Gastrointestinal regulatory peptide storage granule abnormalities in jejunal mucosal diseases

J DAWSON, M G BRYANT,* S R BLOOM, AND T J PETERS

From the Division of Clinical Cell Biology, MRC Clinical Research Centre, Harrow, Middlesex, and Department of Medicine, Royal Postgraduate Medical School, London

SUMMARY   The mucosal concentrations of seven regulatory peptides and the density properties and integrity of their storage granules have been studied in mucosal biopsies from the human jejunum in eight gastrointestinal disease states and compared with normal controls. In diseases with associated mucosal inflammation (coeliac disease, Crohn's disease with jejunal involvement, postinfective tropical malabsorption, and common variable immunodeficiency) there was a selective increase in fragility of the gastric inhibitory polypeptide (GIP) and somatostatin storage granules. The gastrin, motilin, enteroglucagon, secretin, and vasoactive intestinal polypeptide granules had normal properties in these conditions. In diseases in which diarrhoea occurred in the absence of changes in jejunal mucosal histology (irritable bowel syndrome, pancreatic insufficiency, jejuno-ileal bypass for morbid obesity, and purgative abuse) there were no abnormalities of the storage granules. Increased mucosal concentrations of all peptides except vasoactive intestinal polypeptide (VIP) were found in coeliac disease and selective increases of VIP found in Crohn's disease, motilin in the irritable bowel syndrome and gastrin and GIP in pancreatic insufficiency. It is suggested that the storage granule abnormalities in the diseases with abnormal mucosal histology are secondary to the inflammatory changes.

With the major exception of the gut hormone secreting tumours there is as yet little evidence that the gastrointestinal hormones are aetiologically important in gastrointestinal diseases in which they have been studied. Plasma studies, however, have shown marked abnormalities of basal regulatory peptide concentrations and release patterns in a variety of pathological conditions.[1] Quantification of the mucosal endocrine cells[2-4] and morphological studies of peptidergic nerves[5] have also shown abnormalities of some peptides in a variety of gastrointestinal diseases. It might be expected, therefore, that some of these abnormalities will be reflected in altered mucosal concentrations of the peptides and altered properties of the storage granules. In this study we have investigated systematically the mucosal regulatory peptide storage granule properties, as determined by isopycnic sucrose density centrifugation, in the jejunum in conditions with a demonstrable morphological abnormality (coeliac disease, postinfective

Address for correspondence: Dr J Dawson, Department of Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham.

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* Deceased

tropical malabsorption, Crohn's disease, and common variable immunodeficiency) and other conditions in which there is likely to be a functional small bowel abnormality (irritable bowel syndrome, purgative abuse, small bowel bypass, and pancreatic insufficiency). The storage granule properties in these conditions together with the mucosal peptide activities have been compared with normal controls.

Methods

PATIENTS
Jejunal biopsies were obtained from the following group of patients: 12 healthy volunteers with normal jejunal histology and no evidence of gastrointestinal disease; eight patients with untreated coeliac disease associated with subtotal villous atrophy; five patients with Crohn's disease affecting the jejunum in whom biopsies showed moderately severe inflammatory changes; three patients with postinfective tropical malabsorption in whom biopsies showed histological evidence of partial villous atrophy; six patients with common variable immunodeficiency associated with severe diarrhoea and partial villous atrophy; six patients who had undergone jejuno-

ileal bypass surgery for morbid obesity – biopsies were taken seven to 15 months after operation from the functioning jejunum and showed no histological abnormality; three patients with purgative associated diarrhoea and no histological abnormality; six patients with the irritable bowel syndrome associated with diarrhoea and no histological abnormality; and six patients with diarrhoea and steatorrhoea associated with pancreatic insufficiency caused by alcoholic chronic pancreatitis and documented by secretin-pancreozymin or Lundh pancreatic stimulation tests. These studies were approved by the local ethical committees.

TECHNIQUES
Biopsies were obtained with a Crosby capsule from the jejunum, after a 12 hour fast, approximately 10 cm from the ligament of Treitz. The biopsies were divided, half being processed for routine histology and half, approximately 10 mg wet weight, collected in 3 ml of ice cold sucrose solution (0·3 mol/l) containing 1 mmol/l disodium EDTA (pH 7·4) and 22 mmol/l ethanol. The tissue was homogenised as previously described,[6] and, after low speed centrifugation to remove nuclei and cell debris, the postnuclear supernatant was subjected to isopycnic sucrose density centrifugation[6] in a Beaufay small volume automatic zonal rotor.[7] Aliquots of the gradient fractions thus obtained were assayed using radioimmunoassays especially modified and optimised to the small quantities of tissue available[6 8] to detect gastrin, gastric inhibitory polypeptide (GIP), motilin, secretin, somatostatin, enteroglucagon, and vasoactive intestinal polypeptide (VIP).[6 8–11] All homogenisation and fractionation procedures were performed at 0°C to minimise proteolytic degradation of hormones and

immediately after fractionation aliquots of gradient fractions were mixed with an equal volume of 0·2 mol/l hydrochloric acid to stabilise the peptides and the samples were deep frozen at −20°C until radioimmunoassay of hormone content. Protein in the homogenate was assayed by the technique of Lowry[12] and protein in the subcellular fractions were assayed by a micromodification[13] of the fluorimetric technique of Hiraoka and Glick.[14] Bovine serum albumin (Sigma, London) was used as standard. The protein and hormone distribution results were expressed as frequency/density histograms. All calculations, plots, and fractionation recoveries were performed by computer as described previously.[7] Mucosal peptide concentrations in the disease states studied were compared with those in normal controls using Student's *t* test, and the Mann-Whitney U test for non-parametric data.

Results

The Table shows the mucosal peptide concentrations in the eight disease states studied in comparison with normal controls. Significantly raised concentrations of all the regulatory peptides except VIP were found in coeliac disease. In Crohn's disease involving the jejunum significantly increased concentrations of VIP were noted. In pancreatic insufficiency significant rises of GIP and gastrin were found. In the irritable bowel syndrome significantly increased concentrations of motilin were found. In view of the small numbers statistics were not applied to the patients with tropical malabsorption and purgative diarrhoea but inspection indicated that mucosal peptide levels were similar to the normal controls. Figures 1–8

Table   *Peptide hormones in the jejunal mucosa in gastrointestinal diseases associated with normal and abnormal jejunal histology*

	Normal controls	Coeliac disease	Tropical malabsorption	Jejunal Crohn's disease	Immunodeficiency	Pancreatic insufficiency	Jejunoileal bypass	Purgative diarrhoea	Irritable bowel
Number	12	8	3	5	6	6	6	3	6
Somatostatin	9·4±1·6	16·3±1·9†	10·3±1·7	10·9±0·9	9·4±1·6	8·7±1·3	10·2±1·3	10·2±1·2	7·8±1·2
Gastric inhibitory peptide (GIP)	2·5±0·4	4·8±0·2‡	2·9±0·7	1·7±0·4	2·5±0·4	6·1±0·5§	3·1±0·4	2·8±0·8	2·1±0·3
Vasoactive intestinal peptide (VIP)	1·9±0·6	2·9±1·1	1·8±0·4	5·7±0·5‡	1·9±0·6	2·4±0·7	2·4±0·5	2·0±0·4	2·3±0·7
Gastrin	2·6±0·4	5·9±1·0‡	2·2±0·5	2·1±0·5	2·6±0·4	5·7±0·4§	2·4±0·5	2·8±0·3	1·7±0·2
Secretin	3·1±0·6	10·2±2·1§	4·3±1·1	2·4±0·5	3·1±0·6	3·3±1·1	2·0±0·5	2·8±0·8	1·9±0·9
Motilin	3·9±0·7	9·0±0·4‡	5·1±1·8	2·7±0·2	3·9±0·7	3·7±0·4	4·1±0·3	4·1±0·6	7·4±0·8‡
Enteroglucagon	0·6±0·2	0·9±0·2*	0·6±0·3	0·5±0·3	0·6±0·2	0·5±0·2	0·5±0·1	0·4±0·2	0·3±0·2

Mucosal concentrations expressed as pmol/mg protein.
Statistical significance from normal: * $p<0·05$, † $p<0·02$, ‡ $p<0·01$, § $p<0·001$.

show the sucrose density gradient distributions of the seven regulatory peptides studied in the jejunum from patients with disease states in comparison with normal controls. The protein distributions were also recorded. The most striking change is noted in coeliac disease (Fig. 1) in which there is a selective increase in the soluble activity of GIP and somato-

statin. A similar but less marked selective increase in the soluable fractions of GIP and somatostatin was seen in tropical malabsorption (Fig. 2) and Crohn's disease (Fig. 3) and an increase in soluble GIP in common variable immunodeficiency (Fig. 4). It was noted that in all these patients active mucosal inflammation was present. The remaining five
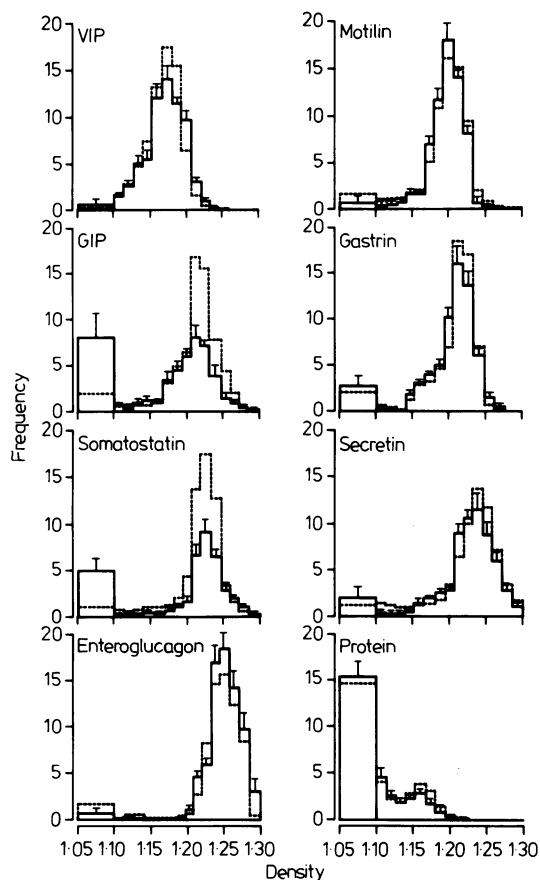
Fig. 1 *Isopycnic centrifugation of postnuclear supernant from jejunal biopsy homogenate in untreated coeliac disease (solid line) in comparison with normal controls (dotted line). Graphs show frequency/density histograms for seven peptides and protein and represent averaged data from eight patients and six controls. Frequency (mean ± SD) is defined as fraction of total recovered activity present in individual fractions divided by density span covered by that fraction. Activity present over density span 1·05–1·10 represents over arbitrary abscissa interval peptide remaining in sample layer and presumed to reflect soluble activity. Percentage recoveries for patients (mean ± SD): VIP 89±12, motilin 103±12, GIP 94±17, gastrin 97±13, somatostatin 101±12, secretin 91±9, enteroglucagon 89±18, protein 88±19.*
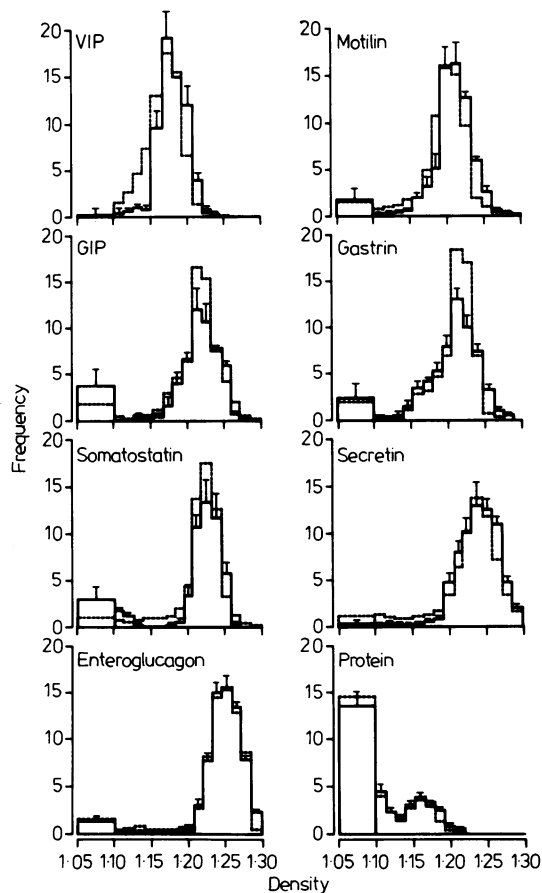
Fig. 2 *Isopycnic centrifugation of postnuclear supernant from jejunal biopsy homogenate averaged from three patients with postinfective tropical malabsorption (solid line) in comparison with six normal controls (dotted line). For details see legend to Fig. 1. Percentage recoveries for patients (mean ± SD): VIP 92±19, motilin 101±13, GIP 88±20, gastrin 99±10, somatostatin 94±12, secretin 87±21, enteroglucagon 103±19, protein 89±13.*

peptides, VIP, motilin, gastrin, secretin, and entero-glucagon, showed no increase in soluble activity, with the exception of a modest increase in soluble VIP in Crohn's disease. None of the peptides studied showed any change in the density gradient distribution in diseases associated with inflammation and each retained its characteristic modal density. This indicates that the granules which remained intact have normal density properties. In patients with pancreatic insufficiency (Fig. 5), jejuno-ileal

bypass (Fig. 6), purgative associated diarrhoea (Fig. 7), and irritable bowel syndrome (Fig. 8) there was no change in the density properties of the secretory granules and no marked changes in soluble activity. It was noted that all these patients had normal jejunal histology.

Discussion

This is the first study in which the properties of the
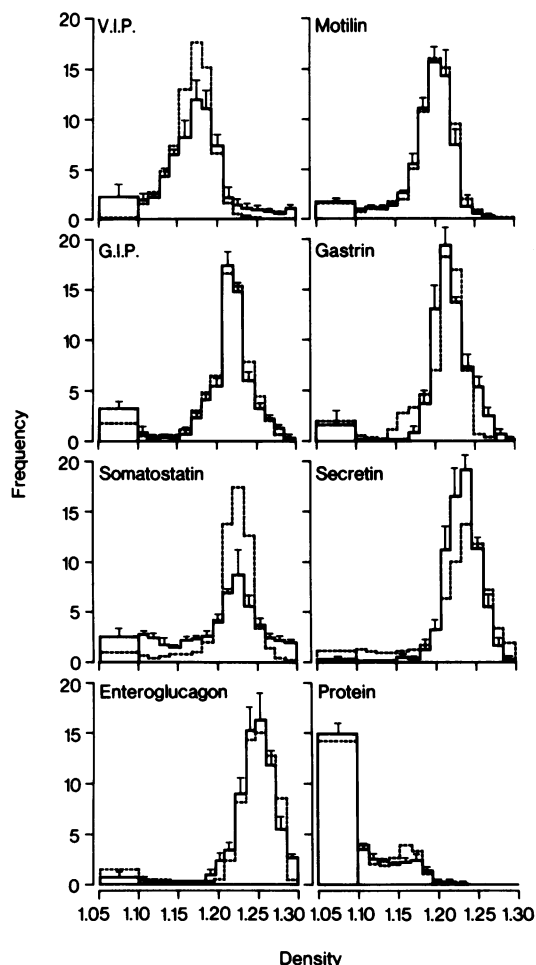

Fig. 3 *Isopycnic centrifugation of postnuclear supernant from jejunal biopsy homogenate averaged from five patients with jejunal Crohn's disease (solid line) in comparison with six normal controls (dotted line). For details see legend to Fig. 1. Percentage recoveries for patients (mean ± SD): VIP 102±12, motilin 89±14, GIP 89±17, gastrin 96±13, somatostatin 98±14, secretin 107±12, enteroglucagon 101±13, protein 89±12.*
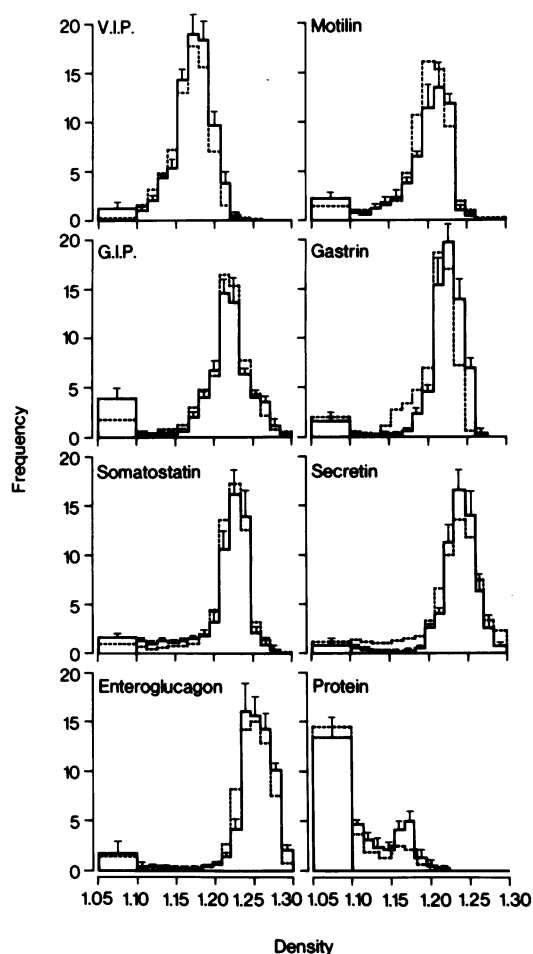
Fig. 4 *Isopycnic centrifugation of postnuclear supernant from jejunal biopsy homogenate averaged from six patients with common variable immunodeficiency (solid line) in comparison with six normal controls (dotted line). For details see legend to Fig. 1. Percentage recoveries for patients (mean ± SD): VIP 89±12, motilin 97±13, GIP 89±14, gastrin 103±19, somatostatin 99±6, secretin 87±19, enteroglucagon 89±12, protein 94±17.*

mucosal regulatory peptide secretory granules and the concentrations of the peptides have been systematically investigated in the human jejunum in a wide range of disease states. The sucrose density gradient experiments indicate a selective increase in the soluble fraction of somatostatin and gastric inhibitory peptide in the four diseases in which diarrhoea is associated with histological jejunal abnormalities. This suggests a selective increase in fragility of the storage granules of these two

peptides.[15] The changes are most marked in coeliac disease where the inflammatory changes are greatest and less marked in tropical malabsorption, Crohn's disease, and common variable immunodeficiency. The pattern of the peptide hormone secretory granule abnormalities does not correlate with the abnormal plasma peptide release reported in these syndromes. Thus in coeliac disease there is a failure of release of GIP and secretin, increased release of enteroglucagon and no change in gastrin release in
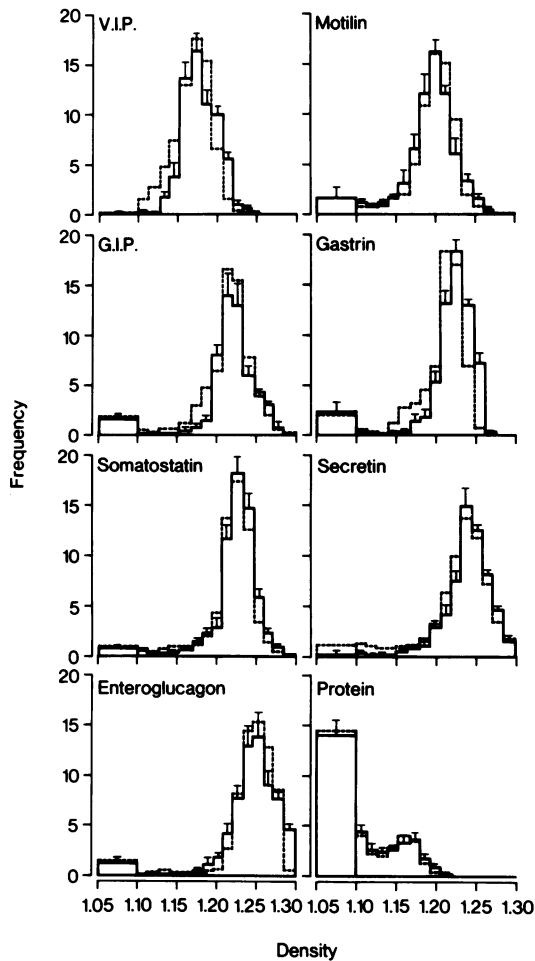

Fig. 5 *Isopycnic centrifugation of postnuclear supernant from jejunal biopsy homogenate averaged from six patients with pancreatic insufficiency (solid line) in comparison with six normal controls (dotted line). For details see legend to Fig. 1. Percentage recoveries for patients (mean ± SD): VIP 96±12, motilin 103±13, GIP 97±8, gastrin 89±12, somatostatin 88±19, secretin 97±12, enteroglucagon 104±13, protein 91±15.*
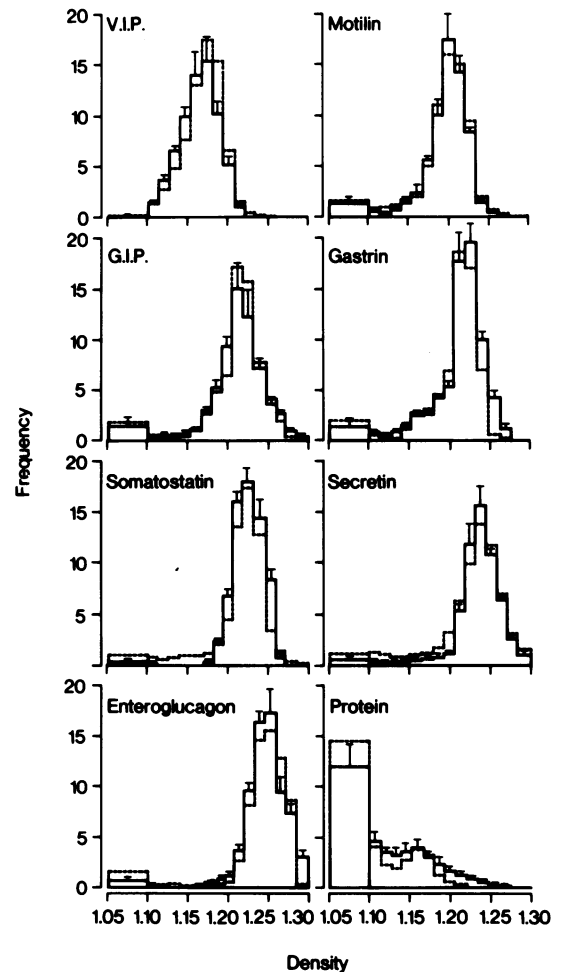
Fig. 6 *Isopycnic centrifugation of postnuclear supernant from jejunal biopsy homogenate averaged from six jejuno-ileal bypass patients (solid line) in comparison with six normal controls (dotted line). For details see legend to Fig. 1. Percentage recoveries for patients (mean ± SD): VIP 87±12, motilin 94±19, GIP 89±14, gastrin 104±12, somatostatin 101±17, secretin 79±18, enteroglucagon 91±12, protein 94±18.*

response to intraluminal stimuli,[16][17] while the secretory granule abnormalities in the present study are confined to somatostatin and GIP. Similarly, in tropical malabsorption[18] and Crohn's disease[19] the characteristically abnormal pattern of peptide release does not reflect the secretory granule abnormalities shown. Furthermore, while a different pattern of abnormality is found in each of these disease states, the secretory granule abnormality is common to all. This, together with the exclusive association of the secretory granule abnormalities with inflammatory diseases, suggests that the granule changes are most likely to be secondary to the final histological insult. The exact mechanism of the secondary changes is uncertain but increased fragility of both lysosomes and brush borders have been noted in coeliac disease[15] and tropical malabsorption.[20] Further it has been argued that the lysosomal abnormalities may be implicated in the enterocyte damage in coeliac disease.[15] It is
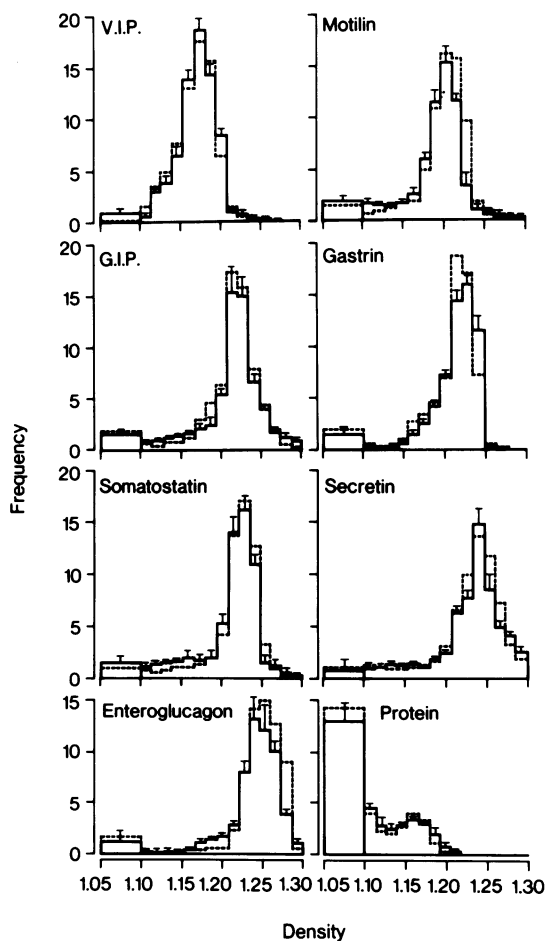

Fig. 7   *Isopycnic centrifugation of postnuclear supernant from jejunal biopsy homogenate averaged from three patients with purgative associated diarrhoea (solid line) in comparison with six normal controls (dotted line). For details see legend to Fig. 1. Percentage recoveries for patients (mean ± SD): VIP 94±12, motilin 97±19, GIP 102±15, gastrin 104±12, somatostatin 97±12, secretin 89±15, enteroglucagon 101±12, protein 93±8.*
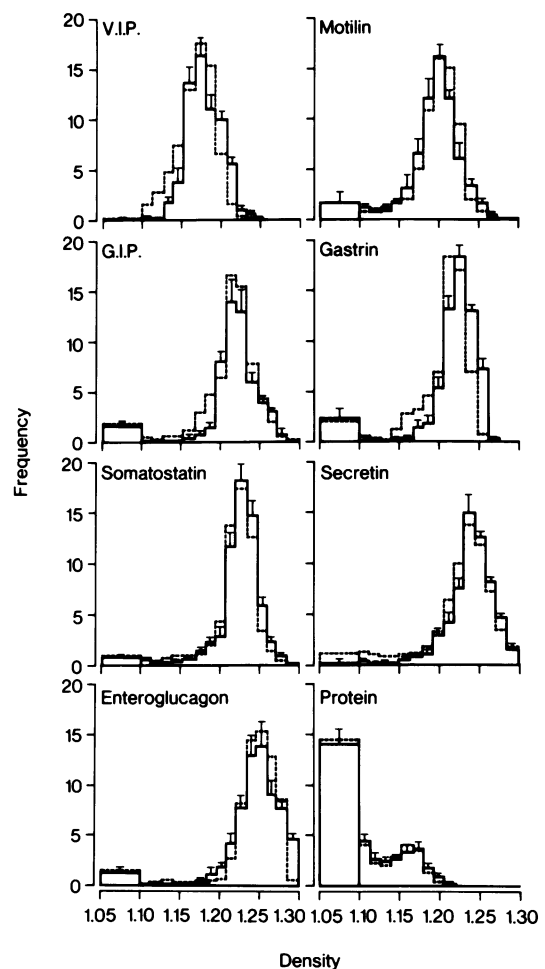
Fig. 8   *Isopycnic centrifugation of postnuclear supernant from jejunal biopsy homogenate averaged from six patients with irritable bowel syndrome (solid line) in comparison with six normal controls (dotted line). For details see legend to Fig. 1. Percentage recoveries for patients (mean ± SD): VIP 87±12, motilin 101±6, GIP 89±15, gastrin 94±12, somatostatin 101±13, secretin 102±8, enteroglucagon 96±17, protein 93±12.*

possible therefore that such lysosomal changes may affect the endocrine cells and that the GIP and somatostatin granules are particularly susceptible to damage.

The abnormalities in mucosal peptide concentrations, although in some instances marked, must be interpreted with more caution. In coeliac disease the well documented failure of release of upper small bowel peptides into the plasma[16] [17] [21] raises the possibility that the high mucosal concentrations found in this study result from an accumulation of peptides in the coeliac mucosa. The gross distortion of mucosal architecture, oedema, and inflammatory changes, however, may produce artificial changes in mucosal peptide concentrations when expressed in terms of protein content. Thus it would be premature to draw firm conclusions. The results do, however, accord with the morphological and immunocytochemical studies which show a generalised hyperplasia of gut endocrine cells[2] [4] in adult coeliac mucosa. Similarly, in Crohn's disease the increased mucosal VIP correlates well with abnormalities of the innervation of the bowel wall,[22] particularly the VIP innervation.[5] [23] Although the current studies do not shed further light on the mechanism of the abnormal peptidergic innervation, the sucrose density gradient experiments do indicate that, despite the gross abnormalities of the neurones, the secretory granules themselves have normal properties.

The increased mucosal concentration of motilin in the irritable bowel syndrome and gastrin and GIP in pancreatic insufficiency likewise do not correlate with the plasma abnormalities reported in these conditions.[24-26] Motilin, however, is known to be concerned with gastrointestinal motility[27] [28] and the increased mucosal concentrations observed in the irritable bowel syndrome may reflect intramucosal paracrine processes which could be concerned with the pathogenesis of this syndrome. The mucosal peptide changes in chronic pancreatitis are not readily explained. Histochemical studies have shown increased numbers of GIP but not gastrin cells in this condition.[3] It has also been reported that experimental pancreatic duct ligation is associated with secondary changes in the small bowel epithelial cells;[29] it is thus possible that the observations in this condition are a secondary morphological phenomenon with no functional sequelae.

In conclusion, these studies give no support to hypotheses that the regulatory peptides studied are aetiologically important in the disease states investigated. They do, however, provide important new insights to the properties of the secretory granules, in particular indicating that the GIP and somatostatin granules differ in some way from other granules being more susceptible to damage by mucosal inflammation. The mechanism of this damage and the reason for the differential susceptibility of these two secretory granules merit further study.

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