Gastric inhibitory polypeptide (GIP)*

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As long ago as 1930, Kosaka and Lim proposed a humoral agent capable of inhibiting gastric acid secretion after a meal. They coined the term entero-gastrone. Using crude preparations of cholecysto-kinin-pancreozymin (CCK PZ) in dogs they were able to inhibit the acid secretion normally stimulated by a meat meal or histamine. They went on to experiment with duodenal extracts, prepared after the instillation into the duodenum of olive oil, and found a similar effect to that seen with crude CCK PZ. The effects of similar crude preparations of CCK PZ in the dog were confirmed by Brown and Pederson in 1970, but further purification of this material led to a diminution of the acid inhibitory effect (Brown and Pederson, 1970). In 1971, Brown and Dryburgh purified and sequenced this gastric inhibitory fraction and found it to be a polypeptide quite distinct from other known peptide hormones. By virtue of its acid inhibitory properties they named it gastric inhibitory polypeptide, GIP.

Chemistry

GIP consists of 43 amino-acid residues in a single chain, with a molecular weight of 5105 daltons. The amino-acid sequence is shown below. Residues which have been underlined correspond to sequence homologies with other members of the secretin-VIP-glucagon family of peptides.

Tyr¹-Ala-Glu-Gly-Thr⁵-Phe-Ile-Ser-Asp-Tyr¹⁰-Ser-Ile-Ala-Met-Asp¹⁵-Lys-Ile-Arg-Gln-Gln²⁰-Asp-Phe-Val-Asn-Trp²⁵-Leu-Leu-Ala-Gln-Gln³⁰-Lys-Gly-Lys-Lys-Ser³⁵-Asp-Trp-Lys-His-Asn⁴⁰-Ile-Thr-Gln

Chromatographic analysis of jejunal extracts and of serum taken after a mixed meal has shown that GIP exists in more than one molecular form (Brown *et al.*, 1975; Sarson *et al.*, 1979). Three distinct peaks of immunoreactive GIP have been shown (Fig. 1).

- (1) A high molecular weight fraction which occurs in the void volume. A significant reduction in this peak is seen following pretreatment of the

sample with 8.0 M urea. Thus, this peak may represent a protein/peptide complex.

- (2) A large molecular form which may correspond to either a 'big GIP' or a precursor pro-GIP.
- (3) A 5000 dalton molecular form. This peak elutes in the same position as porcine standard and ¹²⁵I-labelled GIP.

The exact nature and properties of these different molecular species require further analysis to determine which form or forms of GIP are biologically active.

On the basis of several amino-acid sequence homologies GIP has been placed in the classical secretin-glucagon-VIP family of peptides. Indeed

Fig. 1 Chromatographic analysis of jejunal extract and fasting plasma showing the peaks of immunoreactive GIP. The position of several markers is indicated below the plasma extract.

*Now often referred to as glucose-dependent insulinotropic polypeptide.

these structural similarities are mirrored effectively in shared pharmacological activities. Examples of properties common to these substances are inhibition of gastric acid secretion and the *in vivo* and *in vitro* release of insulin.

CELLULAR LOCALISATION AND DISTRIBUTION

The availability of suitable antisera to GIP has made it possible to localise the GIP-containing cells by indirect immunofluorescence studies. Work reported in 1973 by Polak and colleagues tentatively identified the GIP cell as residing in the middle layer of the upper intestinal mucosa. Recently the site of anti-GIP antiserum activity has been shown to be the K cell, previously classified by electron microscopy according to the appearance of its granules (Buffa *et al.*, 1975).

Radioimmunoassay of GIP in extracts of homogenised gut tissue has confirmed these histochemical findings. Immunoreactive GIP (IR GIP) has been found to be distributed throughout the upper small intestine (Fig. 2), with the greatest concentration in the jejunal mucosa (Bloom *et al.*, 1975).

MEASUREMENT

The crude bioassay employed by Brown and co-workers in the course of purifying GIP is far too insensitive to detect the small quantities in the circulation released by physiological stimuli. This is only possible by the use of a sensitive and highly specific radioimmunoassay. Several such assays have been reported (Kuzio *et al.*, 1974; Lauritsen and Moody, 1978; Morgan *et al.*, 1978; Sarson *et al.*, 1979), but there appears to be little agreement between different authors with regard to basal and postprandial serum concentrations. In some cases ten-fold differences have been reported.

Several factors probably contribute to these differences. Primarily, the affinity of the antisera for each different molecular form of IR GIP needs to be determined, and also whether the forms measured reflect biological activity.

Secondly, but of equal importance, are the difficulties arising from the poor antigenicity of GIP, and the need to conjugate it with a larger molecule. The latter procedure may lead to conformational damage to the molecule, resulting in subsequent problems in the assay. Moreover, the shortage of pure GIP has led to the use of large amounts of impure preparations for the raising of antisera. The major consequence of this is the production of a heterogeneous population of antibodies with low specificity.

Another common difficulty is the preparation of the radio-labelled peptide with high specific activity and full immunogenicity. The reason for this is not

Fig. 2 Distribution of GIP in the gastrointestinal tract of man. The numbers denote the concentration of immunoreactive GIP, and the shading denotes the frequency of GIP-containing cells.

completely clear. A possible factor is the presence of two tyrosine residues which may result in excessive incorporation of 125 iodine. Another is oxidation of the methionine residue during iodination. The resulting damaged peptide is poorly recognised by the antisera and is unstable, with a short shelf life.

Some assayists have also noted non-specific interference by plasma, and plasma extraction techniques have been introduced. Some authors have even been tempted to set up their standard curves in buffer devoid of plasma, thereby effectively hiding the problem. In several cases GIP antibodies have also been shown to be rapidly dissociated from 125 I-GIP by charcoal slurry. This has necessitated the use of alcohol precipitation or double antibody separation techniques, though it is a feature generally found with low affinity antisera.

In spite of the difficulties encountered with GIP radioimmunoassays and the differences in reported values of circulating hormone, there has been considerable agreement on the nature of the stimuli which release GIP. The results so far have provided much useful and exciting information with regard to the physiology and pathology of this new hormone.

Physiology

There have been several actions described for GIP and these are listed below.

- (1) The inhibition of gastric acid secretion.
- (2) The inhibition of pepsin secretion.
- (3) The inhibition of gastric motor activity.
- (4) The stimulation of insulin release.
- (5) The stimulation of glucagon release.
- (6) The stimulation of the flow of jejunal water and electrolytes.

Some of these actions are now considered to be pharmacological rather than physiological as they are only seen with plasma concentrations far higher than those encountered after natural stimuli. Thus, the role of GIP in gastric acid inhibition and as an enteric releaser of insulin have commanded the most attention.

The ingestion of a mixed meal results in an increase of circulating GIP concentration, the peak occurring after about 45 minutes. Separation of the meal into its constituent nutrients showed that the most potent stimuli to GIP release are carbohydrates and fat (Cleator and Gourlay, 1975). Individual amino-acids also increase the plasma concentration but there is no response to oral protein (Fig. 3).

Fig. 3 Plasma GIP concentration after a test breakfast or individual nutrients given orally. The meal and the individual components were given to normal volunteers in isocaloric amounts (2225 kJ).

Intravenous administration of these nutrients does not elicit a GIP response.

INHIBITION OF GASTRIC ACID SECRETION

GIP was originally discovered and named as the acid inhibitory fraction of crude CCK PZ. Its release by fat and glucose, which are both potent inhibitors of gastric acid secretion, further suggested an 'enterogastrone' role. Much of the initial work was carried out in dogs, in which a clear relationship between inhibition of acid secretion and GIP release was seen. Thus, infusions of porcine GIP, to attain circulating levels within the physiological range, inhibited pentagastrin-stimulated acid secretion in dogs with denervated fundic pouches (Pederson and Brown, 1972); there was a good dose/response relationship. The instillation of lipomul, a triglyceride suspension, into the duodenum evoked a rise in circulating plasma IR GIP and a reduction in acid secretion of the order of 70%. However, the hydrogen ion secretion returned to normal while the circulating GIP levels remained substantially raised. This differed from the effect of intravenous GIP, after which acid secretion returned to control levels concurrently with the fall in GIP. It was suggested that the triglyceride mixture released the larger molecular form of GIP which had no enterogastrone activity (Pederson *et al.*, 1978).

The role of GIP in the inhibition of acid secretion in man is less well understood than in dogs, and has given rise to conflicting reports. One group has shown that the amount of exogenous GIP required to inhibit acid secretion after pentagastrin or a peptone meal was of pharmacological proportions (Arnold *et al.*, 1978), the rise in circulating GIP being some five times greater than after a meal. They concluded that although GIP may be of importance in some pathological states it was of doubtful significance in normal man. It is, of course, possible that no single factor is responsible for the physiological inhibition of acid secretion but that GIP is involved with one or more other peptides. Recently, evidence for this has been adduced by Christiansen *et al.* (1978) who found that the instillation of fat into the jejunum caused dramatic inhibition of gastric acid secretion and a significant increase in circulating plasma levels of GIP, enteroglucagon (GLI), and VIP. There was no clear correlation of inhibition of acid secretion with any of the single peptides under review. Further work is required in order to elucidate the role of GIP as a putative 'enterogastrone'.

INSULINOTROPIC ACTION OF GIP

La Barre in 1932 made the suggestion that the duodenum produced a hormone which exerted an effect on the endocrine pancreas. He coined the term

'incretin' or insulin-releasing factor (La Barre, 1932). In 1964 McIntyre made the classical observation that an oral glucose load produced a much greater insulin response than a similar dose given intravenously (McIntyre *et al.*, 1964). Since that time a great deal of work has been devoted to the search for the humoral factor responsible for this increased insulin response to oral carbohydrate.

The realisation that the structure of GIP was similar to that of VIP, secretin, and glucagon, all of which were known to be insulin secretagogues, stimulated speculation on the role of GIP as an incretin. The fact that oral glucose elicited a prompt response of GIP, while intravenous glucose had no such effect, led Dupré *et al.* (1973) to investigate the insulin-releasing properties of this hormone in man. Glucose was infused intravenously with and without exogenous GIP. They reported a significant augmentation of the insulin release when glucose and GIP were infused together. Following this, many attempts have been made to establish that GIP is indeed the gastrointestinal signal for insulin release.

The insulinotropic action of GIP would appear to be demonstrable only when blood glucose is raised. For example, the ingestion of fat by fasting subjects produces a potent GIP response but no rise in plasma insulin. However, if a fat meal is given during a constant glucose infusion a large insulin release occurs *pari passu* with the rise in GIP. Further evidence that hyperglycaemia is necessary for the 'incretin' effect of GIP is to be found in the elegant study of Andersen *et al.* (1978). These authors conclude that a blood glucose threshold mechanism to control the insulinotropic action of GIP is teleologically appropriate as it would prevent the release of large amounts of insulin at a time when there is insufficient substrate present, thereby avoiding the dangers of hypoglycaemia. While this insulinotropic effect is dependent upon raised blood glucose levels when evoked by the administration of fat or glucose, the response to amino-acid ingestion is not hyperglycaemia-dependent, and may be triggered by direct stimulation of the B-cell by the nutrients.

GIP, then, fulfils many of the criteria required for the role of 'incretin', and under the right conditions it is undoubtedly insulinotropic. In view of this, and since its role as a physiological inhibitor of acid secretion is less certain, many workers now refer to this hormone as Glucose-dependent Insulinotropic Polypeptide, thereby retaining the original acronymic form. There are, however, several objections to the suggestion that GIP is the only, or indeed the most important, component of the 'enteroinsular axis' (see paper by Professor Marks, page 38).

The other potential physiological actions of GIP,

such as its stimulation of intestinal juice production, are less well established and are therefore not included in this discussion. Further investigation is required to elucidate the full biological importance of this fascinating peptide.

Pathology

The most striking examples of hypersecretion of gut hormones are those resulting from endocrine tumours. Although such tumours are known to secrete two members of the secretin-glucagon family of peptides, VIP and glucagon, no definite GIPoma has yet been described. The known pharmacology of GIP, however, has prompted a great deal of research into the possible role of this hormone in the aetiology of diabetes mellitus and duodenal ulcer disease.

GIP IN DIABETES MELLITUS

In view of the importance of GIP in carbohydrate metabolism, much speculation has occurred about what part, if any, it plays in diabetes mellitus. Since diabetes is characterised by a pathological oral glucose tolerance curve, often with little or no insulin response, the hyposecretion of an enteric signal could be an important facet of this disease. Measurement of the GIP response after a meal in well-controlled juvenile diabetics, however, has been shown to be indistinguishable from normal (Ebert *et al.*, 1976). In cases of maturity onset diabetes the GIP response has been reported to be augmented after a mixed meal or oral glucose (Crockett *et al.*, 1976). Other workers have found this response to be raised only in obese patients (Bloom, 1976; May and Williams, 1978).

Attempts to explain this phenomenon have revolved around an insulin-GIP interrelationship. It has been postulated that insulin exerts a negative feedback control on GIP release (Brown *et al.*, 1975). The augmented GIP response seen in some diabetics could thus be explained as the result of a defective feedback mechanism. An augmented GIP release has also been reported in some obese patients with hyperinsulinaemia, and it is suggested that in this circumstance the GIP cell becomes insulin resistant (Creutzfeldt *et al.*, 1978). In normal subjects given infusions of insulin, there is little evidence to suggest that this negative feedback control exists (Andersen *et al.*, 1978). The effect of insulin on GIP release in pathological circumstances may, however, be quite different.

In addition to the possible direct involvement of GIP in diabetes, another property of this hormone has been investigated, that of a glucagon secretagogue. It has been shown that infusions of GIP into diabetic subjects resulted in a significant rise in serum

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glucagon levels. This effect was totally absent in the control group (Ross *et al.*, 1974). Thus, it may be an indirect action of GIP via glucagon which is reflected in pathological glucose tolerance.

GIP IN DUODENAL ULCER DISEASE

Duodenal ulcer disease is characterised by acid hypersecretion, which in turn could be the result of the hyposecretion of an inhibitory factor. Since GIP was first discovered by its acid inhibitory properties, it has been proposed as being the agent responsible for duodenal ulcer. Observations made so far have been as equivocal as those on GIP release in diabetes. The GIP response has in fact been found to be exaggerated in some patients with duodenal ulcers (Creutzfeldt *et al.*, 1977). This may be because of the rapid gastric emptying which often occurs in ulcer disease, as it has been shown that acid in the duodenum stimulates GIP release. This augmentation of GIP release in duodenal ulcer fits well with the increased insulin response reported in these patients.

GIP IN OTHER GASTROINTESTINAL DISORDERS

Recently, Besterman and others have investigated the release of GIP and other gastrointestinal hormones in a number of diseases which affect different segments of the gut. As the findings are dealt with in detail by Dr Besterman on page 76, the GIP changes will only be summarised here. In coeliac disease and tropical sprue, the GIP release in response to a standard meal was greatly diminished and accompanied by a significant reduction in the insulin response (Fig. 4, 5). In cases of mal-

Fig. 4 Plasma GIP concentration after a test meal given to patients with active coeliac disease (untreated), patients who responded to a gluten-free diet (GFD), and normal controls (Besterman *et al.*, 1978b). Reproduced by permission of *The Lancet*.

Fig. 5 Plasma GIP concentration after a test meal in 6 patients with acute postinfective malabsorption syndrome (tropical sprue) and 11 normal age- and sex-matched controls (Besterman *et al.*, 1978c).

Fig. 6 Plasma GIP concentration after a test meal in patients with morbid obesity ($225 \pm 7\%$ ideal weight), normal controls ($106 \pm 3\%$ ideal weight), and patients who had undergone jejunioleal bypass surgery ($181 \pm 8\%$ ideal weight) (Besterman *et al.*, 1978d).

absorption because of pancreatic disease the response was normal (Besterman *et al.*, 1978a). In Crohn's disease and ulcerative colitis the GIP response was actually increased.

Studies were also made in cases of morbid obesity. In these the augmented blood glucose and insulin response to the meal were not accompanied by a corresponding change in GIP release. After jejunioleal bypass, however, there was a dramatic five-fold decrease in the incremental response of GIP and diminished release of insulin (Fig. 6). It has been suggested that this lowered response of GIP to a meal may be an important factor in the process of weight loss and that a defective feedback of insulin on GIP may be a major factor in obesity (Creutzfeldt *et al.*, 1978).

The part played by GIP in disease is as yet unclear. The relationships between GIP and insulin release and GIP and inhibition of acid secretion appear to be complex. For the present the assay of GIP has no obvious application in the routine laboratory. In the future, however, the measurement of GIP as a marker for upper intestinal disease, along with other hormone markers for the stomach, pancreas, and distal intestine, may well prove to be a useful tool in the diagnosis and follow-up of treatment in gastro-intestinal disease.

Conclusion

Gastric inhibitory polypeptide has, in the comparatively short time since its discovery, been shown to display a wide range of pharmacological effects. The release of insulin and inhibition of gastric acid secretion may well prove to be physiological effects of this hormone. Further research into these aspects of the biology of GIP may provide valuable insights into the control of alimentary function in both health and disease.

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