The enteroinsular axis

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Rediscovery of the role of the gastrointestinal tract in the control of carbohydrate metabolism and, as we now know, fat and protein metabolism, was made independently in London by two groups of workers (Dupré, 1964a; McIntyre *et al.*, 1964). The major credit undoubtedly belongs to McIntyre and his colleagues (1964, 1965) who, by the simplest of methods, namely comparing the glycaemic and insulinaemic responses to equal amounts of glucose given by intravenous and intrajejunal infusion, and using only two subjects initially, were able to show that the gut mediated an augmentation of the insulinaemic response to hyperglycaemia. Despite an enormous amount of effort by many workers since that time, it is still not possible to attribute, with any degree of certainty, the role of intestinal augmenter of glucose-stimulated insulin release to any one agency. Nervous factors undoubtedly play a role but will not be considered here. Of the many hormonal agents that have been proposed, some meet the bill better than others. For the sake of brevity I shall exclude from further consideration those which I believe play only a minor or non-existent role and concentrate upon those substances that I have personally been most concerned with or consider to be potentially physiologically important, namely glucagon, gut glucagon-cross-reacting material, or GLI (often, perhaps inappropriately, referred to as enteroglucagon), insulin-releasing polypeptide (IRP) and GIP.¹ Several comprehensive reviews are available and interested readers are referred to them (Marks and Samols, 1970; Marks and Turner, 1977).

Glucagon

At more or less the same time that McIntyre and co-workers (1964, 1965) were showing the existence of a hypothetical intestinal factor which was involved

in the stimulation of insulin secretion, and Dupré (1964b) was reviving interest in 'incretin', the purported intestinal hormone responsible for improving oral over intravenous glucose tolerance, my colleagues and I (Samols *et al.*, 1965a, 1966a) were investigating the insulintropic effects of glucagon. We showed, what we had already suspected on circumstantial evidence, that glucagon had a direct stimulatory effect on the β -cells of the pancreatic islets. It was shown, moreover, that the insulintropic effect was enhanced by even minimal hyperglycaemia. Although large doses of glucagon injected rapidly were initially used to show its insulintropic effect, smaller doses, as little as 100 ng per minute, could also be shown to be stimulatory.

These early observations created difficulties and, not to put too fine a point on it, were frankly disbelieved by some investigators. Insulin and glucagon had, for many years, been looked upon as mutual antagonists, insulin lowering and glucagon raising blood glucose levels. This view of their physiological roles was, and still is, difficult to reconcile with the fact that glucagon is one of the most potent direct stimuli to insulin secretion known. It is even more so when the β -cells of the pancreas are, because of their juxtaposition to the α -cells, subjected earlier and to higher concentrations of the hormone than any other cells of the body, including those of the liver. We postulated that in man and other mammals in which α - and β -cells occur in the same islets, glucagon might be one, not necessarily essential, step in the cascade amplification of the augmenting signal to insulin secretion transmitted from the GI tract to the pancreas in response to the ingestion of food. This is still my opinion and is supported by a substantial body of direct and indirect evidence (Marks and Turner, 1977), some of which will be summarised later in this paper. The seemingly damning fact that insulin and glucagon levels in peripheral, and even pancreatic, blood do not always (indeed only rarely) change in the same direction in response to the various stimuli to their secretion is explicable on the basis of modulation of the hormonal responses to humoral factors by the sympathetic and parasympathetic nervous systems, and the fact that

¹Abbreviations. Many of the gut hormones are known by their initials and in some cases these have become their semi-official names. GIP originally stood for gastric inhibitory polypeptide, but as its possibly greater significance as a stimulator of insulin release became appreciated, it was used to denote its glucose-potentiated insulin releasing properties. (Glucose-dependent Insulintropic Peptide). VIP stands for vasoactive intestinal peptide, GLI for glucagon-like immunoreactivity.

changes in plasma hormone levels observed in blood reflect the nett effect of a number of stimulatory and inhibitory agents acting simultaneously and possibly independently (Samols and Harrison, 1976) upon individual cells which are capable of reacting in only a very limited number of ways.

Gut glucagon-like immunoreactivity (GLI)

It was known from the work of Sutherland and his colleagues (Sutherland and De Duve, 1948; Makman and Sutherland, 1964) that in some animals at least the intestine contained a substance with properties similar in many respects to glucagon. It was knowledge of this fact that made us look, with what are now recognised to be the crude immunoassay techniques available to us at that time, first at the plasma glucagon response to oral glucose (Samols *et al.*, 1965b) and then at the gut itself (Samols *et al.*, 1966b). We reported for the first time that a rise in plasma immunoreactive glucagon followed the ingestion of a glucose load but later, in view of the fact that it was also observed in patients who had undergone pancreatectomy (Samols and Marks, 1967), suggested that the material measured originated from the gut rather than from the pancreas. On the basis of cross-reactivity studies we (Samols *et al.*, 1966b) suggested that the glucagon immunoreactive material released from the gut was not, as Makman and Sutherland (1964) had suggested, identical with (pancreatic) glucagon, but only shared some of its immunological determinants.

The glucagon-like immunoreactive material of the gut has not yet been obtained in pure form (if indeed, as seems unlikely, it is a single substance) and its biological properties have consequently not been ascertained. There is, however, suggestive evidence that it can, at least under certain circumstances, augment glucose-stimulated insulin secretion from β -cells *in vitro* and that this effect can be blocked by anti-glucagon antisera.

Investigations revealed that other sugars which stimulated insulin release when taken orally but not when given intravenously, notably galactose, also stimulated GLI release (Marks and Samols, 1969), whereas those, such as fructose, which did not stimulate GLI release, had no insulin-stimulating properties at all, whether taken intravenously or orally. Further support for involvement of GLI in insulin secretion came from the observation that subjects who had undergone partial gastrectomy, and experienced signs and symptoms of reactive hypoglycaemia as a result of stimulated hyperinsulinaemia, exhibited greater than normal rises in plasma GLI levels after oral glucose administration (Samols and Marks, 1967). While admitting that

the case for the participation of gut GLI in the stimulation of insulin secretion has not yet been made conclusively, it must, I submit, remain under consideration and not be dismissed, as 'incretin' itself once was, for want of conclusive proof.

Insulin-releasing polypeptide (IRP)

Turner, one of the co-rediscoverers of the enteroinsular axis, was not satisfied that any of the gastrointestinal hormones known up till that time had the properties suitable to fit it for the role of intestinal mediator of insulin release. Consequently, he applied himself to the purification and identification of the hypothetical 'incretin', and in 1972 reported the isolation, partial purification, and pharmacological characterisation of an insulin-releasing polypeptide (IRP) from hog intestine (Turner, 1972; Turner and Marks, 1972). Unlike crude extracts of intestinal mucosa, IRP regularly stimulated insulin release both in the rat (Turner and Marks, 1972) and the baboon (Turner *et al.*, 1974a), but only in the presence of at least minimal hyperglycaemia. The insulinotropic effects of an IRP-containing infusion were maintained for as long as the infusions continued and were markedly attenuated by simultaneous adrenaline administration (Shabaan *et al.*, 1974). Shortly after his description of IRP, Turner obtained small amounts of each of three newly isolated intestinal hormones from their respective discoverers and tried them out in his bioassay system. Whereas motilin was ineffective, and VIP only very weakly effective, in stimulating insulin release, GIP was extremely active exhibiting a dose response curve similar in potency to that of glucagon, hitherto the most powerfully insulinotropic agent known, and almost 1000 times as potent as IRP (Turner *et al.*, 1974b). Simultaneously and independently, Dupré and his colleagues (1973) described the insulinotropic effects of GIP in man. Examination by radioimmunoassay (Morgan *et al.*, 1978) of some of the IRP preparations used by Turner and co-workers (1973, 1974b) showed that many of them contained sufficient GIP to account for the insulinotropic effects. While it is impossible to be certain why cruder extracts of porcine mucosa than those prepared by Turner, and called IRP, are usually ineffective in stimulating insulin release, it may be related to the presence, in the unpurified mixture, of the inhibitor of insulin release somatostatin, whose existence in mucosal extracts was, until recently, quite unsuspected.

Glucose-dependent insulinotropic peptide (GIP)

The availability of a radioimmunoassay for the measurement of GIP in plasma (Kuzio *et al.*, 1974)

soon led to the discovery that GIP was released from the gut in response to the ingestion of not only glucose, but also triglycerides (Brown *et al.*, 1975). The latter observation explained earlier reports that while fats taken alone by mouth had no stimulatory effect upon insulin release, when taken with glucose either by mouth or by intravenous infusion, they markedly improved glucose tolerance and stimulated insulin release (Marks and Samols, 1969).

Galactose, like glucose, with which it shares an active intestinal transport system, is a potent stimulus to GIP release and, in the presence of induced hyperglycaemia, insulin secretion. Fructose, on the other hand, which has no insulin stimulatory properties whether taken by mouth or intravenously, apart from that attributable to a small rise in blood glucose that sometimes follows its parenteral or oral administration, does not stimulate GIP release (Watts *et al.*, 1978).

The insulintropic effects of exogenous GIP are, like those of glucagon, barely discernible at 'physiological' doses, except in the presence of mild to moderate hyperglycaemia. This raises the possibility that GIP might in fact exert its effect mainly, or even exclusively, by promoting glucagon release from pancreatic α -cells.

Glucagon, as we have seen, is a potent stimulus to insulin secretion and its liberation by the α -cells ensures that it is ideally placed to amplify any signal to insulin release coming from the intestinal tract. GIP, it transpires, not only stimulates the release of insulin, but also that of glucagon, which may escape detection unless great care is taken (Dupré *et al.*, 1978). Under experimental conditions (synthetic) GIP administered to fasting rats produced a relatively much larger rise in peripheral plasma glucagon than in plasma insulin levels, a situation that was completely reversed when induced hyperglycaemia was present (Taminato *et al.*, 1977). Under these circumstances the insulintropic effects of GIP were dominant and the glucagon stimulatory effect of the GIP was manifested only as a failure to observe the usual fall in plasma glucagon levels produced by intravenous glucose alone, which is probably a consequence of the direct inhibitory effect of glucose-induced insulin release on α -cell function (Samols and Harrison, 1976).

Further, admittedly indirect, evidence of the role played by pancreatic glucagon in mediating insulin release by enteric, and possibly other, stimuli to β -cell function includes observations that an increase in glucagon secretion precedes the rise in insulin secretion during evolution of the secretory responses to many, possibly the majority of, stimuli to insulin secretion (Dencker *et al.*, 1975; Pek *et al.*, 1976). Unfortunately, it has not yet proved possible in the

experimental situation to produce selective α -cell destruction, that is, glucagon deficiency analogous to the selective β -cell damage, or insulin deficiency, produced by alloxan and streptozotocin. Cobalt chloride and neutral red, two substances which have been claimed on standard histological evidence to damage α -cells selectively, are, in our experience, incapable of doing so; indeed, cobalt chloride-treated rats have persistently higher rather than lower plasma glucagon levels than controls. Nevertheless, the insulintropic effects of arginine, normally a potent stimulus to both insulin and glucagon secretion, can be abolished or greatly reduced by immunising rabbits with glucagon, thereby producing high titre antibodies capable of binding exogenous (and presumably, therefore, endogenous) glucagon, and thereby abolishing its insulintropic effects (Al-Tamer, 1978).

The evidence implicating GIP, secretin, and gastrin in insulin secretion has recently been reviewed by Brown and Otte (1978).

Enteroinsular interactions

We have recently confirmed, using our own non-glucagon cross-reacting GIP antiserum, the immunohistochemical observations of Smith *et al.* (1977) and Garaud *et al.* (1978) that, in some species, glucagon immunoreactive cells in the pancreatic islets react also with GIP antiserum, even in the presence of excess exogenous glucagon. These observations suggest that the α -cells contain both hormones, though whether the immunoreactive GIP is produced by the α -cells or transported from the gut and absorbed onto receptors on the surface of the α -cells is uncertain. The second alternative is an attractive one but must be considered to be no more than an unproven idea which will undoubtedly shortly be put to the test.

In contrast to the effort put into proving and investigating the mechanism of the stimulatory effect of gut hormones on insulin and glucagon secretion, remarkably little has been devoted to unravelling the role of the insular hormones on intestinal endocrine function. There is some (disputed) evidence that both insulin and glucagon exert negative feedback control on GIP secretion, especially in the basal state. An inhibitory effect of either insulin or glucagon on GIP secretion in the stimulated state need not, as has generally been assumed, be a direct one, but could be mediated indirectly by inhibition of absorption of GIP secretagogues. A hint that such a mechanism might exist, and thereby explain some of the hitherto inexplicable inconsistencies in the unravelling of the enteric-insular relationship, comes from a seldom quoted study on the role of insulin in

galactose absorption in rabbits (Beyreiss *et al.*, 1964). Further studies on other species using different GIP secretagogues have not been carried out hitherto but are currently under way in our own laboratory.

The Figure summarises, and grossly simplifies, my current views on the nature and structure of the enteroinsular axis. It ignores for the sake of convenience and lack of incontrovertible evidence the undoubtedly essential role played by the δ -cells, the source of intra-islet somatostatin, in insulin and glucagon secretion. It also omits any reference to the part played by the two divisions of the autonomic nervous system in initiating and, even more significantly, in modulating insulin, glucagon, and possibly somatostatin secretion.

Fig. Grossly simplified and highly schematic conception of relationship between gastrointestinal and pancreatic hormone secretions. A and B represent pancreatic cell types producing glucagon and insulin, respectively. IRP represents all the intestinal mediators of insulin secretion, AA represents insulinotropic amino-acids; +ve denotes stimulatory, -ve denotes inhibitory activity.

Briefly, the proposition is that glucose, possibly alone among nutrients, has a direct effect upon the β -cells of the pancreas, especially those which are situated in the middle of the islets and consequently do not adjoin either α - or δ -cells. Other nutrients in addition to glucose do, however, stimulate the release of one or more of the intestinal insulin releasing hormones. These substances also possess the ability to stimulate glucagon secretion by direct action. Pancreatic glucagon released in response to such stimulation would be an extremely potent local (paracrine) stimulus to insulin release, especially in the presence of the mild to moderate postprandial hyperglycaemia produced by the ingestion of a meal. The hyperinsulinaemia would in turn act as a

powerful inhibitor to further glucagon and possibly GIP release. It is further suggested that in man and most other mammals the hepatic, or hyperglycaemic, effect of glucagon is of secondary importance and becomes relevant in body energy economy only when the insulinotropic effect of glucagon is abolished in response to sympathetic nervous activity, for example, during fasting, fight, or flight, and in response to injury.

Physiological significance

The physiological importance of the enteroinsular axis and the consequences of its disordered function are poorly understood. Early suggestions that the delayed release of insulin in response to the ingestion of a meal that characterises maturity-onset diabetes might be the result of defective secretion of the intestinal mediator has neither been established nor disproved. There is some evidence that GIP secretion is abnormal in some types of obesity and might, therefore, be responsible for the hyperinsulinaemia and consequent excessive deposition of adipose tissue and conservation of energy which is characteristic of this disorder. Whether the abnormality of GIP secretion is a result of the hyperplasia of the GIP secreting cells seen in some types of obesity, and itself possibly a secondary consequence of hyperalimentation because of hyperphagia, has still to be established. Clearly the enteroinsular axis impinges upon a number of metabolic and dietary problems encountered in clinical practice, but at present hard data are too few to permit anything more than speculation.

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