Effects of porcine gastric fundic factor, somatostatin, substance P, glucagon, neurotensin, bombesin, VIP, motilin, and pentagastrin on jejunal glucose absorption in the rat

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SUMMARY The effects of a porcine gastric fundic mucosal extract (molecular weight <10 000) has been compared with the effects of eight candidate gastrointestinal peptides on glucose absorption from the jejunum in a rat model. Bolus injection of the extract produced immediate and marked depression of glucose absorption. None of the candidate peptides tested produced this response, although somatostatin and substance P depressed absorption as a late phenomenon after 30 minutes. We conclude that the effects of the fundic extract are not reproduced by any of these candidate peptides. This strengthens the evidence for a novel gastrointestinal peptide, resident in fundic mucosa, which affects absorption from upper small bowel.

There is evidence for a peptide factor, secreted from gastric fundic mucosa, which affects jejunal mucosal absorption.¹⁻⁴ Stimulation of gastric fundic mucosa by distension or raised pH significantly reduced luminal loss of glucose, ¹⁴C-labelled 3-O-methyl glucose and tracer ions ²²Na and ^{99m}Tc, from the jejunum. An extract of porcine gastric mucosa also reduced jejunal absorption when infused intravenously in a rat model.⁴

Samples of the porcine gastric fundic mucosal extract contained only small and variable amounts of some of the known gastrointestinal peptides (Bloom, personal communication; Table 1). The present series of experiments was designed to see if the effects of the fundic extract on jejunal absorption could be because of the action of a specific candidate gastrointestinal peptide.

Methods

Pure synthetic peptides (Table 2) were dissolved in distilled water in siliconised containers and dilutions prepared with 1% bovine albumin (Sigma Chemical Co.) in 0.15M saline. Aliquots were taken and kept

at -20°C until used. Candidate peptides were selected according to their known distribution in the gastric mucosa or their identification in the crude porcine gastric fundic extract.

In previous experiments porcine gastric fundic extract has been administered by constant intravenous infusion.¹ In the present series, however, the extract, and each candidate peptide, was given by a single bolus injection to allow observation of the time course of any effect. As each peptide dose was given by bolus injection dilution would occur, initially in the rat blood volume (10-12 ml) and later, almost certainly in a larger theoretical volume of distribution. It is, therefore, likely that effective

Table 1 Concentrations of gastrointestinal peptides in sample of porcine gastric fundic mucosal extract

Peptide	Maximum (ng/ml)	Minimum (ng/ml)
Pancreatic glucagon	42.5	0
Gastrin	2.7	0.14
VIP	314.0	1.3
Pancreatic polypeptide	0	0
Somatostatin	7.9	0
Enteroglucagon	16.3	3.5
Motilin	23.0	0.54
Substance P	32.0	0.40

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Table 2 Candidate peptides tested: sources and doses used

Peptide	Molecular weight	Doses* ng/rat bolus injection	
Somatostatin (Cal)	1620	100	1000
Substance P (Pen)	1348	100	1000
Glucagon (Nov)	3485	100	1000
Neurotensin (Cal)	1673	10	100 1000
Bombesin (Cal)	1620	10	100 1000
VIP (Pen)	3326	10	100 1000
Motilin (Pen)	2700	10	100 1000
Pentagastrin (ICI)	768	10	100 1000

Sources: Cal = Cal Biochem, Nov = Nova Pharmaceuticals, Pen = Peninsula Labs, ICI = ICI.

* To convert peptide doses from ng to molar equivalents divide ng figure by the appropriate molecular weight.

Peptides from Cal, Biochem, ICI, and Peninsula Labs are guaranteed at least 98% pure.

concentrations were obtained of a smaller order of magnitude than the initial bolus dose. This technique was used in order to allow several peptides to be tested over a wide dose range, and to permit comparison with the effects of the unknown concentration of active agent in the gastric fundic extract.

Fundic extract was prepared from gastric fundic mucosa dissected from fresh pig stomachs as described elsewhere.¹ In essence, the method consisted of extracting lyophilised, acetone-extracted fundic mucosa with water at 80°C. The aqueous extract was mixed with an equal volume of absolute ethanol, centrifuged, and the supernatant lyophilised to produce a powder designated as 'crude extract'.

The crude extract was dissolved in water and passed through an Amicon filter with a cut-off point molecular weight <10 000, and freeze dried. Later, solutions were prepared containing either 30 mg % or 300 mg % protein in 0.15M saline.

Male Sprague-Dawley rats, weight 250 g, were fasted for 12 hours, water allowed *ad libitum*, and anaesthetised under nitrous oxide/halothane/oxygen mixture after ether induction. A midline abdominal incision was made and a caval infusion line sited to give 0.15M saline 3.0 ml/h. A segment of proximal jejunum about 10 cm long, 1 cm from the ligament of Treitz was cannulated, washed gently with warm saline, emptied by air injection and perfused with 10 mM glucose in 0.15M saline at 1.0 ml min⁻¹ from a reservoir 6–8 cm above the bowel at 37°C (Fig. 1). Fifteen seconds before perfusion was started, 0.5 ml of candidate peptide, porcine fundic extract or control solution of 1% albumin saline was injected over 10 seconds *via* the caval line. One hundred

Fig. 1 Representation of rat model used. For sake of clarity stomach has not been shown.

microlitre samples of perfusate were taken at 0, 15, 30, and 60 minutes after perfusion was started.

At the end of the experiment each animal was killed, the jejunal segment excised and its length measured and recorded. Fluid loss was estimated by titrating perfusate back to the initial level. Water absorption was assumed to occur at a constant rate throughout the experiment. Perfusate samples were prepared in 100 µl 4% perchloric acid before glucose estimation⁵ using a Beckman glucose analyser. Glucose absorption was calculated as µmol luminal loss for 15, 30, and 60 minutes of perfusion after correction for volumes removed by sampling. Results are presented as µmol glucose luminal loss per 10 cm jejunum.

One hundred and seventy-two rats were used in all. Fifty-four received albumin/saline with no peptide and acted as controls; 12 received the porcine gastric fundic extract, 10 somatostatin, eight substance P, 12 glucagon, 12 neurotensin, 12 bombesin, 14 VIP, 20 motilin, and 18 pentagastrin. The perfused bowel segments measured 9.9 cm on average for both control and peptide groups.

Results

Mean glucose luminal losses for each peptide are compared with control groups in Figs 2–10. Data for pentagastrin and motilin appear with their own relevant controls because these experiments were performed separately from the others.

The porcine fundic mucosal extract, injected at a concentration of 30 mg % protein, reduced glucose absorption by 22.7% in the first 15 minutes ($p < 0.0125$, $n = 6$ (Fig. 2)). This effect was sustained up to 30 minutes but thereafter the rate of absorption was the same as in the control group. Fundic extract injected at a concentration of 300 mg % protein reduced glucose absorption throughout the hour; by 48% in the first 15 minutes ($p < 0.001$, $n = 6$), by 8% from 15–30 minutes, and by 21% from 30–60 minutes ($p < 0.005$). Overall reduction of absorption over the 60 minute period was 22.3% ($p < 0.001$).

The various doses of somatostatin had no obvious effect on jejunal glucose absorption in the first 30 minutes (Fig. 3). Glucose absorption at 60 minutes does not differ significantly from controls, but on comparison with the gradients for the 30–60 minute period somatostatin 100 ng and 1000 ng reduced glucose absorption by 36% ($p < 0.002$, $n = 4$) and 43% ($p < 0.001$, $n = 6$) respectively.

Substance P 100 ng produced no significant change in glucose absorption but 1000 ng reduced absorption by 20.2% ($p < 0.001$, $n = 4$ (Fig. 4)) over 60 minutes. Comparison between the 15–30 and 30–60 minute groups, however, show no significant differences.

Fig. 2 Glucose (μmol) luminal loss after injection of porcine fundic extract 30 mg % protein and 300 mg % protein 0.5 ml per rat. ** $p < 0.02$; *** $p < 0.001$.

Fig. 3 Glucose (μmol) luminal loss after injection of somatostatin 100 ng and 1000 ng/rat.

Glucagon 100 ng produced no significant effect on glucose absorption (Fig. 5). One thousand nanograms glucagon increased jejunal absorption by 19% from 30–60 minutes ($p < 0.01$, $n = 4$) but the effect was not significant over the whole hour. Ten thousand nanograms glucagon increased absorption by 53% from 15–30 minutes ($p < 0.002$, $n = 4$) and by 30% from 30–60 minutes ($p < 0.02$), an increase over the whole 60 minute period of 28.5% ($p < 0.001$).

Fig. 4 Glucose (μmol) luminal loss after injection of substance P 100 ng and 1000 ng/rat. *** $p < 0.001$.

Fig. 5 Glucose (μmol) luminal loss after injection of glucagon 100 ng, 1000 ng, and 10 000 ng/rat. * $p < 0.02$; ** $p < 0.005$.

Fig. 7 Glucose (μmol) luminal loss after injection of bombesin 10 ng, 100 ng, and 1000 ng/rat.

Fig. 6 Glucose (μmol) luminal loss after injection of neurotensin 10 ng, 100 ng, and 1000 ng/rat.

Fig. 8 Glucose (μmol) luminal loss after injection of VIP 10 ng, 100 ng, and 1000 ng/rat.

Fig. 9 Glucose (μmol) luminal loss after injection of motilin 10 ng, 100 ng, and 1000 ng/rat.

Fig. 10 Glucose (μmol) luminal loss after injection of pentagastrin 10 ng, 100 ng, and 1000 ng/rat.

Neurotensin 10 ng and 1000 ng produced near control rates of glucose absorption (Fig. 6), neurotensin 100 ng produced a 14% increase in absorption over 60 minutes ($p < 0.02$, $n = 4$ (Fig. 6)), but comparison with different parts of the hour, 0–15 minutes, 15–30 minutes, and 30–60 minutes, showed no significant differences from controls.

In this model the various doses of bombesin, VIP, motilin, and pentagastrin produced no significant effects on jejunal glucose absorption as compared with controls (Figs 7–10).

Discussion

During experiments on gastric mucosal permeability in the rabbit Newman⁶ recorded that raised gastric fundic pH by instillation of human bile caused marked reduction of uptake of the tracer ion ^{99m}Tc from the gastric antrum. The effect on antral absorption was transferable by injection of portal serum taken during the experiment, into a second rabbit.³ Further strong evidence for a fundic humoral mechanism affecting ^{99m}Tc and glucose absorption was obtained in experiments on conscious dogs with antral pouches containing tracer substances and autotransplanted gastric fundic pouches exposed to alkaline buffer pH 8.² Preliminary evidence has been reported for this phenomenon in man.⁷

A rat model was devised to observe absorption of tracer substances from jejunum and antrum. This model exhibited reduced jejunal absorption of tracers after fundic distension or alkalinisation. Both these stimuli are well known mechanisms for

release of humoral factors from gut mucosa.^{8–10} The model also responded to caval infusion of the porcine gastric fundic mucosal extract, and has been used to test different fractions of the fundic extract for biological activity. Preliminary evidence from gel filtration chromatography, together with loss of activity by trypsin digestion, suggests that the active substance is a peptide with a molecular weight between 1000 and 2000. Extracts of porcine antral and jejunal mucosa, brain, lung, liver, and pancreas, prepared in the same way as the fundic mucosal extract, have failed to show the same biological activity.¹¹

At least seven types of APUD cells have been localised in the gastric mucosa by immunocytochemical methods.¹² Four samples of the porcine fundic extract (mol wt $< 10\,000$) have been examined by radioimmunoassay (Bloom, personal communication; Table 1). These have been shown to contain small amounts of glucagon, gastrin, somatostatin, motilin, and enteroglucagon, variable amounts of VIP and substance P, and no pancreatic polypeptide. In the present study pure VIP, motilin, bombesin, and pentagastrin had no effect on jejunal glucose absorption. Glucagon, and possibly neurotensin, increased absorption. Somatostatin and substance P reduced absorption, but their effects were apparent only at 30 minutes after injection. In contrast, the fundic extract reduced absorption within 15 minutes. This pattern of response, together with the negligible amount (4.8 pmol/ml) of somatostatin detected in only one of four samples of the extract, suggests that the active peptide is not somatostatin. The largest concentration of

substance P detected in the extract was 32.2 ng/ml whereas it required 1000 ng of the pure peptide to produce any significant effect on absorption in the rat model.

Our results suggest that the biological effect is not due to the action of any single peptide tested in the present study. These observations strengthen our hypothesis that the responsible factor is a novel peptide released from the gastric fundic mucosa. Any theories concerning possible physiological actions of a gastric fundic factor must remain speculative until more is known about its chemistry, distribution, and actions. As the detection of the factor depends, for the present, upon its effect on small bowel absorption it may be that the factor is involved in the regulation of absorption just as other gastrointestinal peptides contribute to the neuro-endocrine control of the other gut functions of secretion, digestion and motility. Some delay in absorption from upper small bowel may help to prevent too rapid a rise in blood glucose which could produce a large output of insulin resulting in hypoglycaemia if absorption is completed abruptly. This phenomenon is well recognised as a complication of gastric surgery.

Debnam reported that perfusion of rat terminal ileum with monosaccharide solutions markedly increased jejunal absorption of tracer substances.¹³ It is known that L-cells occur most densely in the mucosa of the terminal ileum and caecum.¹² Furthermore, L-cells are known to release enteroglucagon when exposed to sugars, amino acids, and food residues in the bowel lumen.¹⁴ It is, therefore, possible that the phenomenon described by Debnam depends upon the release of enteroglucagon and that this may form part of a mechanism controlling the rate of absorption in the jejunum according to the concentration of unabsorbed products of digestion reaching the terminal ileum.¹⁵

Poulakos and Kent reported that marker substances rapidly passed through upper small bowel in the rat.¹⁶ If the animals were fasted before observations were made, the leading edge of the marker moved significantly faster, traversing 75% of the small bowel length in 15 minutes. Read *et al* observed rates of food transit through the bowel in conscious human subjects using marker dyes, radioactive particles and measuring breath hydrogen concentration as oligosaccharides in the meal were degraded by colonic bacteria. They recorded an early peak in breath hydrogen concentration within 30 minutes of food ingestion with a larger peak some hours later.¹⁷ The early peak in their data is consistent with the arrival of a small sample of the test meal in the distal bowel within 30 minutes of eating.

Thus one may speculate that food, by distension of the stomach and buffering of its acid, would stimulate release of gastric fundic factor. The factor would act to inhibit absorption during the passage of gastric chyme through the jejunum to the terminal ileum. The presence of chyme in the terminal ileum would stimulate enteroglucagon release and so prepare the jejunum to absorb the digested products of the main bulk of the meal. So, it is possible that gastric fundic factor and enteroglucagon, together with other factors may serve to regulate the rate and distribution of absorption along the small bowel.

* This work was reported in preliminary form as a paper to the BSG meeting April 1981 at Bristol.

The authors wish to thank the North West Regional Research Grants Committee for financing the purchase of the pure synthetic peptides tested. We would also like to thank Dr S R Bloom for allowing us to reproduce Table 1, Mrs A E Tomlin and Mrs D Fleet for their technical assistance, the Department of Medical Illustrations, Manchester Royal Infirmary, for producing the tables and figures, and Miss M Wray for typing the manuscript.

Figures 2-7

A full table of results of glucose absorption for each peptide dose, including overall fluid absorption, is available from the authors on request.

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the less common stains is commented upon in the explanatory paragraphs which give a clear description of the condition illustrated and emphasise important diagnostic features.

A short bibliography groups the major standard texts according to discipline and refers to more general texts and review articles. The atlas ends with a simple clear index. It is a work which will prove invaluable to the diagnostic histopathologist, and should amply succeed in its intention of extending his experience.

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Books received

Mucus in health and disease – II Edited by E N Chantler, J B Elder, and Max Elstein. (Pp 441; illustrated; \$49.50.) New York: Plenum Press. 1982.

Acute renal failure Edited by H E Eliahou. (Pp. 310; illustrated; £24.00.) London: Libbey. 1982.

Basic science in gastroenterology. Structure of the gut Edited by J M Polak, S R Bloom, N A Wright, and M J Daly. (Pp. 488; illustrated; UK £7.00; Overseas £11.00.) Hertfordshire: Glaxo. 1982.

News

BSG Research Award 1983

A three page summary of personal research work is invited by the Awards Committee who will recommend to Council the recipient of the Award for 1983. A bibliography may also be submitted if desired. The Award consists of a medal and £100 prize. Entrants must be 40 years or less (on 31 December 1983) but need not be a member of the BSG. All (or a substantial part) of the work must have been performed in the UK or Eire. The recipient will be required to deliver a 40 minute lecture at the Plenary Session of the Spring meeting in 1984. Applications (six copies) should be made to: The Honorary Secretary, BSG, The Rayne Institute, 5 University Street, London WC1E 6JJ, by 1 December 1983.

Dr V S Chadwick

Dr Chadwick will shortly be taking up a new appointment as Professor of Experimental Medicine, and Director of the Wellcome Research Institute at the University of Otago, Dunedin, New Zealand.

World Organization of Gastroenterology

The World Organization of Gastroenterology has established a Young Investigator Scholarship. These awards are intended to encourage investigators under the age of 35 in countries with developing academic programmes. Further information can be obtained from the Secretary-General of OMGE, Professor I A D Bouchier, University Department of Medicine, Ninewells Hospital and Medical School, Dundee DD1 9SY.

Mechanisms of gastrointestinal motility and secretion

A NATO advanced study institute on the above subject will take place on 5–16 September 1983 in Erice, Sicily. Further information from Professor Alan Bennett, Department of Surgery, King's College Hospital Medical School, The Rayne Institute, 123 Coldharbour Lane, London SE5 9NU.

International Society for Diseases of the Esophagus

The second International Congress of this Society will take place in Rome, Italy, from 3–6 October 1983. Further details may be obtained from the Organising Secretary, Masson Italia Congressi, Via G Pascoli 55, 20133, Milan, Italy.

Fourth International Symposium on Neonatal Diarrhoea

This symposium will be held at the University of Saskatchewan, Saskatoon, Saskatchewan, Canada, from 3–5 October 1983. Further details from Programme Chairman, VIDO, 124 Veterinary Road, Saskatoon, Saskatchewan, Canada S7N 0W0.

Correction

Effects of porcine gastric fundic factor, somatostatin, substance P, glucagon, neurotensin, bombesin, VIP, motilin, and pentagastrin on jejunal glucose absorption in the rat: N J Andrews, S Rinno-Barmada, K Burdett, and J B Elder (April issue, *Gut* 24: 326–32). The dose ranges in Figs. 6 and 8 should read: 10, 100, and 1000 ng/rat on the actual Figures. The legends for the Figures and the doses stated in Table 2 are correct.