Gut hormones in tropical malabsorption

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Summary and conclusions

Concentrations of various gut hormones were measured after a test breakfast in eight patients with severe tropical malabsorption and 12 controls. The patients with tropical malabsorption had greatly raised basal plasma motilin and enteroglucagon concentrations, but their post-prandial release of both gastric inhibitory polypeptide and insulin was significantly reduced. The pattern of gut hormone release differed from that found in coeliac disease.

The measurement of gut hormones, each of which has a specific site and function, thus throws new light on the pathophysiology of tropical malabsorption and may suggest approaches to treatment.

Introduction

Tropical malabsorption is characterised by the onset of diarrhoea, usually acute, in patients with no history of gastrointestinal disease while they are exposed to a tropical environment.¹ The diarrhoea progresses to a malabsorptive state with associated steatorrhoea and weight loss. The term "tropical malabsorption" is used in preference to "tropical sprue," which covers a wide variety of syndromes.² The cause of tropical malabsorption is

poorly understood, though abnormal bacterial colonisation of the small intestine is considered to be a major factor in the pathogenesis.³⁻⁵ Whereas small intestinal motility is usually increased by the presence of bacteria and is important for maintaining the normal milieu of the intestinal lumen,^{6,7} relative stasis may, however, occur in severe tropical malabsorption.⁸⁻¹⁰ This may play a part in the bacterial overgrowth of the upper small intestine.

The factors controlling gut motility have not been fully elucidated. Two gut hormones, enteroglucagon and motilin, may have an important influence. Enteroglucagon probably slows transit^{11,12} while motilin enhances the rate of gastric emptying¹³ and can initiate the onset of interdigestive myoelectric complexes.¹⁴ We therefore studied the gut hormone response to a test breakfast in patients with tropical malabsorption.

Patients and methods

Eight consecutive patients with severe tropical malabsorption¹⁰ presenting at the Hospital for Tropical Diseases were studied. They were selected on clinical and biochemical grounds (see table) before their hormone concentrations were measured. Twelve healthy controls (eight men, four women; mean age 29, range 23-49 years; weight $104 \pm 3\%$ of ideal) who had no history of gastrointestinal disease were also studied. Informed consent was obtained from all subjects, and after an overnight fast they ate a standard test breakfast consisting of two eggs, orange juice, toast, butter, and marmalade (containing 18 g protein, 22 g fat, and 66 g carbohydrate). Blood samples, before and for three hours after the test breakfast, were taken into heparin tubes containing 400 KIU aprotinin/ml, immediately centrifuged, and the plasma stored at -20°C until assay.

Gut hormone radioimmunoassays—Radioimmunoassays were carried out by conventional methods using antisera raised to synthetic human gastrin,¹⁵ natural human pancreatic polypeptide,¹⁶ natural porcine motilin,¹⁷ gastric inhibitory polypeptide (GIP),¹⁸ and glucagon¹⁹ and to natural bovine insulin and neurotensin.²⁰ The assays could detect the following plasma changes with 95% confidence: gastrin 2 pmol/l; pancreatic polypeptide 4 pmol/l; motilin 3 pmol/l; GIP 5 pmol/l; enteroglucagon 8 pmol/l; insulin 6 pmol/l, and neurotensin 5 pmol/l. No significant cross reaction was detectable between any of the peptides assayed, with the exception of enteroglucagon, which was measured by subtracting pancreatic glucagon from total glucagon.¹⁹ Blood glucose was measured by the alkaline ferricyanide method adapted for the autoanalyser.²¹ The plasma forms

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Details of patients with tropical malabsorption

Case No	Age and sex	Weight loss (kg)	Country of origin	Country of onset of tropical malabsorption	Length of history (months)	Mean faecal fat/24 h (mmol/l)	Mean urinary xylose (mmol/5 h)	Schilling test		Jejunal biopsy grade*	Hydrogen breath test (min)
								Urinary vitamin B ₁₂ /24 h (μg)	Plasma vitamin B ₁₂ /8 h (μg)		
1	26 M	15.9	New Zealand	Thailand	3	ND	2.9	1.3	ND	II	90
2	19 M	19.1	England	Afghanistan	4	105	2.4	1.2	0.1	II/III	120
3	39 F	7.3	"	Indonesia	5	116	1.5	ND	ND	III	170
4	26 M	15.9	"	Brazil	3	64	4.4	ND	ND	III/IV	50
5	57 M	19.1	"	India	3	38	3.5	2.3	0.1	II	60
6	20 F	19.1	"	Nepal	5	20	3.8	ND	ND	III/IV	90
7	28 M	15.9	"	Nepal	3	55	2.8	0.1	0.1	III	110
8	31 M	9.5	"	Afghanistan	3	80	1.4	0.1	0	III	190
Mean...	31 5 M; 4 F	15.2			3.6	68.3	2.8	1.0	0.1		110
SE of mean or range	19-57	1.6			0.3	13.1	0.4	0.4	0.03		17.4
Normal values						11-18	8-16	>10	>0.6		53 $\pm$ 6

ND = Not done.

*Jejunal biopsy graded using classification of Ridley and Ridley.²⁴

of glucagon and motilin were analysed by gel permeation chromatography of plasma on Sephadex G50 superfine (Pharmacia). Internal markers (dextran blue, cytochrome C, and sodium iodide 125) were included with each plasma sample.

In addition, patients underwent a Schilling test, xylose tolerance test, three-day faecal fat collection, a lactulose breath hydrogen test,²²⁻²³ and a jejunal biopsy.²⁴

Results

Patients with tropical malabsorption had a flattened glucose response to the meal. Their mean glucose concentration ($\pm$ SE of mean) rose from a basal level of 3.8 ± 0.1 to a peak of 4.5 ± 0.2 mmol/l, whereas that of the control group rose from 4.3 ± 0.2 to 5.8 ± 0.2 mmol/l ($P < 0.001$). The patients' insulin response was similarly significantly lower rising to a peak of 117 ± 18 pmol/l compared with a peak of 240 ± 26 pmol/l in controls ($P < 0.005$; fig 1). The incremental rise of GIP after the meal in the patients with tropical malabsorption was half (11 ± 1 pmol/l) that seen in the healthy controls (22 ± 2 pmol/l; $P < 0.001$; fig 2). In contrast, basal plasma motilin was more than four times higher in the patients than in the controls ($P < 0.005$), but the postprandial rise, while greater in absolute terms, was proportionately similar (fig 3). Analysis by gel permeation chromatography showed this increase to be due to increased levels of both small and large circulating forms of plasma

FIG 1—Plasma insulin response to test meal in patients with tropical malabsorption (○—○) and controls (●—●).

FIG 2—Plasma gastric inhibitory polypeptide response to test meal in patients with tropical malabsorption (○—○) and controls (●—●).

FIG 3—Plasma motilin response to test meal in patients with tropical malabsorption (○—○) and controls (●—●).

FIG 4—Plasma enteroglucagon response to test meal in patients with tropical malabsorption (○—○) and controls (●—●).

FIG 5—Relationship between fasting enteroglucagon and time of appearance of breath hydrogen after lactulose ingestion in patients with tropical malabsorption.

motilin immunoreactivity. The basal plasma enteroglucagon was raised almost tenfold in patients with tropical malabsorption ($P < 0.01$) but showed little response to the test breakfast (fig 4). Chromatography showed that most of the enteroglucagon immunoreactivity was in the large molecular weight elution position, with a relatively minor proportion eluting in the position of pancreatic glucagon. There was no difference in pattern between patients and controls. Fasting and postprandial plasma concentrations of gastrin (controls: basal 3.9 ± 0.8 , peak 26 ± 4 pmol/l; patients: 7.6 ± 1.6 and 20 ± 4 pmol/l), pancreatic polypeptide (controls: basal 29 ± 8 , peak 293 ± 69 pmol/l; patients: 44 ± 10 and 261 ± 56 pmol/l), and neurotensin were similar in the two groups.

The 24-hour faecal fat output, urinary xylose excretion, vitamin B₁₂ absorption, and jejunal biopsy were abnormal in all the patients with tropical malabsorption (see table). The breath hydrogen appearance time in the patients was considerably prolonged and correlated well with the raised fasting enteroglucagon levels ($R = 0.92$, $P < 0.001$; fig 5).

Discussion

Over the past decade several new gut hormones have been isolated, including motilin,²⁵ pancreatic polypeptide,²⁶ neurotensin,^{27, 28} and GIP,²⁹ and the existence of a further moiety, enteroglucagon, has been inferred by its cross reaction with pancreatic glucagon antibodies.³⁰ Together with the more classical hormone gastrin these substances are released by food and can all be reliably detected in human plasma. They each have a characteristic site in the gut and a different physiological function. Thus gastrin, which is released from the antrum and upper intestine, acts to stimulate acid secretion and maintain growth of the mucosa.³¹ Pancreatic polypeptide, derived from endocrine cells found in both islets and parenchyma of the pancreas, has recently been shown to inhibit gall-bladder contraction and pancreatic enzyme secretion.³² GIP, which is secreted by endocrine cells in the mucosa of the upper small intestine, was originally isolated because of its ability to inhibit gastric acid secretion. Subsequent work has suggested that this action is pharmacological and that GIP's potent stimulation of insulin release reflects its most likely physiological role.²⁹ Because GIP stimulates insulin release only when glucose concentrations are raised, the name glucose-dependent insulinotropic peptide has been suggested, thus retaining the initials GIP. Release of GIP is thought to be the mechanism whereby glucose taken by mouth produces release of insulin several times greater than that produced by intravenous glucose—that is, the mediator of the enteroinsular axis. Motilin also comes from the upper small intestine and, like many other hormones, is found in the circulation in two forms, large and small.³³ As both always behave similarly after any stimulus, the large form is probably merely a synthetic precursor of the small and they are probably both produced and released together by the same cell. Several forms of enteroglucagon, a hormonal peptide originating from endocrine cells in both ileal and colonic mucosa, have also been found in the circulation. Unlike motilin, however, only one of the forms responds to physiological stimuli, and it has been proposed therefore that this is the biologically active molecule. As these peptides are now easy to measure we have investigated the use of a standard ordinary breakfast as a dynamic stimulus to their release, a stimulus which also reflects the form of the patient's everyday nutrition at home. Changes in the gut hormone profile are thus likely to indicate both the area of digestive tract affected, because of the different locations of each of the gut hormone peptides, and also the nature of the functional disturbance.

The basic pathological process in tropical malabsorption is not fully understood. There are important differences, however, from another cause of sprue—coeliac disease. Although the mucosal atrophy in tropical malabsorption is generally milder than in coeliac disease it usually affects the entire small intestine uniformly.^{34, 35} Malabsorption is therefore more severe, and nutritional deficiencies occur earlier. The pattern of gut hormone

release has been studied after the same standard test breakfast in patients with untreated coeliac disease. Normal plasma motilin levels and only slightly increased basal plasma enteroglucagon concentrations were found. The postprandial rise in enteroglucagon was, however, enormous.³⁶ Thus the gut hormone response was clearly different from that seen in this study. The patients with coeliac disease had a reduced postprandial release of the insulinotropic hormone GIP and, in this respect, were similar to those with tropical malabsorption. The observed reduction in postprandial insulin release may, in part, have been the result of this reduction of GIP, but was probably also related to the reduced rise of glucose after the breakfast.

The mean time of hydrogen appearance after oral lactulose is significantly prolonged in patients with tropical malabsorption,¹⁰ which suggests that small-intestinal transit is delayed in these patients. The patients with the highest enteroglucagon concentrations had the longest time to breath hydrogen appearance and thus the slowest small-intestinal transit rate.¹⁰ Little is known of the physiology of enteroglucagon, nor has this material ever been purified in sufficient quantities to study its pharmacology. Indirect evidence of its actions comes from a patient with an enteroglucagon-secreting tumour who had extremely slow intestinal transit and increased intestinal mucosal growth, changes which reverted to normal immediately after resection of the tumour had lowered enteroglucagon concentrations to normal.¹¹ Enteroglucagon concentrations are also raised in patients who have had small-intestinal resection³⁷ and after jejunal ileal bypass³⁸⁻⁴⁰ operations, both circumstances when a compensatory reduction of intestinal transit speed and an increased enterocyte turnover occur. The raised enteroglucagon concentrations might therefore reflect a compensatory mechanism in the patients with tropical malabsorption and might be responsible for delaying small-intestinal transit, a change expected to increase efficiency of nutriment absorption. It is tempting to speculate that this action of enteroglucagon may, in some individuals, have the deleterious effect of causing sufficient stasis to allow bacterial colonisation and result in a vicious circle of further mucosal damage and greater increase of enteroglucagon. From this model, one would predict that therapeutic manoeuvres which overcame the effect of enteroglucagon by stimulating gut motility might effect a permanent cure.

Motilin causes powerful contractions in isolated strips of human stomach and upper small intestine, but its physiological role has not been fully elucidated. Exogenous motilin, infused to produce plasma concentrations within the physiological range, has been shown to accelerate the rate of gastric emptying in man,¹³ but other experiments using somewhat higher concentrations of motilin have suggested an impairment of gastric emptying.⁴¹ The plasma motilin concentrations found in the patients we studied are of a similar order of magnitude to those produced in the latter investigation. The measurement of gut hormones in tropical malabsorption thus helps our understanding of its pathophysiology and may also indicate new therapeutic approaches.

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Pathogenesis of neonatal hyperbilirubinaemia after induction of labour with oxytocin

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Summary and conclusions

To determine the pathogenesis of neonatal hyperbilirubinaemia after oxytocin-induced labour venous cord blood from 95 healthy newborn infants was examined. Of these, 15 were delivered by elective caesarean section, 40 after spontaneous labour, and 40 after oxytocin-induced labour. There was no significant difference in any haematological or biochemical variable between the first two groups. Infants born after oxytocin-induced labour, however, showed clear evidence of increased haemolysis associated with significantly decreased erythrocyte deformability ($P < 0.001$). In-vitro studies showed a time- and dose-related reduction in erythrocyte deformability in response to oxytocin.

The findings suggest that the vasopressin-like action of oxytocin causes osmotic swelling of erythrocytes leading to decreased deformability and hence more rapid destruction with resultant hyperbilirubinaemia in the neonate.

Introduction

The association between oxytocin-induced labour and neonatal hyperbilirubinaemia is well documented,¹⁻⁸ suggested causes including hepatic glucuronyltransferase immaturity,⁵ anoxic

liver damage,⁶ enhanced placentofetal transfusion,⁷ increased erythrocyte fragility,⁸ and mechanical trauma to erythrocytes.⁴ I began the following study after the observation that fetal erythrocytes were less deformable after oxytocin-induced labour than after spontaneous labour. Erythrocyte deformability is an important determinant of erythrocyte life span,^{9,10} and in several conditions decreased deformability results in increased haemolysis.^{11,12} I therefore investigated the effect of oxytocin on erythrocyte deformability both in vivo and in vitro and sought evidence of fetal erythrocyte destruction during both spontaneous and induced labour.

Patients and methods

The study group comprised 95 healthy newborn infants (44 boys, 51 girls) with birth weights of 3005-4108 g (mean 3420 g) and Apgar scores exceeding 7 at one minute. With use of a wide-bore needle and gentle suction to avoid haemolysis 20 ml venous cord blood was collected as soon as the cord had been clamped and divided and before placental separation. The blood was anticoagulated with lithium heparin.

Of the 95 infants, 40 had been delivered after spontaneous labour and 40 after induction of labour by amniotomy and intravenous oxytocin (Syntocinon). The average dose of oxytocin used was 4500 mU (range 3000-7000 mU). In the cases selected for study the duration of labour was between six and 14 hours and analgesia was achieved with intramuscular pethidine. No patient was given epidural analgesia. In none of the cases was there clinical or cardiotocographic evidence of fetal distress, and all mothers had spontaneous vertex deliveries. The remaining 15 infants were delivered by elective caesarean section.

Within two hours after delivery the following procedures were carried out on the cord blood: packed cell volume was measured with a Hawksley microhaematocrit; plasma osmolality was measured with an advanced osmometer; and erythrocyte deformability was measured by a microfiltration technique¹³ whereby the erythrocyte deformability index (EDI) was calculated as the volume of erythrocytes contained in a 20% suspension in their native plasma that

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