Glucagon-Like Peptide-I and the Control of Insulin Secretion in the Normal State and in NIDDM

BERNARD THORENS AND GERARD WAEBER

Potentiation of glucose-induced insulin secretion by intestinal factors has been described for many years. Today, two major peptides with potent insulinotropic action have been recognized: gastric inhibitory peptide and truncated forms of glucagon-like peptide I, GLP-I(7–37) or the related GLP-I(7–36)amide. These hormones have specific β-cell receptors that are coupled to production of cAMP and activation of cAMP-dependent protein kinase. Elevation in intracellular cAMP levels is required to mediate the glucoincretin effect of these hormones: the potentiation of insulin secretion in the presence of stimulatory concentrations of glucose. In addition, circulating glucoincretins maintain basal levels of cAMP, which are necessary to keep β-cells in a glucose-competent state. Interactions between glucoincretin signaling and glucose-induced insulin secretion may result from the phosphorylation of key elements of the glucose signaling pathway by cAMP-dependent protein kinase. These include the ATP-dependent K^+ channel, the Ca^{++} channel, or elements of the secretory machinery itself. In NIDDM, the glucoincretin effect is reduced. However, basal or stimulated gastric inhibitory peptide and glucagon-like peptide I levels are normal or even elevated, suggesting that signals induced by these hormones on the β-cells are probably altered. At pharmacological doses, infusion of glucagon-like peptide I but not gastric inhibitory peptide, can ameliorate postprandial insulin secretory response in NIDDM patients. Agonists of the glucagon-like peptide I receptor have been proposed as new therapeutic agents in NIDDM.
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Glucose ingestion induces a considerably greater elevation in plasma insulin levels compared with an intravenous glucose load. This augmented β-cell secretory response to an oral glucose challenge was suggested to result from the release of gut hormones into the blood that, at the level of the β-cells, potentiate glucose-induced insulin secretion (1–3). This effect, referred to as the glucoincretin effect, has been attributed to several insulinotropic peptides purified from intestinal extracts, such as secretin, cholecystokinin, gastrin, gastrin-releasing peptide, VIP, or enteroglucagon (1–3). However, most of these peptides display weak insulinotropic activity at high pharmacological concentrations. The principal glucoincretins that have been characterized are GIP, now more commonly called glucose-dependent insulinotropic polypeptide, and truncated forms of GLP-I: GLP-I(7–37) or GLP-I(7–36)amide.

GIP is a 42 amino acid–long peptide produced by K-cells from the duodenum and the upper jejunum and is secreted in response to both oral absorption of glucose and fatty acids (2,4). It has an insulinotropic action at physiological concentrations, and its effect on insulin secretion requires the presence of glucose at concentrations slightly above the normal value of ~5 mM. GIP apparently stimulates glucagon secretion. To assess the extent of the glucoincretin effect accounted by GIP, gut extracts were depleted of this hormone by immunoabsorption. The resulting extracts nevertheless still contained ~50% of the initial insulinotropic activity, indicating the existence of additional incretin hormones in the gut (5).

More recently, GLP-I, a product of the preproglucagon

From the Institute of Pharmacology and Toxicology, Lausanne, Switzerland; and the Department of Internal Medicine B, University Hospital, Lausanne, Switzerland.

Address correspondence and reprint requests to Dr. Bernard Thorens, Institute of Pharmacology and Toxicology, Bugnon 27, 1005 Lausanne, Switzerland.

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NIDDM, non-insulin-dependent diabetes mellitus; GLP-I, glucagon-like peptide I; GIP, gastric inhibitory peptide or glucose-dependent insulinotropic polypeptide; VIP, vasoactive intestinal polypeptide; GRPP, glicentin-related pancreatic polypeptide; MPF, major proglucagon fragment; GLUT, glucose transporter; IP1, intervening peptide 1; IP2, intervening peptide 2; STZ, streptozocin.

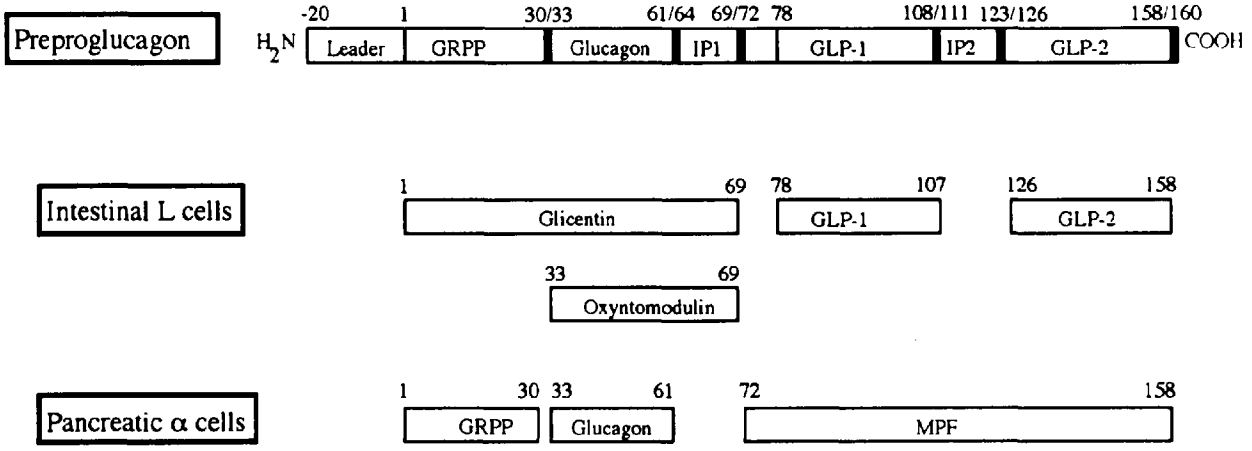

FIG. 1. Schematic representation of the preproglucagon molecule and of its major proteolytic fragments produced in intestinal L-cells and in pancreatic α-cells. Sequence from position 1 to 160: proglucagon. Heavy vertical bars, site of dibasic amino acid residues where proteolytic processing of proglucagon takes place. At position 77, a single Arg residue is the recognition signal for proteolytic cleavage of GLP-I in the active form GLP-I(7–36)amide.

gene secreted by intestinal endocrine cells, has been characterized and recognized as a new glucoincretin. In this perspective, we will focus on the physiological action of GLP-I.

GLP-I

Molecular cloning of the hamster preproglucagon cDNA (6) and of the human glucagon gene (7) revealed the presence of peptides other than glucagon (Fig. 1). These sequences are separated by dibasic amino acid residues, which are potential sites for cleavage by proteolytic enzymes involved in the maturation of peptidic hormones (8). The preproglucagon molecule is composed of 180 amino acids. The first 20 amino acids form the leader sequence. The glucagon sequence is present at position 33–61 of proglucagon. Two segments containing sequences homologous to glucagon are located at position 72–108 and 126–158 and are called GLP-I and GLP-II, respectively.

In pancreatic α-cells, the major preproglucagon-derived products are glucagon (corresponding to preproglucagon 33–61), GRPP (proglucagon 1–69), and MPF (proglucagon 72–158), which contains both the GLP-I and GLP-II sequences (9,10). No physiological role for GRPP and MPF have yet been demonstrated.

In intestine, analysis of proglucagon-derived peptides revealed the presence of glicentin (proglucagon 1–69, or enteroglucagon) and oxyntomodulin (proglucagon 33–69) (10,11). Although the physiological role of glicentin is not clearly established, oxyntomodulin has been shown to have an inhibitory effect on acid secretion by parietal cells from the stomach (12) and also to stimulate insulin secretion by pancreatic β-cells (13). However, these effects are observed at elevated, probably supraphysiological, concentrations of this hormone. In insulinomas, oxyntomodulin binding to the GLP-I receptor has been demonstrated, but with a KD 100-fold higher than for GLP-I (14). Both GLP-I and GLP-II can be detected in intestine but not in pancreas extracts (9). Purification of GLP-I from pig and human intestine and its analysis by amino acid sequence and mass spectroscopy revealed that the naturally occuring peptide corresponded to proglucagon (78–107)amide or GLP-I(7–36)amide (15).

BIOLOGICAL ACTIONS OF GLP-I

The only biologically active forms of GLPs are the truncated forms of GLP-I, GLP-I(7–37), or GLP-I(7–36) amide. For the rest of this perspective, we will use the term GLP-I to refer to GLP-I(7–36)amide, which is the predominant natural form of the peptide (15).

The stimulatory action of GLP-I on glucose-induced insulin secretion was first demonstrated in the perfused rat pancreas system (16,17). In these experiments, GLP-I(7–37) or GLP-I(7–36)amide were shown to have identical effects on the β-cell secretory response. The stimulation of insulin secretion was detected at concentrations of the peptide as low as 1–10 pM (18). The stimulatory effect of these peptides was shown to be glucose dependent. No insulin secretion could be elicited by GLP-I with 2.8 mM glucose in the perfusate. However, with 6.6 mM glucose in the perfusate, a strong potentiation of insulin secretion was induced by GLP-I. A similar glucose dependency also has been described for GIP action (1,2).

A glucoincretin effect of GLP-I in man also has been demonstrated. Increase in circulating insulin levels and inhibition of glucagon secretion have been observed in the fasted state as a result of GLP-I infusion. The insulin secretory effect was further enhanced after an intravenous glucose infusion (19). In the same set of experiments, the efficiency of GIP as an incretin was shown to be less than that of GLP-I.

Other important effects of GLP-I on β-cells are the stimulation of insulin gene transcription and insulin accumulation in secretory granules, as measured on insulinoma cell lines (20,21). The transcriptional regulation of the insulin gene by GLP-I may be mediated by cAMP and a cAMP-responsive element binding protein (22). Therefore, these additional effects of GLP-I are probably im-

portant to help replenish the insulin stores within the β-cells.

In extrapancreatic tissues, GLP-I induces inhibition of hydrochloric acid secretion by parietal cells from the stomach in in vivo experiments (23). In rat isolated parietal cells, however, GLP-I has been reported to stimulate acid secretion (24). The inhibitory action of GLP-I in vivo has been suggested to be indirect and to result from the stimulation of somatostatin release from stomach D-cells; this, in turn, inhibits the secretory activity of parietal cells (14). Binding sites for GLP-I have been described in lung membranes (25) and an mRNA for the receptor has indeed been detected in lung RNA preparations (26). The role of GLP-I in lung physiology is not presently known. In adipocytes, GLP-I binding sites have been characterized (27), and the addition of GLP-I or GIP to explants of rat adipose tissue has been reported to stimulate fatty acid synthesis (28). The presence of GLP-I binding sites in different regions of the brain also has been reported (29,30). The role of this peptide in the brain is not yet clear.

INTRACELLULAR SIGNALING BY GLP-I

The GLP-I receptor.

Structure of a cloned receptor. A recent advance in the glucoincretin field was the cloning of the GLP-I receptor (26). A cDNA for this receptor was isolated by a strategy that used expression cloning (31) from a rat pancreatic islet cDNA library. A human cDNA clone that is 90% identical to the rat receptor sequence also has been characterized (32). Characterization of GLP-I receptor in transfected Cos cells (31) or Chinese hamster lung fibroblasts (14) has shown that the receptor displays a high affinity for GLP-I (0.2–0.6 nM), whereas peptides of related structure, such as GIP, VIP, or secretin, do not bind to the receptor even at concentrations up to 1 mM. Oxyntomodulin and glucagon bind to the receptor but with affinities 100 and >1000 times lower than GLP-I, respectively.

Analysis of the nucleotide and deduced amino acid sequences revealed several important features. The receptor contains eight hydrophobic segments, seven of which are most probably transmembrane domains, whereas the first amino terminal hydrophobic segment represents a leader sequence. This receptor thus belongs to the superfamily of heterotrimeric G-protein–coupled receptors. Interestingly, however, the GLP-I receptor shows significant sequence similarity to only the receptors for glucagon (33), secretin (34), VIP (35), parathyroid hormone (36), calcitonin (37), and growth hormone–releasing factor (38), which form a newly described subfamily of G-coupled receptors. These receptors have a <10% sequence identity with the other members of the superfamily. One particular feature of these receptors is the presence of a relatively long (120 amino acids) amino terminal extracellular domain. Although the sequence of the amino terminal domain is poorly conserved among the different receptors, they all contain six cysteines distributed at identical locations. These cysteines may form disulfide bonds important for the proper three-dimensional structure of this region.

Signaling by the receptor. Signal transduction by the cloned GLP-I receptor has been studied after transient expression in Cos cells or in stably transfected Chinese hamster lung fibroblasts. Binding of GLP-I induces a dose-dependent accumulation of intracellular cAMP with a threshold at 10 pM and an EC_{50} at about 0.5 nM, a value close to the KD of GLP-I binding to the receptor. In these cells, no coupling to inositol phosphate production nor mobilization of calcium from intracellular stores have been recorded (Christian Widmann and B.T., unpublished observations). Similar stimulation of cAMP production in insulinoma cells has been reported (20,39). Different authors have also suggested that production of cAMP by glucoincretins is also glucose dependent. A possible explanation for this observation is that the β-cell adenylate cyclase may be regulated by calcium and calmodulin (40). Therefore, increase in intracellular Ca^{++} concentration consequent to glucose stimulation of β-cells may provide a link between glucose signaling and increased cAMP production.

In summary, these evidences indicate that the incretin effect of GLP-I is probably only mediated by activation of adenylate cyclase with a consequent increase in cAMP production and activation of cAMP-dependent protein kinase.

cAMP as a competence factor. Elevated intracellular cAMP levels have been proposed as important for two different aspects of β-cell function: *1*) the stimulation of glucose-induced insulin secretion, as already discussed; and *2*) the maintenance of β-cells in a glucose-competent state. The concept of cAMP as a competence factor was first introduced in elegant studies performed by Pipeleers et al. (41,42). These authors determined that isolated β-cells, which have a reduced cAMP concentration compared with intact islets, display a low secretory response to glucose. Elevation of intracellular cAMP by addition either of isobutylmethyl xanthine (an inhibitor of phosphodiesterase), glucagon, or by reaggregating β-cells with α-cells, enhanced considerably the glucose-dependent secretory response. They therefore concluded that cAMP was necessary to allow β-cells to respond to glucose stimulation. They further suggested that glucagon, secreted within the islet, was responsible for maintaining an adequate cAMP level in β-cells.

In physiological conditions, however, glucoincretins are probably more important than glucagon in maintaining the competence state of β-cells. Indeed, within pancreatic islets, blood flow reaches β-cells first, then α-cells, and finally δ-cells (43). Therefore, β-cells, except possibly those within close proximity of α-cells, are probably not directly influenced by paracrine glucagon secretion (44). Rather, most β-cells are exposed to only circulating levels of this hormone, whose concentration varies between 20 and 50 pM. At this concentration, glucagon displays only a very small stimulatory effect on insulin secretion (18) and a small or undetectable effect on intracellular cAMP in purified β-cells or intact islets (42). In contrast, circulating levels of GLP-I or GIP, which may vary between 10 and 100 pM (45,46), are sufficient to markedly stimulate cAMP production and glucose-induced insulin secretion (18,20,47). These hormones

may therefore be of crucial importance to maintain the glucose competence of β-cells in the basal state.

The glucose competence state of single β-cells can also be measured by recording the depolarization of the plasma membrane potential that follows exposure to glucose and/or GLP-1 (48) or by microfluorimetry recordings of intracellular Ca^{2+} in isolated β-cells exposed to 20 mM glucose (49). For instance, Holz et al. (48) determined that single β-cells can be divided into three subgroups: one composed of cells that can never be made responsive to glucose, a second group in which cells responded well to 10 mM of glucose, and a third group of cells that did not respond to 10 mM of glucose but could be made responsive by GLP-1. Moreover, GLP-1 could prime the cells to respond to subsequent exposure to glucose, provided that the time interval between the application of the two components was not too long. Inversely, glucose pre-exposure to glucose rendered β-cells responsive to subsequent exposure to GLP-1. A similar priming effect by GLP-1 and GIP also was described by Fehman et al. (47). These authors first perfused pancreases with GLP-1 (or GIP) at concentrations within the normal physiological range (10–1000 pM) and in the presence of substimulatory glucose concentrations (2.8 mM). After a 10-min washout period, they could show that the response to a high glucose challenge was markedly stimulated by pre-exposure to glucoincretins. Interestingly, the first phase of secretion was much more sensitive to the priming effect than the second phase.

Cross talk between GLP-1 and glucose signaling pathways. How does GLP-1 potentiate glucose-induced insulin secretion? To understand the possible sites of interaction between these two signaling systems, we must first describe briefly glucose signaling in β-cells. Figure 2 schematically presents the different steps in glucose-induced insulin secretion. These include *1*) glucose uptake into the β-cells by the facilitated diffusion glucose transporter GLUT2; *2*) phosphorylation of glucose by glucokinase and production of glucose metabolites, the most important of which is probably ATP; *3*) closure of an ATP-dependent K^+ channel, probably as a consequence of a change in the ATP:ADP ratio, and depolarization of the plasma membrane; *4*) opening of a voltage-gated Ca^{++} channel; and *5*) increase in the intracellular Ca^{++} concentration that initiates a cascade of events leading to exocytosis of insulin granules (50–52).

Phosphorylation of the ATP-dependent K^+ channel by cAMP-dependent protein kinase may be a site of interaction between glucose and GLP-1 signaling. Electrophysiological measurements in the cell-attached patch configuration have demonstrated that closure of the ATP-dependent K^+ channel could be induced by GLP-1 in a subgroup of cells that were responsive to glucose after priming with glucoincretin (48). In addition, depolarization in the presence of 10 mM of glucose but in the absence of GLP-1 could be induced by Sp-cAMPS, an agonist of cAMP. Conversely, the effects on depolarization induced by glucose and GLP-1 were blocked by Rp-cAMPS, an antagonist of cAMP. Thus, the GLP-1

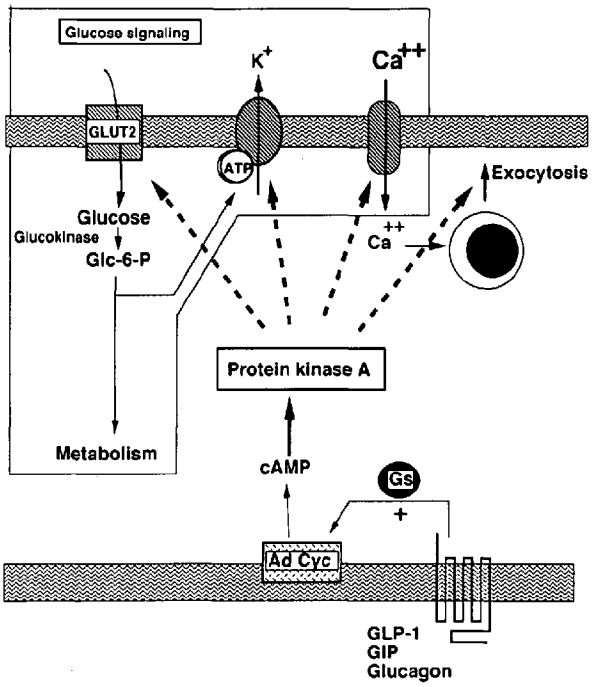

FIG. 2. Schematic representation of the possible interactions between glucose and glucoincretin hormones signaling. Glucose uptake by GLUT2 is followed by its catalytic phosphorylation by glucokinase. ATP or a change in the ATP:ADP ratio consequent to glucose catabolism induces the closure of ATP-dependent K^+ channels. The consequent depolarization of the plasma membrane induces the opening of dihydropyridine-sensitive Ca^{2+} channels. The increase in intracellular Ca^{2+} concentration initiates a cascade of events resulting in insulin secretion. Glucoincretins binding to their receptors stimulate the production of intracellular cAMP, probably by stimulating adenylate cyclase via activation of the α-subunit of the heterotrimeric GTP-binding protein Gs. The consequent activation of cAMP-dependent protein kinase (protein kinase A) can modulate by phosphorylation several elements of the glucose signaling pathway, as indicated by the broken arrows and discussed in detail in the text.

priming effect on β-cells may, at least in part, be mediated by a sensitization of the K^+-ATP channel resulting from activation of protein kinase-A. It is, however, not yet clear whether the channel itself can be phosphorylated. A previous electrophysiological study (53) on RINm5F insulinoma cells indeed failed to detect any direct effect of either increased intracellular cAMP levels or of the catalytic subunit of protein kinase-A on K^+-ATP channel activity.

Because an increase in intracellular Ca^{2+} is required for insulin secretion (54), a direct effect of GLP-1 on cytosolic Ca^{2+} was sought. Several reports have demonstrated an increase in intracellular Ca^{2+} concentration, measured with the indicator Fura-2, in the HIT hamster insulinoma cells after exposure to cAMP analogues, GIP, or GLP-1 (55–57). This increase in intracellular Ca^{2+} concentration was dependent, however, on the presence of glucose at stimulatory levels and was blocked by depletion of extracellular calcium with EGTA or by the L-type calcium channel blockers nimodipine, verapamil, and nifedipine. In subpopulations of isolated rat β-cells that did not respond to 20 mM of glucose by an increase in intracellular Ca^{2+}, theophylline or glucagon could induce a strong response (49). Also, the elevation in

intracellular Ca^{2+} of responsive β-cells could be further enhanced by an increase in cAMP (49). In mouse β-cells, analogues of cAMP induced a threefold increase in Ca^{2+} channel activity (58). Therefore, activation of protein kinase-A by glucoincretins can modulate the intracellular Ca^{2+} response. Phosphorylation of the voltage-dependent Ca^{2+} channel is a likely way to regulate its activity and the consequent insulin secretion.

The secretory machinery itself can be sensitized by glucoincretins. In digitonin-permeabilized islet cells (59) or RINm5F cells (60), insulin secretion stimulated by fixed concentrations of Ca^{2+} could be further increased by addition of cAMP. In a recent study (53a) that used isolated β-cells exposed to 5 mM of glucose, potentiation of insulin secretion by an increase in intracellular cAMP resulted mostly from an effect on the exocytotic machinery itself rather than from an effect on Ca^{2+} uptake by the L-type channels. Therefore, activation of protein kinase-A by GLP-I or GIP may also directly phosphorylate elements of the secretory machinery, thereby augmenting exocytosis of insulin secretory granules in response to glucose signaling.

To what extent each of the above-described phosphorylation events contribute to the glucoincretin effect is not yet clear. Whether other elements of the glucose signaling pathway, such as the glucose transporter GLUT2 or glucokinase, can be modulated by phosphorylation also is not clear. A further complication in the interpretation of these results is that they have been obtained in different experimental situations, with purified islets or with isolated β-cells, in the presence of various glucose concentrations, and with or without distinguishing different β-cell subpopulations. However, a well-established fact is that a subpopulation of isolated β-cells already responds to glucose without requiring in vitro exposure to agents elevating intracellular cAMP. Why other β-cells require pre-exposure to such agents to become glucose responsive is not known. One can speculate that glucoincretin receptor expression is heterogenous among β-cells in vivo. This could render subpopulations of β-cells variably glucose competent as a result not only of acute effects on insulin secretion but also because of long-term effects mediated by transcriptional activation of cAMP-responsive genes such as the insulin gene.

GLP-I IN NIDDM

Pancreatic β-cells from NIDDM patients are characterized by a defect in insulin secretion. The best characterized abnormality is a decreased sensitivity to glucose with, however, a normal secretory response to nonglucose secretagogues such as Arg (61–64). The variation of the glucoincretin effect in NIDDM patients is less understood. However, available data indicate that this effect might be reduced in NIDDM (1,65,66). For example, in a study of NIDDM patients, insulin and C-peptide values increased strongly in plasma of control individuals receiving an oral glucose challenge, whereas in diabetic patients, increases in circulating insulin levels were blunted with virtually no enhancement of C-peptide values (67). These small increases in insulin could have been attributable to a decreased hepatic insulin extraction resulting from the effect of gut hormones on liver. This study convincingly demonstrated a reduced glucoincretin effect in these patients. Furthermore, the reduced glucoincretin effect was not correlated with decreased basal or stimulated levels of immunoreactive GIP. The lack of correlation between GIP levels and the glucoincretin effect has also been reported in other studies (66). A similar impaired sensitivity of pancreatic β-cells to GIP and GLP-I has been demonstrated in an animal model of NIDDM, the neonatal STZ rat. This decreased sensitivity to the glucoincretins was not improved by previous insulin treatment (68).

Altogether, these studies suggest that the diminished incretin effect does not result from a decreased secretion of circulating glucoincretins. Instead, signaling by these hormones at the β-cell level may be impaired in NIDDM because of either a decreased expression of their receptor or to an inefficient coupling of their respective receptors to cellular second messengers. Whether a decreased glucoincretin effect in β-cells follows or precedes development of diabetes is not known. It is, however, conceivable that impaired signaling by GIP or GLP-I receptors may lead to a loss of glucose competence and a decreased expression of genes important for the normal functioning of β-cells, thereby causing the dysfunctions associated with NIDDM.

GLUCOINCRETINS AS THERAPEUTIC AGENTS IN NIDDM

The incretin effect of GLP-I and GIP is glucose dependent. Therefore, these hormones or agonists of their β-cell receptor could be used to stimulate insulin secretion in diabetic patients without the risk of inducing hypoglycemia, a complication often associated with the antidiabetic sulfonylureas (69). Indeed, infusion of GLP-I, but not of GIP, at pharmacological concentrations has been shown to stimulate postprandial insulin secretion, decrease glucagon release, and increase glucose disposal (70–72). These studies therefore led to two conclusions: *1*) even if the total incretin effect in diabetic patients is reduced, pharmacological doses of GLP-I can still display an insulinotropic action; and *2*) in diabetes, the signaling pathway of the β-cell GIP receptors is probably impaired to a greater extent than that of the GLP-I receptors.

CONCLUSIONS

The glucoincretin hormones discussed in this perspective are probably required to maintain β-cells in a glucose-competent state. They also potentiate glucose-induced insulin secretion, and their effect is probably uniquely mediated by controlling intracellular cAMP levels. The exact physiological importance of these hormones in the general control of glucose homeostasis and in the development of the β-cell dysfunctions associated with NIDDM is, however, not yet completely understood. Physiological studies have shown that the incretin effect of both GIP and GLP-I were reduced in NIDDM patients. This did not result from decreased circulating levels of these peptides but, rather, from a diminished sensitivity

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