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Review Articles

The Incretin Concept Today*

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Summary. 1. The insulinogenic factor of the gastrointestinal mucosa named *"incretin"* is only one part of the complex enteroinsular axis. – 2. Of the chemically defined gastrointestinal hormones GIP is the strongest *incretin* candidate. – 3. Because of the dual function of GIP as gastrone and insulinotropic substance several safeguards against GIP-mediated insulin hypoglycaemia exist. – 4. No pathological condition has yet been found which is causally related to hyper- or hyposecretion of GIP. However, an exaggerated GIP response (usually secondary to the disease) may participate in the pathogenesis of hyperinsulinaemia of patients with obesity and duodenal ulcer. – 5. The injection of GIP antibodies only partially abolishes the *incretin* effect. Therefore, GIP, although important, is not the only *incretin.*

Key words: Incretin, entero-insular axis, gastrointestinal hormones, insulin secretion, GIP.

1. Historical Background

It has been known for a long time that much larger amounts of glucose can be given orally than intravenously without production of glucosuria. Claude Bernard in his "Leçons sur le diabète" [1] which were published in 1877, one year before his death, explained this superiority of oral over intravenous glucose tolerance by the fact that the liver takes up most of the glucose during the first portal circulation, thus acting as a barrier and preventing hyperglycaemia. As late as 1954, Scow and Cornfield [2] held the same view.

Long before the discovery of insulin extracts from the intestine were tested for possible metabolic effects. Moore, Edie and Abram [3] suggested in 1906 that the duodenum "supplies a chemical excitant for the internal secretion of the pancreas", by analogy with secretin controlling its external secretion. They tested the hypothesis that "in certain cases of diabetes the appearance of sugar in the urine might be due to functional disturbance occasioned by the absence of such an intestinal excitant of the internal secretion" and administered duodenal extracts to diabetics. Their results were not conclusive.

Twenty years later, La Barre and his colleagues [4, 5, 6] performed cross-circulation experiments and demonstrated that the intravenous injection of crude secretin produced hypoglycaemia in dogs via stimulation of the endocrine pancreas. They concluded that crude secretin contained two active principles: *"incretin"* stimulating the internal secretion of the pancreas and "excretin" stimulating the exocrine pancreas. At the same time, Heller [7, 8] prepared a duodenal extract which lowered the blood glucose mainly during postprandial hyperglycaemia and named it "duodenin".

For the next thirty years *"incretin"* and "duodenin" were forgotten. Revival of interest in the differences of oral and intravenous glucose tolerance commenced with the availability of the radioimmunoassay for insulin and the application of insulin estimation to this problem in 1964 by McIntyre and colleagues [9] and Elrick and colleagues [10]. A much greater insulin response was observed after an oral load than after intravenous injection of the same amount of glucose, despite a smaller increase of the blood glucose level. Perley and Kipnis [11] estimated that half of the insulin secreted after an oral glucose load was released by gastrointestinal factors.

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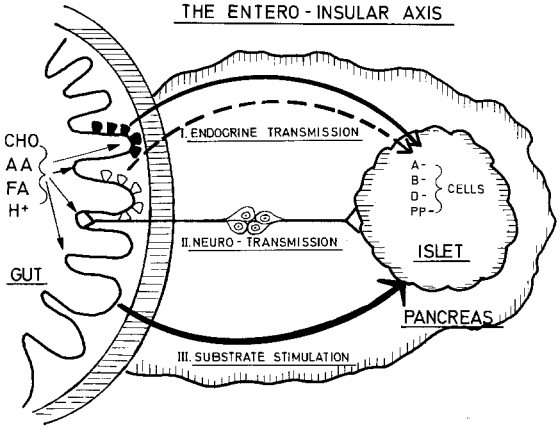

Fig. 1. The term entero-insular axis comprises all stimuli from the gut to different cell types of the pancreatic islets. CHO, carbohydrate; AA, amino acids, FA, fatty acids

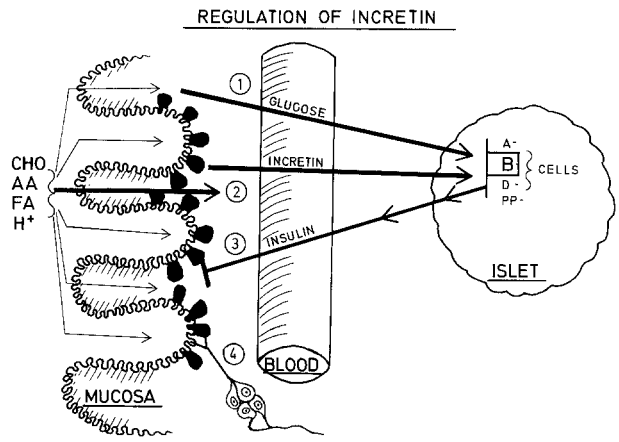

Fig. 2. The endocrine transmission of stimuli from the gut to the B-cells through an "incretin" is safeguarded by four devices preventing hypoglycaemia. The numbers refer to Table 2. Abbreviations as in Fig. 1

2. The Design of the Entero-Insular Axis (Fig. 1)

Until recently, only hormonal factors were regarded as possible transmitters of signals from the gut to the pancreatic islets, the so-called "entero-insular axis", a term coined by R. Unger [11a]. Neural influences on blood glucose homoeostasis, as first demonstrated by Claude Bernard in 1849 [12], have received very little consideration in this context.

While numerous reviews about effects of hormones on insulin secretion appear each year, the first review on neural control of the pancreas was written as late as 1974 [13]. In this context it is of interest that La Barre who coined the term *"incretin"* for the insulin releasing humoral factor of the intestinal mucosa also provided evidence that insulin can be released by vagal stimulation; he used the technique of pancreato-duodenal-jugular anastomosis in his studies [14].

Progress in the field of gastrointestinal endocrinology over the last decade paradoxically has led to a reconsideration of the importance of the neural system as a control mechanism for the gastrointestinal tract. It has been realised [15] that many gastrointestinal peptides (some with insulin-releasing potency, i. e. incretin candidates) are present in both the central nervous system and the gut, such as bombesin, cholecystokinin (CCK) enkephalin, gastrin, neurotensin, somatostatin, substance P, thyrotropin releasing hormone (TRH) and vasoactive intestinal peptide (VIP). Some of the gut peptides are found only in nerves and not in endocrine cells; some are found in both. Evidence is accumulating that these peptides may function as neurotransmitters. Consequently, in addition to the established adrenergic and cholinergic nerves, a peptidergic nervous system has to be considered [16]. If peptides with *incretin* activity can act as neurotransmitters, changes of plasma levels cannot be used to assess the physiological rôle of such peptides, as has been suggested for the acceptance of a gut peptide as a hormone [17]. It may well be that a peptide present in the nerves of the gut and the pancreatic islets like VIP [18], which has in vivo [19] and in vitro [20, 21] insulin-releasing potency, is an important component of the entero-insular axis without showing alterations of its plasma levels.

The gastrointestinal neural system may participate in the entero-insular axis in two ways. Firstly, by regulating the release of an *incretin* into the blood stream and, secondly, by direct stimulation of the islets via nerve fibres. Evidence for this stems from the observation that after pancreatectomy and orthotopic transplantation of a denervated pancreatic graft oral glucose tolerance and insulin response deteriorate while the intravenous glucose tolerance and insulin response are unchanged [22]. However, this finding needs confirmation because in another study after heterotopic transplantation of the pancreas the *incretin* effect was not significantly reduced [23].

3. Definition of Incretin (Fig. 2)

If a peptide is the neurocrine transmitter of insulin release via peptidergic fibres, this substance would not qualify as an *incretin.* Therefore, VIP can be taken off the list of *incretin* candidates despite its insulin-releasing potency. However, this does not exclude a physiological function of peptidergic ("VIPergic") innervation during food induced insulin

release. *Incretin* in the original sense refers only to *endocrine* transmitter(s) for *insulin* release, i. e. to *one* part of the complex neuro-endocrine system called "enteroinsular axis", which comprises multiple factors influencing the release of all hormones produced in the islets of Langerhans.

The search for *incretin* is the search for an *endocrine* transmitter produced in the gastrointestinal tract which

1. is released by nutrients, especially carbohydrates, and
2. stimulates insulin secretion in the presence of glucose if exogenously infused in amounts not exceeding blood levels achieved after food ingestion.

The dependency of the *incretin* effect upon the glucose level – at least in physiological concentrations of the hormone – is an important prerequisite of this principle for prevention of hypoglycaemia. Thus, an *incretin* can be released by different intestinal contents, but elevated *incretin* blood levels stimulate insulin secretion only in the presence of elevated blood glucose levels, i. e. when insulin is needed.

The endocrine (and paracrine) cells of the gastrointestinal tract respond to neural and/or blood borne stimuli as well as chemicals in the gut content. For this they are equipped with microvilli exposed to the gut lumen, possibly serving as sensors. Due to the wide distribution of the endocrine cells along the gut, the concentration of hormones released is dependent on the food load entering and passing through the gut. At least in the case of GIP, it has been shown that only the absorption and not the mere presence of foodstuffs releases this peptide. From a teleological viewpoint this also can be regarded as a safeguard against hypoglycaemia. *Incretin* and consequently insulin release is only commenced when food is being absorbed.

A third safeguard against hypoglycaemia is a feedback control of *incretin* release by insulin, as established for GIP, and a fourth security is the neural control of *incretin* release (see below).

4. Incretin Candidates

Established gut hormones, i. e. all peptides with effects on gastrointestinal functions, have been tested for their insulin releasing potency. Many of them stimulate insulin secretion in vivo and in vitro [cf. 24–28]. However, a peptide qualifying for *incretin* must stimulate insulin release in physiological doses and must respond to nutrients, especially glucose. Most gut hormones do not fulfil these two conditions. Table 1 summarizes the response of the known hormones to food ingestion. The only established gut

Table 1. Release of gut hormones with insulin releasing potency into the blood by different food components

Hormone	Released by			
	Carbo-hydrate	Protein	Fat	Acid
Gastric inhibitory polypeptide (GIP)	+	+	+	+
Gastrin	(+)	+	0	0
Cholecystokinin (CCK)	0	+	+	+
Secretin	0	+	0	+
Vasoactive intestinal polypeptide (VIP)	0	0	+	+
Glycentin (GLI-l)	0			

Table 2. Safeguards against GIP mediated insulin hypoglycaemia

1. Glucose dependence of insulin release by GIP
2. Dependence of GIP release upon absorption of nutrients
3. Feedback control of GIP release by insulin
4. Neural inhibition of GIP release

polypeptides released by glucose are GIP and gastrin. Compared to GIP the gastrin response to glucose ingestion is small [32]. Cholecystokinin (CCK) is released by protein, fat and acid; secretin only by protein and acid. Increased circulating VIP levels after fat and acid [29] do not prove an endocrine role of this peptide, which has been found to be localized in gut nerves and not in endocrine cells. Crude gut extracts containing glucagon-like immunoreactivity (GLI) release insulin and increase after glucose and fat ingestion. The presence of known insulin releasing peptides in these extracts has not been investigated. The only chemically characterized gut GLI is glycentin (GLI-1) [30]. Plasma glycentin levels do not increase after glucose ingestion in man [31].

It can be concluded from Table 1 that GIP and gastrin are the only *incretin* candidates left from the group of chemically defined gut peptides. This conclusion does not exclude the possibility that other peptides augment insulin secretion if released during the digestive phase by other stimuli or that other uncharacterized gut peptides exist with *incretin* activity.

Several gut hormones are released by protein or acid (Table 1). Also their participation in the augmentation of insulin release during food ingestion must be considered. For this it is necessary to demonstrate an insulin releasing potency with physiological serum concentrations. So far, this has been investigated for gastrin [32], secretin [33] and GIP [34, 35, 36]. Exogenous gastrin does augment glucose induced insulin release in concentrations attained after a protein meal [32]. However, during an oral

glucose load gastrin is only a minor insulin secretagogue [32, 37] and adds little if anything to amino acid stimulated insulin secretion, i. e. during ingestion of protein [38]. It may be that gastrin influences insulin secretion under pathological conditions, i. e. in hypergastrinaemic states.

Secretin can be taken off the list of *incretin* candidates because injection of exogenous secretin to achieve physiological concentrations does not release insulin or augment glucose induced insulin release in man [33]. Exogenous GIP does not release insulin in the normoglycaemic state in dog [39] or man [36, 40] even if given in doses resulting in serum concentrations several times higher than those attained after glucose or fat ingestion. However, exogenous GIP, producing physiological levels, potentiates glucose induced insulin release in the dog [39] and in man [34, 36].

Whether CCK is insulinogenic or augments glucose induced insulin secretion has not been established because most CCK preparations contain GIP [35, 41]. In one study GIP-free CCK in high concentration augmented the glucose induced insulin release in vitro [42]. At present CCK cannot be dismissed as a possible member of the *incretin* family because of the identical amino acid sequence at the C-terminal end of gastrin and CCK and the fact that caerulein [43, 44] and tetragastrin [45] stimulate insulin secretion. However, caerulein does not augment glucose induced insulin release [44]. In addition, by intraduodenal perfusion of different amino acids GIP release could be separated from CCK release (measured by pancreatic enzyme secretion) and thus demonstrated that the augmenting effect of endogenous CCK on amino acid induced insulin release is minimal [46]. The final decision about the role of CCK or CCK-like peptides awaits a reliable CCK radioimmunoassay to assess whether the observed insulinogenic effects are obtained with physiological plasma CCK levels.

5. GIP Regulation – a Link between Digestion and Metabolism

The effect of GIP on the pancreatic B-cell and the release of this peptide are regulated in a way which links gastrointestinal function and metabolic need closely together. The connection of the two is not only the result of the incretin concept (a gut factor releasing an anabolic hormone) but also due to the bifunctional potency of GIP as a gastrone and an insulinogenic substance, two completely unrelated functions. A possible third function, the stimulation of glucagon release, has been questioned recently because exogenous GIP in physiological and supraphysiologial doses did not stimulate glucagon release in man [36].

5.1. Glucose Dependence

It has been demonstrated in vitro [47, 48] and in vivo [35, 36] that the insulinogenic effect of GIP is strictly glucose dependent, i. e. elevated serum GIP levels do not release insulin in the euglycaemic state. A threshold glucose concentration above which GIP exerts an insulinotropic action exists in the isolated perfused rat pancreas [48]. At a fixed concentration of GIP, increased glucose concentrations stimulate IRI release in more than an additive manner. Brown suggested that endocrinologists should interpret GIP as "*G*lucose dependent *I*nsulinotropic *P*olypeptide" [49]. This glucose dependency is of great importance for the protection of the brain against hypoglycaemia and must be added to the long list of defence mechanisms the organism has against insulin hypoglycaemia.

The necessity of such a device is obvious because GIP is released not only by the ingestion of glucose but also by fat [35, 50, 51], protein [52] and in some species by intraduodenal instillation of hydrochloric acid, i. e. lowering of the duodenal pH [53]. Obviously, the GIP response to fat and acid serves only gastrointestinal processes. It may well be that the gastric inhibitory activity is a relict from an earlier phylogenetic state and does not operate any longer under physiological conditions [40]. However, the stimuli for GIP release in this ancient control system of acid secretion, fat or acidic duodenal pH, are still effective and would endanger the organism if GIP were insulinogenic during normoglycaemia. The GIP secreted in response to fat [35, 50, 51] and acid [53] is fully active as an insulinotropic agent because it potentiates the effect of a simultaneous intravenous glucose infusion (Fig. 3). Accordingly, the distribution of serum GIP components after glucose or fat ingestion is similar [54].

The demonstration of GIP release by acid fully explains older observations which led to the now obsolete concept of secretin as being a major incretin. A glucose dependent hypoglycaemic effect of intraduodenal HCl infusion was described in 1938 [55] and it was shown later that intraduodenal infusion of HCL and stimulation of gastric acid secretion by betazole enhanced insulin secretion during intravenous infusion of glucose in man [33, 55a]. These observations can now be related to acid induced GIP release.

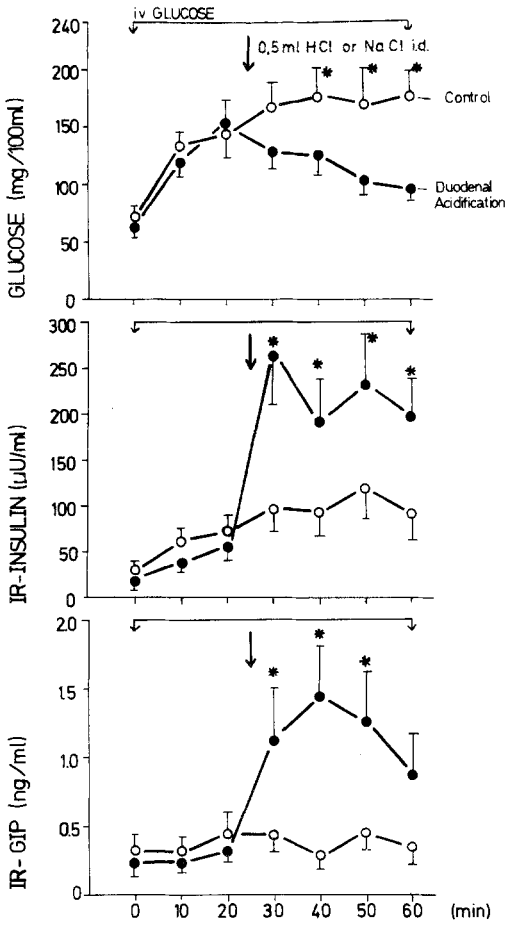

Fig. 3. Enhancement of glucose induced IRI release by intraduodenal infusion of hydrochloric acid in rats. Glucose was infused at a rate of 650 mg/kg/h for 60 minutes. Twenty-five minutes after start of the glucose infusion either 0.5 ml of 0.1 mol/l HCl (●—●) or 0.5 ml of 0.154 mol/l NaCl (○—○) was given intraduodenally within 5 minutes. Mean ± SEM of 8 experiments. The asterisks indicate significant differences between the control rats and the rats receiving the acid load ($p < 0.05$ or less). i. d., intraduodenal

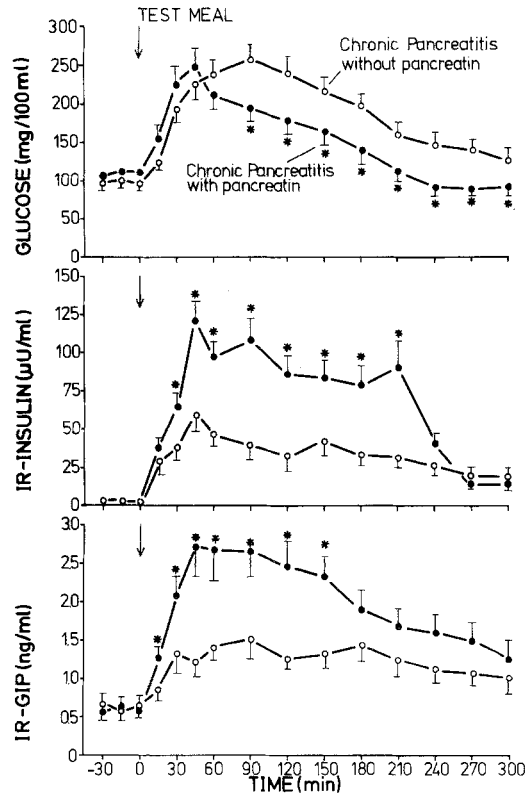

Fig. 4. Response of serum levels of immunoreactive GIP (IR-GIP), insulin (IRI) and glucose to a high caloric mixed liquid test meal without (○—○) and with added "pancreatin" (●—●) in 16 patients with chronic pancreatitis and massive steatorrhoea (daily fat excretion > 25 g)

5.2. *Dependence on Absorptive Function*

In patients with untreated coeliac disease and marked malabsorption a test meal evokes only a weak response of GIP compared to control subjects [56]. Since the number of GIP cells is not reduced in coeliac disease, defective absorption might explain the diminished GIP response. This conjecture ist supported by the observation that in patients with severe pancreatogenic steatorrhea the GIP response to a test meal increases if pancreatin is added to the mixed liquid test meal. This greater GIP response after pancreatin is accompanied by a greater insulin response and an improved glucose tolerance (Fig. 4).

Experimentally induced malabsorption of carbohydrates results in a flattening of the GIP curve.

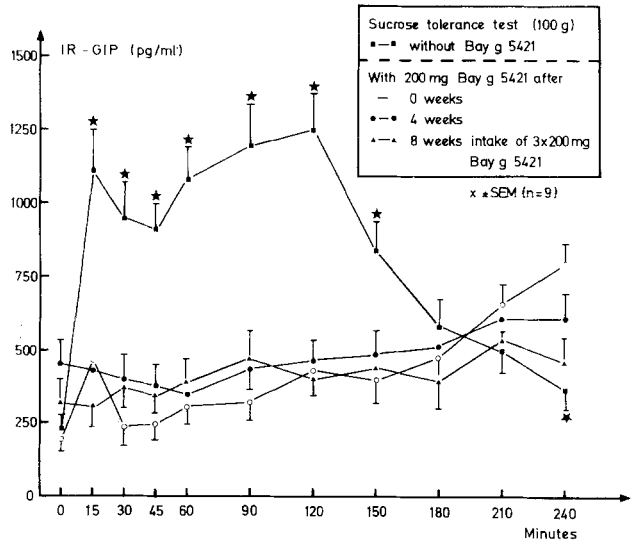

Fig. 5. Response of serum levels of IR-GIP to ingestion of 100 g sucrose without (■—■) and with the addition of 200 mg of the glucosidase inhibitor, Bay g 5421. The effect of the inhibitor on the sucrose test was investigated at the first, the thirtieth and the sixtieth day of a long-term intake of 3 × 200 mg daily of the glucosidase inhibitor by nine volunteers. * $p < 0.05$ between test and control experiments

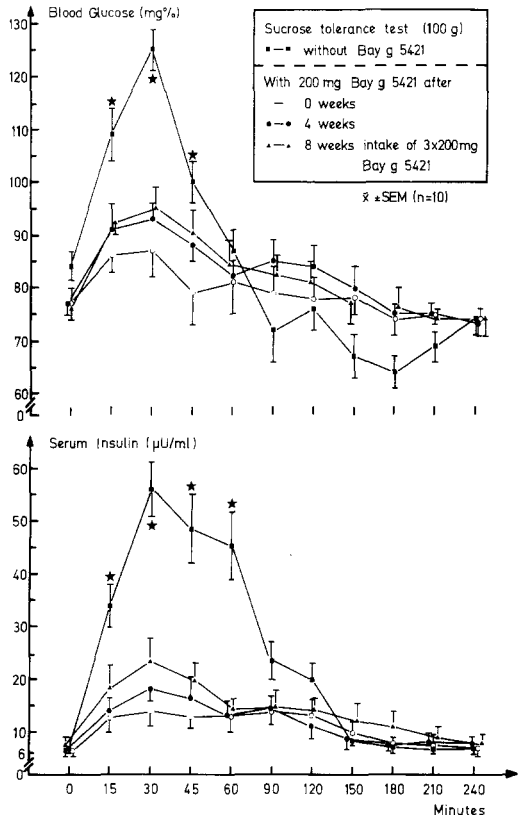

Fig. 6. Response of serum levels of glucose and insulin to 100 g sucrose without and with a previous dose of 200 mg glucosidase inhibitor. Same experiment as shown in Fig. 5 (n = 10). * $p < 0.05$ between test and control experiments

This has been shown in acute and chronic studies in man and rat. Thus, phlorizin nearly abolishes the GIP response to glucose in rats [57]. TRIS buffer which inhibits intestinal brush border sucrase reduces sucrose absorption and GIP release after a sucrose load in man [58]. A similar effect can be achieved in man by adding 200 mg of glucosidase inhibitor [59, 60] to an oral sucrose load. Repeated sucrose tolerance tests during a long-term treatment with the glucosidase inhibitor showed the GIP response to be absent and only small increases occurred in the blood levels of glucose, and insulin (Fig. 5 and 6). In this situation only small amounts of sucrose are digested and absorbed.

These findings in spontaneous malassimilation syndromes (coeliac disease and pancreatic steatorrhea) and experimentally induced malabsorption and impaired digestion (phlorizin, TRIS buffer and glucosidase inhibitor) demonstrate that absorption of nutrients and not their mere presence in the gut is the stimulus for GIP. This finding establishes a second protective mechanism against hypoglycaemia: only when insulin is needed is GIP released. It is not known exactly how absorption stimulates the GIP-producing cells. The microvilli protruding into the gut lumen cannot be the sensor.

Since dietary fibre also operates via modifying absorption the GIP response to fibre supplemented food can be expected to be decreased and by this also insulin release. Hence the favourable effect of dietary fibre may partially be interpreted via the incretin mechanism.

5.3. Feedback Control of GIP Release by Insulin

The response of serum GIP levels to oral fat is decreased by the simultaneous intravenous infusion of glucose [50, 57, 61]. Since exogenous insulin also diminished the increase of serum levels of GIP following ingestion of fat [35] it is reasonable to assume that the effect of intravenous glucose is due to the release of endogenous insulin and to propose a feedback loop between GIP and insulin [35]. The physiological implication of such a control system would again be protection of the organism against hyperinsulinaemia after food ingestion. No suppressive effect of endogenous or exogenous insulin on *glucose* induced GIP release was observed when using the glucose clamp technique [62].

Glucose infusion always results in increased serum levels of endogenous insulin in normal subjects; exogenous insulin can only be given together with glucose to normal subjects. Therefore, the question whether insulin or glucose is the suppressor of GIP release can be settled only in a situation of complete lack of endogenous insulin, i. e. in long – standing insulin dependent juvenile diabetics [63]. Intravenous insulin infusion in a dose which normalizes elevated blood glucose levels of diabetics but not an increase of blood glucose by intravenous glucose infusion suppresses the GIP response to fat ingestion (Fig. 7). The same insulin infusion does not suppress the GIP response to glucose ingestion. Thus, insulin feedback control of GIP release only exists for fat but not glucose induced GIP release. A teleological explanation for this difference could be that the organism needs protection against an insulin releasing agent only in the normoglycaemic state, i. e. after fat but not after glucose ingestion. One has to assume that GIP producing cells have separate glucose and fat receptors and that only the fat receptor responds to insulin.

5.4. Nervous Control of GIP Release

The exaggerated GIP response observed after duodeno-pancreatectomy has been related to the accelerated passage of food and by this an increasing

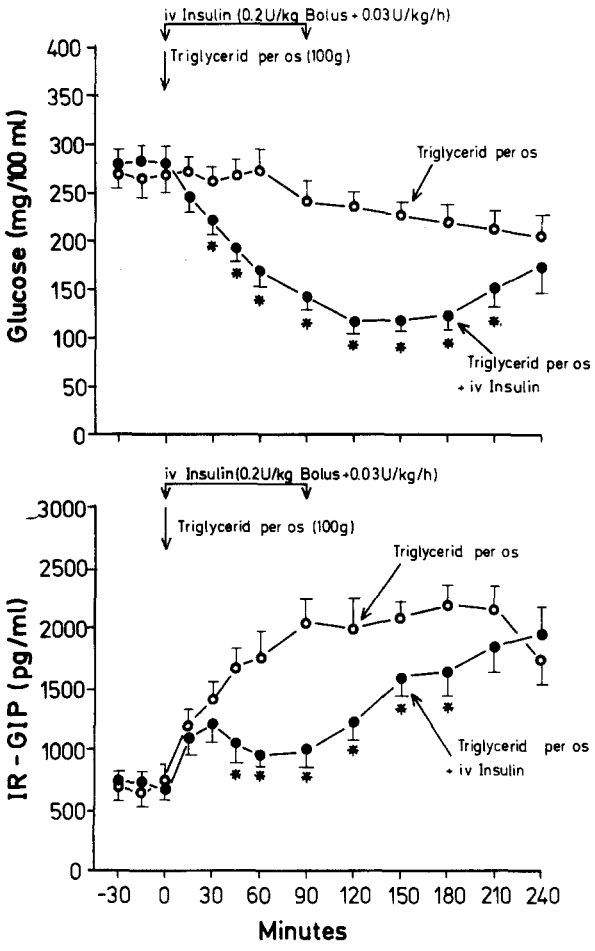

Fig. 7. Changes of serum levels of glucose and IR-GIP after ingestion of 100 g triglyceride without (○—○) and with (●—●) an IV infusion of insulin in 7 normal weight patients with insulin-dependent juvenile-type diabetes of at least 5 years duration. The last insulin injection was given 24 hours before start of the experiment. * $p < 0.05$

load stimulating more GIP cells [56]. However, such changes can also be explained by partial denervation of the small bowel during this operation. A first indication for nervous control of GIP release was provided by the demonstration of a decreased GIP response to intraduodenol glucose perfusion after atropine [64]. Since atropine seems not to alter glucose absorption [65] the accompanying reduction of the insulin increment may be the consequence of the decreased GIP response. Preliminary investigations in our laboratory revealed an exaggerated GIP response to glucose ingestion after β-receptor blockade (propranolol) and a suppression of the GIP response after β-receptor stimulation (orciprenaline). The involvement of the nervous system in the regulation of *incretin* release certainly needs further exploration.

6. Pathology of GIP

The availability of a reliable GIP radioimmunoassay prompted several laboratories to investigate whether known gastrointestinal or metabolic diseases are associated with a disturbed secretion of GIP or whether a pathological secretion of GIP may even account for one of these diseases. The findings have been summarized recently [58].

To date, no pathological condition has been found which is causally related to hyper- or hyposecretion of GIP. However, the described changes (elevated basal serum levels and decreased or exaggerated response to food ingestion) may account for some symptoms of the respective diseases, especially those related to hyperinsulinaemia. Elevated fasting levels of GIP have been found in obese subjects and patients with maturity-onset diabetes. Prolonged starvation is accompanied by extremely elevated GIP levels and so is ketosis in juvenile diabetics and uremia [58].

A decreased GIP response could be a pathogenetic factor in *duodenal ulcer* disease, if the, gastrone effect of GIP is of physiological relevance. However, it has been demonstrated that patients with active duodenal ulcer disease release significantly more GIP after ingestion of a mixed meal [66, 67] or glucose [69] than controls. This finding might be related to the acid hypersecretion or the more rapid stomach emptying in duodenal ulcer patients and would explain the long known hyperinsulinaemic response of ulcer patients [69, 70].

Moore's hypothesis [3] that certain cases of *diabetes mellitus* are related to the absence of an *incretin* has been evaluated by several authors. A lack of GIP or a decreased GIP response to food ingestion was not found indicating that the primary defect of the B-cell leading to delayed and insufficient insulin response cannot be overcome by GIP. Well controlled insulindependent juvenile diabetics have a normal [74] and maturity onset diabetics an exaggerated GIP response to glucose [71, 72, 73] and a mixed test meal [74]. It has been suggested that the exaggerated GIP response is due to the defective insulin response leading to a diminished feedback inhibition of GIP secretion by insulin [73]. It is doubtful that this suggestion is valid since insulin feedback control of glucose-induced GIP secretion does not seem to exist [62, 63].

Starvation for three weeks and refeeding for three days almost normalized the exaggerated GIP response of maturity onset diabetics to a test meal, while the insulin response and the glucose tolerance markedly improved (Fig. 8). A similar effect occur-

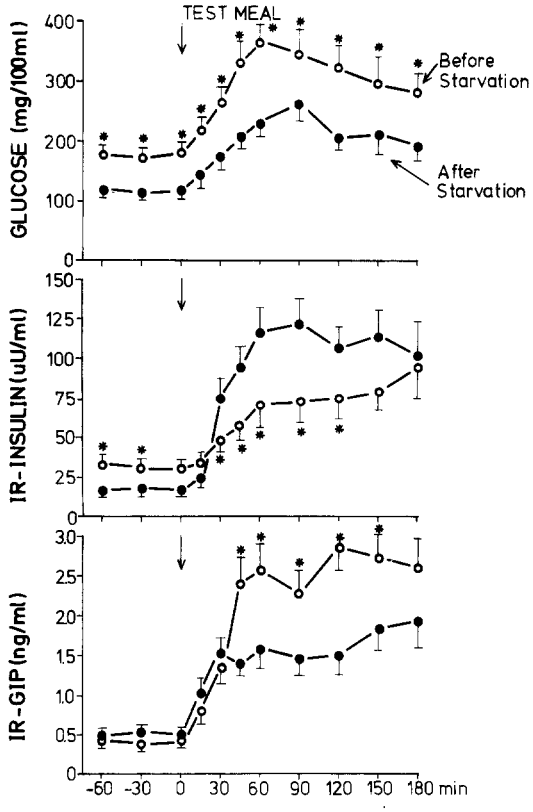

Fig. 8. Response of serum levels of IR-GIP, IRI and glucose to a mixed liquid test meal in 14 patients with maturity-onset diabetes before (○—○) and after (●—●) starvation for 3 weeks and refeeding for 3 days with increasing amounts of calories. * $p < 0.05$

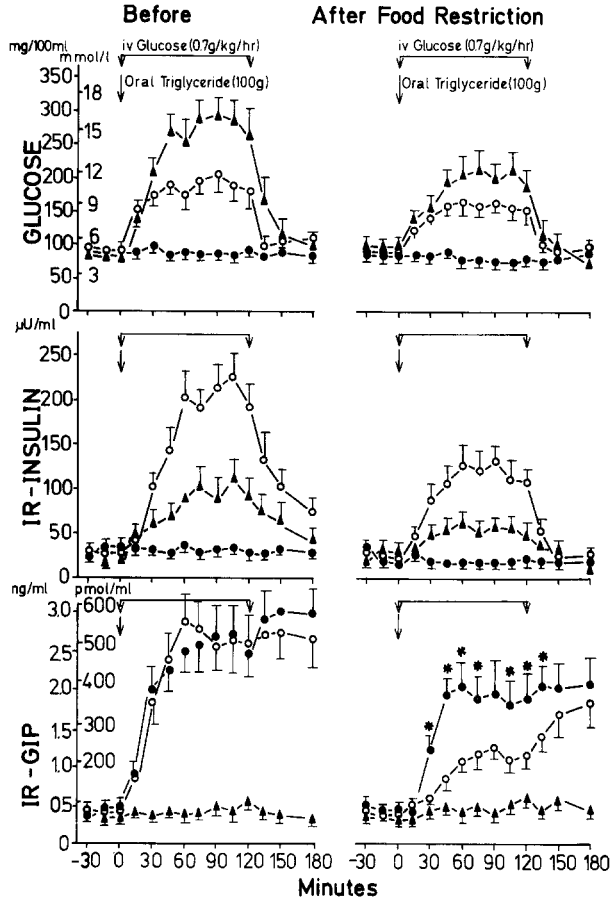

Fig. 9. Response of serum levels of glucose, IRI and IR-GIP to oral fat (100 g) and/or IV glucose (0.7 g/kg/h) in six obese subjects with glucose intolerance before (left panels) and after (right panels) food restriction for three weeks. ●—● = oral triglyceride; ▲—▲ = IV glucose; ○—○ = IV glucose plus oral triglyceride. Significant differences between the serum IR-GIP levels following oral fat and oral fat plus IV glucose are indicated (* = $p < 0.05$ and less)

red following treatment with glibenclamide for three weeks [57].

During the past years our laboratory has concentrated on the question whether an overactive enteroinsular axis is involved in the pathogenesis of hyperinsulinaemia of *obesity* [75–77]. It has been shown that obese subjects have an exaggerated GIP secretory response to fat and a mixed meal. The response to glucose is abnormal only in obese subjects with pathological glucose tolerance. The feedback control of fat induced GIP release by insulin is defective in obesity and can be restored by food restriction or starvation, together with a normalization of the GIP response to a test meal (Fig. 9). Since the GIP response to fat is abnormal also, when no insulin is released the greater responsiveness of the GIP cells in obese persons cannot be solely due to insulin resistance. It is suggested that the high caloric intake of obese persons conditions the GIP producing cells for greater hormone release. This would also explain the reversibility of the greater responsiveness after food restriction. The data suggest that increased GIP secretion participates in the development of the hyperinsulinaemia of obesity and that attempts to decrease GIP response, e. g. inhibitors of absorption, can be of value in the treatment of obesity and hyperinsulinaemic obese maturity onset diabetes.

7. Is GIP the Only *Incretin*?

It has been claimed from experiments with crude and partially purified gut extracts that three possible *incretins* other than GIP exist [27]. We have tested this suggestion using an immunochemical approach. The binding of endogenously released hormones by

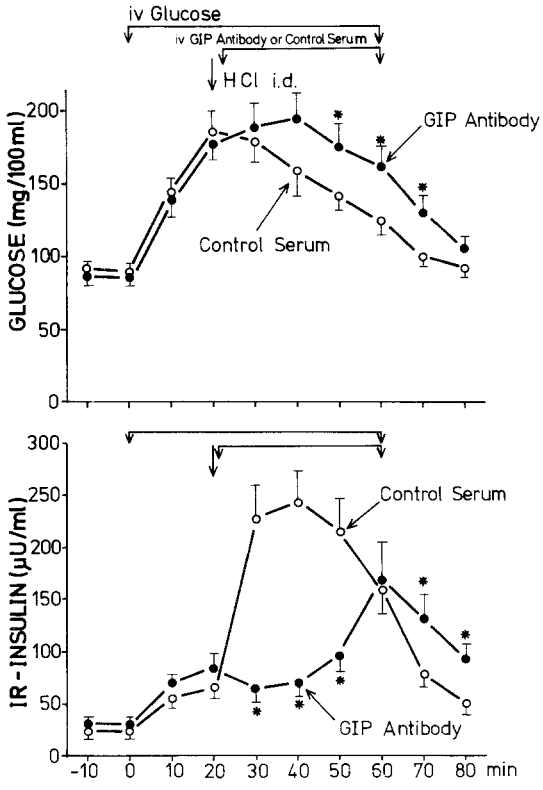

Fig. 10. Effect of IV application of GIP-antiserum on serum levels of glucose and IR-insulin during IV infusion of glucose and intraduodenal instillation of HCl. Twenty minutes after the start of the glucose infusion (650 mg/kg/h) 0.5 ml of 0.1 mol/l HCl was given intraduodenally within 5 minutes. Simultaneously, either 0.3 ml/kg undiluted antibody solution was given as a bolus injection followed by a continuous infusion of 3.5 ml/kg 1:20 diluted antiserum within 40 minutes (●—●) or corresponding amounts of control serum (○—○) were given. Each curve represents the mean ± SEM of seven experiments. The asterisks indicate significant differences between control serum and GIP-antiserum ($p < 0.05$ or less)

injection of specific antisera has been used to create hormone deficiency and to demonstrate through this the physiological role of the respective hormone [78, 79]. A potent GIP antiserum completely abolished the initial *incretin* effect of hydrochloric acid in anaesthesized glucose infused rats (Fig. 10). Therefore this initial effect of acid is mediated by GIP [53, 80]. A late *incretin* effect of HCl must be attributed to additional gut factors. The *incretin* effect of intraduodenal glucose was reduced, but not abolished, by GIP antiserum in anaesthesized rats [80]. Oral glucose tolerance tests in conscious rats were not altered by previous injections of GIP antiserum, despite an antibody titre binding in vitro of more than 95% of added ^{125}I-GIP.

These results support the contention that *incretins* other than GIP exist awaiting their isolation and purification. Until these *incretins* can be assayed in blood Moore's hypothesis that certain cases of diabetes are related to the absence of an *incretin* cannot be completely ruled out.

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