentrations of pancreatic polypeptide and both basal and postprandial responses of motilin, enteroglucagon, and vasoactive intestinal polypeptide (VIP) were also significantly greater than controls. No abnormalities in plasma concentrations of gastrin, gastric inhibitory polypeptide (GIP) or pancreatic glucagon were found. The suggested physiological actions of the raised hormones may be relevant to the pathophysiology of diarrhoea.

The acute, spontaneous onset of diarrhoea in a previously healthy individual is generally considered to be the result of an enteritis which may be caused by ingestion of a bacterial toxin, or by direct viral or bacterial infection.¹ Often the causative agent is not identified and treatment consists of supportive measures only and complete recovery follows within a short period of time.

Major abnormalities in water and electrolyte transport exist in infectious diarrhoea owing to raised adenylyclase and cAMP activity, diminished Na⁺, K⁺-ATPase activity, loss of epithelial integrity, rapid transit and disorders of intestinal motility.² The relative importance of these mechanisms probably varies depending on the aetiology.

A number of gut hormones are thought to affect intestinal secretion and motility. The jejunal hormone, motilin, has pronounced effects on gastrointestinal motility,³ affecting the interdigestive migrating complexes.⁴ Indirect evidence suggests that enteroglucagon is trophic to the mucosa of the small intestine and has the effect of slowing intestinal transit.⁵

In order to ascertain whether motilin and enteroglucagon or any other gut peptide was altered in acute diarrhoea, we measured the plasma concentrations of these peptides in patients with an acute attack of severe transient diarrhoea.

Method

PATIENTS

A test breakfast was given to 12 patients, five women and seven men with acute diarrhoea whose mean age was 37 years (range 19-67 years). All had been previously in good health and none had a past history of gastrointestinal disease. They had all required admission to hospital for severe diarrhoea (at least eight motions per day) and had been treated on admission with intravenous fluid and electrolyte replacement. In addition, a further four patients had fasting samples taken between admission and discharge (but no test breakfast). Thus in all, 16 subjects were studied on admission and during recovery.

Salmonella agona and *saint-paul* were isolated from stool specimens in two patients. In one patient's mother, who had also had acute diarrhoea, *Salmonella tennessee* was isolated but no organism was found in the patient studied. *Entamoeba coli* were identified in two further patients. No salmonella, shigella or other organisms were cultured in the other 10 patients, nor were ova, cysts, or pathogenic parasites observed on stool microscopy. Barium enema radiology was performed in two patients with *Entamoeba coli* but was normal in each case. All patients subsequently made complete and lasting recoveries.

Thirteen healthy subjects, four women and nine men were used as controls, whose mean age was 33 years (range 23-59 years). None had past or present

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history of gastrointestinal disease.

Informed consent was obtained from all subjects and the study had been approved by the relevant ethical committee.

INVESTIGATIONS

Blood samples were taken in the fasting state soon after admission and serially until discharge from hospital. Blood was sampled both before and for three hours after a standard test breakfast. This was given at the earliest appropriate time of their recovery, when their appetite was normal and they felt well in themselves. At this stage although the motion was still only a thin liquid, the frequency of their diarrhoea had diminished to two or three motions per day. The test breakfast consisted of two medium sized boiled eggs, 60 g bread as toast, 10 g butter, 35 g marmalade, and 150 ml unsweetened orange juice (this breakfast containing a total of 18 g protein, 22 g fat and 66 g carbohydrate, equivalent to 530 Calories). Samples for hormone assays were taken into heparinised tubes containing 400 Kallikrein-inactivating units of aprotinin (Trasylol) per ml of blood and the plasma separated within 15 minutes of sampling and stored at -20°C until assay.

Blood glucose estimation was carried out using standard glucose oxidase/peroxidase methodology as adapted for the auto-analyser.⁶ Plasma hormone concentrations were measured by specific radio-immunoassays, which have previously been described in detail, and were carried out by conventional methods with antisera raised to pure human gastrin,⁷ pancreatic polypeptide,⁸ and insulin⁹ to pure porcine gastric inhibitory polypeptide (GIP),¹⁰ motilin,¹¹ and vasoactive intestinal

polypeptide (VIP).¹² Glucagon was measured using two separate systems, one using a C-terminal reacting antibody specific for pancreatic glucagon¹³ and a second reacting with the mid to N-terminal sequence of glucagon which also measured gut glucagon immunoreactivity, and which showed complete crossreactivity with porcine glicentin.¹⁴ Enteroglucagon was derived by subtraction of the specific pancreatic glucagon values from those obtained using the N-terminal glucagon assay. The assays were capable of detecting the following plasma changes with 95% confidence: gastrin 2 pmol/l, pancreatic polypeptide 4 pmol/l, GIP 3 pmol/l, motilin 3 pmol/l, VIP 1.5 pmol/l, entero-glucagon 10 pmol/l, and insulin 6 pmol/l and showed no crossreaction with each other or other relevant hormones. Statistical analysis was made using Student's *t* test for unpaired data for parameters with normal distribution and using non-parametric (Whitney Mann U Test) methodology for parameters with a known skewed distribution.

Results

Mean fasting levels, peak postprandial rise and total integrated postprandial responses for blood glucose and for the gut hormones measured are given in the Table. Values are given as mean $\pm$ standard error of the mean.

BLOOD GLUCOSE

Patients with acute diarrhoea had significantly reduced fasting levels of blood glucose with a diminished postprandial rise, compared with control subjects. The postprandial rise was more prolonged,

Table Fasting concentrations, peak postprandial rises and total integrated responses after the breakfast for blood glucose and all peptide measured (mean $\pm$ SEM)

	Gastrin pmol/l	Blood glucose mmol/l	Insulin pmol/l	GIP pmol/l	HPP pmol/l	Pancreatic glucagon pmol/l	Motilin pmol/l	Entero- glucagon pmol/l	VIP pmol/l
Controls n=13									
Basal	5.0 $\pm$ 1.0	4.6 $\pm$ 0.2	19.0 $\pm$ 1.0	17.0 $\pm$ 4.0	22.0 $\pm$ 5.0	4.2 $\pm$ 0.8	41.0 $\pm$ 8.0	19.0 $\pm$ 2.0	5.5 $\pm$ 0.9
Peak rise	17.0 $\pm$ 5.0	2.1 $\pm$ 0.2	125.0 $\pm$ 11.0	43.0 $\pm$ 5.0	181.0 $\pm$ 31.0	3.4 $\pm$ 0.7	25.0 $\pm$ 6.0	21.0 $\pm$ 4.0	3.2 $\pm$ 1.2
TIR	2.9 $\pm$ 0.7	895.0 $\pm$ 39.0	13.0 $\pm$ 1.5	8.3 $\pm$ 1.1	23.1 $\pm$ 3.4	0.8 $\pm$ 0.1	7.9 $\pm$ 1.7	5.2 $\pm$ 0.5	1.2 $\pm$ 0.3
Acute diarrhoea patients n=12									
Basal	10.0 $\pm$ 3.0	3.5 $\pm$ 0.1	25.0 $\pm$ 2.0‡	20.0 $\pm$ 4.0	41.3 $\pm$ 6.0†	4.1 $\pm$ 0.5	138.0 $\pm$ 26.0	64.5 $\pm$ 12.0	16.5 $\pm$ 1.6
Peak rise	24.0 $\pm$ 6.0	1.2 $\pm$ 0.2§	105.0 $\pm$ 18.0	40.0 $\pm$ 5.0	250.0 $\pm$ 47.0	2.2 $\pm$ 0.6	63.0 $\pm$ 13.0†	41.0 $\pm$ 16.0	9.3 $\pm$ 2.3‡
TIR	4.6 $\pm$ 1.3	722.0 $\pm$ 38.0§	13.6 $\pm$ 1.9	8.5 $\pm$ 0.7	26.2 $\pm$ 3.6	0.8 $\pm$ 0.1	27.9 $\pm$ 5.5	15.5 $\pm$ 3.0	3.2 $\pm$ 0.4

Total integrated responses (TIR) for peptides in nmol/l/180 min and for blood glucose in mmol/l/180 min.

* = $p < 0.05$ vs controls. † = $p < 0.02$ vs controls. ‡ = $p < 0.01$ vs controls. § = $p < 0.005$ vs controls. || = $p < 0.001$ vs controls.

however, and levels were still greater than basal at 120 and 180 minutes compared with controls who had returned to basal by 120 minutes.

INSULIN

Fasting insulin concentrations were significantly greater than normal in the patients with diarrhoea ($p < 0.01$). Postprandial insulin concentrations, however, were similar to controls. Basal concentrations of insulin in the diarrhoeal patients were 26.8 ± 3.6 pmol/l soon after admission and 24.4 ± 2.7 pmol/l, before discharge from hospital.

PANCREATIC POLYPEPTIDE (HPP) (Fig. 1)

Fasting levels of PP were significantly greater than normals ($p < 0.01$) in the diarrhoeal patients. After the breakfast, PP concentrations were increased compared with controls ($p < 0.05$) only at 60 minutes. Otherwise the peak rise and total integrated response of HPP was similar to normal in the patients with diarrhoea. Their fasting concentrations of PP after admission were 45.2 ± 6.3 pmol/l and 44.8 ± 7.2 pmol/l before discharge from hospital.

MOTILIN

Fasting plasma motilin concentrations in the 16

patients on admission were 202 ± 30 pmol/l falling to 136 ± 23 pmol/l during recovery, ($p < 0.005$ using *t* test for paired samples) (Fig. 2). Fasting plasma motilin concentrations in the healthy controls were 41 ± 8 pmol/l ($p < 0.001$ against recovery samples). Ingestion of the breakfast resulted in a small rise in plasma motilin (Fig. 3) which was greater in absolute terms in the patients with diarrhoea, but was proportionally similar in both groups.

ENTEROGLUCAGON

The patients with diarrhoea also had significantly greater fasting and postprandial plasma entero-glucagon concentrations than the healthy controls (Fig. 4). Fasting entero-glucagon concentrations in the diarrhoeal patients shortly after admission were 78.5 ± 91.6 pmol/l and had fallen significantly to 46.7 ± 6.1 pmol/l ($p < 0.02$) before their discharge from hospital.

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP)

Fasting concentrations of VIP were significantly greater than controls in the patients with diarrhoea ($p < 0.01$), they also had a peak postprandial rise which was higher than normal ($p < 0.01$). Fasting VIP concentrations showed no significant change in

Fig. 1 Plasma pancreatic polypeptide responses to test breakfast in 12 patients with acute diarrhoea and in 13 healthy controls.

Fig. 2 Fasting plasma motilin concentrations in patients with acute diarrhoea on admission and during recovery.

Fig. 3 Plasma motilin responses to test breakfast in 12 patients with acute diarrhoea and 13 healthy controls.

Fig. 4 Plasma enteroglucagon responses to test breakfast in 12 patients with acute diarrhoea and in 13 healthy controls.

sequential samples from admission to discharge from hospital (13.3 ± 1.7 and 11.1 ± 1.3 pmol/l, respectively on admission and before discharge).

GASTRIN, GIP AND PANCREATIC GLUCAGON

Fasting and postprandial concentrations of gastrin, GIP, and pancreatic glucagon did not differ significantly from the control subjects. Fasting levels of these hormones shortly after admission were 11.3 ± 2.5 , 23.3 ± 2.5 , and 2.0 ± 0.9 pmol/l and before discharge from hospital were 7.2 ± 1.6 , 20.8 ± 3.3 , and 3.1 ± 0.9 pmol/l, respectively.

Discussion

Motilin is an upper small intestinal hormone which has been shown to affect gut motility in a number of different ways. *In vitro* studies have shown that the colon of rabbits is very sensitive to the synthetic analogue of motilin, 13-norleucine motilin.¹⁵ Similar studies with synthetic motilin have shown dose dependent contractions on isolated rabbit intestinal

segments, with ileum and colon showing similar responses.¹⁶ Although 13-norleucine motilin had little effect *in vitro* on human colonic circular muscle it did stimulate contraction of the taenia coli.¹⁵ *In vivo* studies in healthy subjects have shown that exogenous motilin, infused to produce physiological plasma concentrations, caused a significant increase in electrical and pressure activities in the descending colon.¹⁷ Furthermore, 13-norleucine motilin infused in man reduced small intestinal transit time by 50%.¹⁸ Synthetic motilin, when infused in man in the interdigestive state, induced a series of strong contractions of the stomach, similar to the natural gastric interdigestive migrating contraction. These contractions then propagated distally along the small intestine.⁴ Thus the finding of significantly raised endogenous plasma motilin in patients with diarrhoea may have relevance to the motor changes that occur.^{2, 19-22} In particular, it is of interest that fasting levels tended to fall with the resolution of the diarrhoeal illness.

Fasting and postprandial plasma concentrations of

motilin were also significantly raised compared with normal in patients with Crohn's disease,^{23 24} ulcerative colitis,²³ tropical malabsorption,²⁴ and small intestinal resection.²⁵ Patients with untreated coeliac disease²⁶ and chronic pancreatitis²⁷ had only a slight rise of plasma motilin compared with control subjects. In contrast, patients with irritable bowel syndrome either with diarrhoea or with constipation, but having no demonstrable organic pathology of the intestinal tract, had totally normal concentrations of plasma motilin.^{28 34} From these observations it would appear that motilin concentrations are raised in conditions associated with diarrhoea or steatorrhoea secondary to organic disease of the intestinal tract or pancreas. It is unlikely that this is purely a chance association, and it is of interest to speculate that abnormalities of motilin secretion in diarrhoeal states may be primarily involved in their pathogenesis or may represent secondary or adaptive responses to the disordered motility and/or secretion.

Mucosal atrophy has been reported to occur with infective diarrhoea in children,²⁹ and with diarrhoea caused by viruses.^{30 31} In piglets infected with transmissible gastroenteritis virus, mucosal changes after 40 hours varied from mild partial to complete villous atrophy.³² These changes were accompanied by a significant degree of crypt hyperplasia. Similar studies in man have shown an acceleration of the rate of normal migration of enterocytes from the crypts, with failure of differentiation so that the cells of the gut epithelium had the functional characteristics of normal crypt cells.³¹

Plasma concentrations of enteroglucagon were significantly raised both in the fasting state and after the meal in the patients with acute diarrhoea. Concentrations were higher shortly after admission compared with those after symptomatic recovery, before discharge. These concentrations, however, were still significantly greater than those in fasting normal subjects, suggesting that either the process of mucosal regeneration continues for longer than the manifest symptoms or that the stimulatory signal, if such, takes a longer time to return to normal.

The physiology of enteroglucagon, which is principally located in the ileum and colon,³³ still awaits definitive elucidation. Indirect evidence from an enteroglucagon secreting tumour suggests that it may stimulate villous growth and slow intestinal transit.⁵ Plasma concentrations are increased in pathological states, where there is a decrease in small intestinal mucosal surface area. Thus raised enteroglucagon concentrations have been reported in patients with coeliac disease,³⁴ tropical malabsorption,²⁴ after ileal resection,²⁵ and in

Crohn's disease.³³ Further, more recent direct evidence, supporting a trophic role for enteroglucagon has come from studies using partially purified enteroglucagon in rodents. The rate of jejunal mucosal DNA synthesis was increased by approximately 50% compared with controls.³⁵ As in patients with coeliac disease, it is possible that the degree of enteroglucagon rise, found in patients with acute diarrhoea, may relate to the nature of any changes in the mucosa of the small intestine.

A surprising finding was that of significantly lower blood glucose concentrations in the diarrhoeal patients compared with controls. One possibility is that this relative hypoglycaemia may in part result from the higher basal insulin concentrations found in these patients. After the breakfast the diarrhoeal patients had a significantly reduced peak rise in blood glucose, but in both groups, maximal concentrations were attained by 30 minutes. In controls blood glucose concentrations had returned to basal by two hours, whereas in the diarrhoeal patients concentrations were still sustained above basal at three hours. Similar obtunded but sustained postprandial glucose responses were found in patients with untreated coeliac disease,³⁴ and in patients with severe tropical malabsorption.²⁴ This would suggest diminished absorption reflecting possible mucosal damage typical of these two latter conditions and also reported in patients with diarrhoea.

Fasting concentrations of pancreatic polypeptide were significantly raised in patients with acute diarrhoea, compared with normal. The physiology of this hormone is still uncertain. Recent evidence on studies of fluid flux in the rat small intestine has shown that pancreatic polypeptide caused a significant increase in net absorption in the distal ileum.³⁶ Whether pancreatic polypeptide has a similar effect in man is unknown. If this is a physiological effect, then increased concentrations of pancreatic polypeptide stimulating fluid absorption would be an appropriate compensatory response in diarrhoea. Pancreatic polypeptide has no detectable action on motor events, for example it has no influence on the migratory motor complex.³⁷

Fasting and postprandial concentrations of VIP were significantly greater in the diarrhoeal patients than in control subjects. These concentrations were, however, of an order of magnitude less than the plasma concentrations found in the Verner-Morrison or VIPoma syndrome,³⁸ where VIP released from a pancreatic tumour passes into the circulation and causes diarrhoea by increasing net fluid loss in the small intestine. VIP is localised in neurons in the wall of the entire alimentary tract, with some of the greatest tissue concentrations in

the ileum and colon.³³ VIP when infused in pigs, albeit achieving supra-physiological plasma concentrations, causes diarrhoea.³⁹ Known biological actions of VIP on the small and large intestine include inhibition of absorption, stimulation of water and ion secretion, stimulation of adenylate cyclase activity, and relaxation of colonic smooth muscle.⁴⁰ Furthermore VIP has recently been described to be released from the feline small intestine when exposed to cholera toxin. It was proposed that the increased secretion might be secondary to the activation of intramural nervous reflexes in the gut.⁴¹ Thus the finding of mildly raised VIP concentrations in patients with acute diarrhoea may indicate a local role in the pathophysiological mechanisms of diarrhoea.

It is unlikely that the raised basal concentrations of many of the gut hormones measured are because of haemoconcentration. There were no significant differences from normal in the diarrhoea patients' haemoglobin, sodium, and urea concentrations. Nor were there any significant differences in the plasma concentrations of all but motilin and enteroglucagon of the peptides measured, between the initial sample taken while still suffering from diarrhoea and the final sample when symptomatically recovered.

The effect of taking nothing by mouth and total parenteral nutrition on fasting and meal stimulated gut hormone release has been studied after considerably longer time periods than the few days of intravenous therapy necessary in the diarrhoeal patients.⁴² There were no differences on the fasting concentrations of gastrin, motilin, GIP, secretin, pancreatic polypeptide, pancreatic glucagon, enteroglucagon, or VIP during prolonged total parenteral nutrition. The peak postprandial response of enteroglucagon was higher and that of insulin was lower after the test breakfast and after prolonged total parenteral nutrition than that when repeated after normal eating. As the patients with acute diarrhoea were only on intravenous alimentation for a matter of only a few days (compared with weeks in the study above) but were allowed oral fluids as soon as these could be tolerated, it is very unlikely that any of the alterations in gut hormones found in the diarrhoea patients could be ascribed to prolonged fasting.

The importance of these preliminary findings of raised plasma concentrations of pancreatic polypeptide, motilin, enteroglucagon, and VIP in patients with diarrhoea must await further study.

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