Insulinotropin: Glucagon-like Peptide I (7-37) Co-encoded in the Glucagon Gene Is a Potent Stimulator of Insulin Release in the Perfused Rat Pancreas

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Abstract

Insulin secretion is controlled by a complex set of factors that include not only glucose but amino acids, catecholamines, and intestinal hormones. We report that a novel glucagon-like peptide, co-encoded with glucagon in the glucagon gene is a potent insulinotropic factor. The glucagon gene encodes a proglucagon that contains in its sequence glucagon and additional glucagon-like peptides (GLPs). These GLPs are liberated from proglucagon in both the pancreas and intestines. GLP-I exists in at least two forms: 37 amino acids GLP-I(1-37), and 31 amino acids, GLP-I(7-37). We studied the effects of synthetic GLP-Is on insulin secretion in the isolated perfused rat pancreas. In the presence of 6.6 mM glucose, GLP-I(7-37) is a potent stimulator of insulin secretion at concentrations as low as 5×10^{-11} M (3- to 10-fold increases over basal). GLP-I(1-37) had no effect on insulin secretion even at concentrations as high as 5×10^{-7} M. The earlier demonstration of specific liberation of GLP-I(7-37) in the intestine and pancreas, and the magnitude of the insulinotropic effect at such low concentrations, suggest that GLP-I(7-37) participates in the physiological regulation of insulin secretion.

Introduction

Pancreatic glucagon and intestinal glicentin are synthesized in the form of a 180-residue protein, preproglucagon encoded in a single gene (1). The precursor contains in addition to glicentin and glucagon the sequences of two glucagon-like peptides (GLPs)[1], GLP-I and GLP-II, separated by an intervening peptide (IP-II) (2–5). The posttranslational processing of preproglucagon differs in pancreas and intestine (1, 6). In the pancreas the precursor is processed to glucagon and GLP-I, and in both large and small intestines glicentin, GLP-I, GLP-II and IP-II–leucine–amide are found. Both pancreas and intestine contain GLP-I in at least two forms—31 and 37 residues long (1).

The close similarity of the amino acid sequence of GLP-Is and GLP-II with glucagon and the other peptides related in structure to glucagon (secretin, vasoactive intestinal peptide, gastric inhibitory peptide, growth hormone–releasing hormone) suggests that the GLPs might have a role in metabolic regulation. The specific liberation of GLP-I and GLP-II in the intestine indicates that these peptides may be components of the entero-insular axis (7), which comprises multiple intestinal factors influencing the release of hormones produced in the pancreatic islets. Further, they may be incretins, endocrine transmitters produced in the gastrointestinal tract that are released by nutrients and stimulate insulin secretion in the presence of elevated glucose if exogeneously infused in amounts not exceeding blood levels achieved after food ingestion (8). Detection of both GLP-I(1-37) and GLP-I(7-37) in pancreas and intestines raises the possibility that GLP-I(1-37) is itself a prohormone that undergoes a proteolytic cleavage at the single arginine residue at position 6 to release the biologically active GLP-I(7-37). In these studies we used synthetic GLP-I(1-37) and GLP-I(7-37) to investigate their effects on insulin secretion in the perfused rat pancreas and find that GLP-I(7-37) has uniquely potent insulinotropic actions.

Methods

Synthesis of peptides. Glucagon and GLP-Is were synthesized by the stepwise solid-phase method (9). Because the assembly of the peptide chain proceeds in the carboxyl- to the amino-terminal direction, GLP-I(1-37) and GLP-I(7-37) were prepared in the same synthesis by separating the peptide resin after incorporation of a protected histidyl residue at position 7 and continuing the assembly of amino acids on the other aliquot of the peptide resin to obtain protected GLP-I(1-37) peptide resin. Peptides were purified by preparative reverse-phase C-18 chromatography. Purified peptides were shown to be homogeneous by amino acid analysis, preview-sequence analysis, and high performance liquid chromatography (HPLC) on reverse-phase C-18 and ion-exchange DEAE-52 columns.

Radioimmunoassays. Development of the antisera and competitive binding radioimmunoassays for glucagon and GLP-I are described elsewhere (1). In brief, samples were incubated with the antisera in borate buffer (pH 8.1) for 24 h at 0°C, followed by addition of ^{125}I-labeled peptide for an additional 24 h in a total volume of 0.5 ml. Separation of the antibody bound from the free peptide was accomplished with dextran-coated charcoal. Assay sensitivity with all three antisera was 10 pg/ml. The antiserum against GLP-I was obtained by immunization with GLP-I(1-37) and is directed against both the amino-terminal (1-6) part of the molecule and to 7-37 determinants. Therefore, the amount of GLP-I(7-37) may be over or underestimated with respect to GLP-I(1-37) in the assay. The assay for insulin (10) used charcoal separation and rat insulin standards (Novo Research Institute, Copenhagen, Denmark).

Rat-perfused pancreas experiments. The preparation of the in situ isolated rat pancreas has been described previously (11, 12). The perfusate contained bicarbonate buffer (pH 7.4) and 120 mg/dl glucose, 4% dextran T-70, and 0.2% bovine serum albumin, and was equilibrated with 95% O_2 and 5% CO_2. The first 20 min of each perfusion was an equilibration period and is not represented in the data graphs.

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1. *Abbreviation used in this paper:* GLP, glucagon-like peptide.

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After the initial 20-min equilibration period, aliquots of perfusate were removed every 2–4 min for additional 20 min, thus allowing the system to equilibrate for a total of 40 min. The perfusion, with GLP-I(1-37) or GLP-I(7-37), was for 6 min and samples were collected at 1-min intervals. The peptide perfusions were followed by equilibration periods of 20 min, during which four samples 5 min apart were collected. A second 6-min perfusion followed with the same peptide as the first perfusion only at 100 times higher concentration of peptide. Again, samples 1 min apart were collected. The entire perfusion time was between 70 and 85 min.

In each aliquot of perfusate obtained, insulin was determined by radioimmunoassay. In addition the efficiency of delivery of the GLP-Is was confirmed by radioimmunoassay of corresponding aliquots of perfusate in which insulin was measured (1).

Results

To optimally study the effects of GLP-I(7-37) and GLP-I(1-37) on insulin secretion we used separate perfusions with each peptide, perfusing twice at two different concentrations of peptides and allowing 20-min time intervals between the two perfusions. In perfusions of two separate pancreases using this protocol, GLP-I(7-37) was a potent stimulator of insulin secretion, giving about a 20-fold stimulation at 5×10^{-9} M and a sixfold stimulation at 5×10^{-11} M (Fig. 1). In comparison, GLP-I(1-37), also studied in two pancreases, showed no effect on insulin secretion at either 5×10^{-9} or 5×10^{-7} M (Fig. 2). At the latter concentration no effect was observed even during a 15-min perfusion period (Fig. 2 *B*).

Using a slightly different perfusion protocol than that described above (Figs. 1 and 2) we gave alternate 5-min infusions of the peptides at concentrations ranging from 5×10^{-7} to 5×10^{-12} M to five additional individual pancreases. We reproducibly observed insulin release in response to GLP-I(7-37) at concentrations as low as 5×10^{-11} M, and little if any insulin responses to GLP-I(1-37) at concentrations as high as 5×10^{-7} M. Thus, the potent insulinotropic actions of GLP-I(7-37) have been observed in studies of seven separate pancreases.

Effects of glucagon on insulin secretion in the perfused pancreas have been established previously (13). We also compared the effects of glucagon to that of the GLP-Is. We used synthetic glucagon in the concentration range of 10^{-11}–10^{-7} M and found it to be less potent than GLP-I(7-37).

Discussion

The results of these studies clearly indicate that GLP-I(7-37) has potent insulinotropin activity. The liberation of this peptide from proglucagon in the intestine, and to a lesser extent in the pancreas (1), raises the possibility that GLP-I(7-37) has a role in endocrine regulation in the entero-insular axis (7). Our data, taken together with earlier observations that glucagon-like immunoreactivity in crude gut extracts released insulin after ingestion of glucose and fat, (8) suggest that GLP-I(7-37) could potentially be an incretin. Of all the known intestinal hormones tested for their insulin-releasing potency in the past, gastric inhibitory peptide has been considered as a possible incretin (14, 15). However, the concentrations of gastric inhibitory peptide required to stimulate insulin secretion exceed the physiologic levels of the peptide achieved after a meal. In the rat-perfused pancreas in the presence of 8.9 mM glucose, gastric inhibitory peptide (10^{-9} M) increased insulin secretion sixfold (16). We find a comparable increase in insulin secretion with GLP-I(7-37) at concentrations 100-fold lower than those required for an insulinotropic response to gastric inhibitory peptide. By radioimmunoassay we have measured both GLP-I(1-37) and GLP-I(7-37) levels of ~ 150 pg/ml (50 pM) in rat portal blood and 50 pg/ml (15 pM) in peripheral blood (S. Mojsov, unpublished results). Therefore, the insulinotropic effect that we have observed at concentrations of GLP-I(7-37) of between 5 and 50 pM are well within the physiological levels of GLP-I(7-37) found in the circulation.

There has been considerable interest in the potential intra-islet relationships which might occur between A, B, and D cells, such that the secretory product of one cell type might influence the function of a neighboring cell (17). Interaction could take place via a paracrine mechanism or through a local intra-islet portal system. Glucagon can stimulate both insulin and somatostatin secretion (13, 18), but because there appears to be a functional compartmentalization between islet cells, it is unclear whether glucagon can actually reach B and D cells (19). Taking into account the vascular arrangement of the rat islet, the glu-

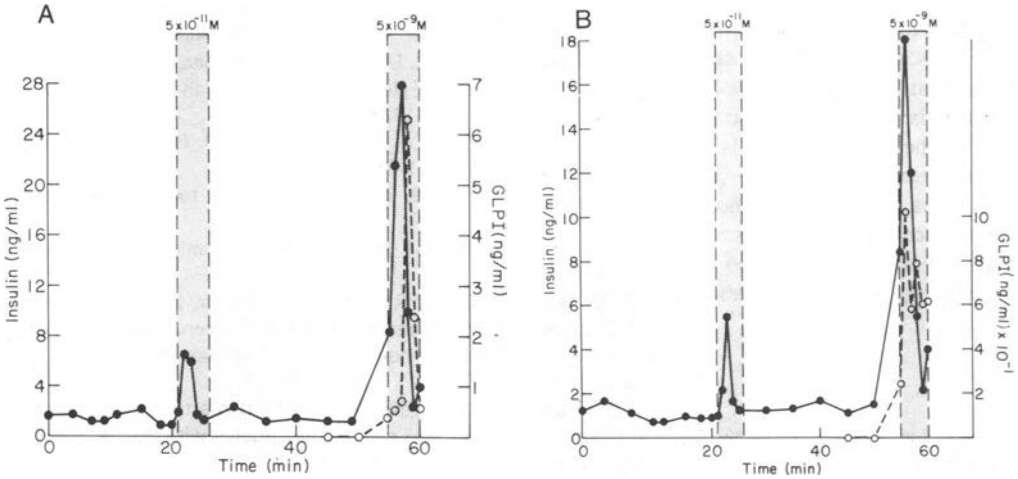

Figure 1. The effects of separate perfusions in two representative pancreas GLP-I(7-37) at two concentrations, 5×10^{-11} and 5×10^{-9} M. *Solid lines,* insulin values determined by radioimmunoassay. *Dashed lines,* amount of peptide perfused as determined in a competitive binding radioimmunoassay with antisera against GLP-I(1-37). The amount of GLP-I(7-37) at 5×10^{-11} M is beyond the detection sensitivity of the radioimmunoassay. Each graph represents a perfusion of a separate pancreas with a given peptide.

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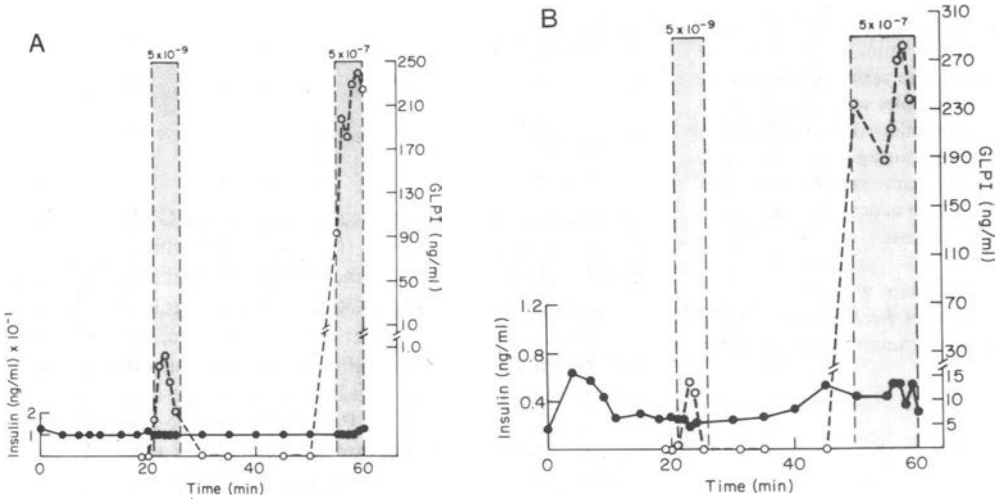

Figure 2. The effects of separate perfusions in two representative pancreas with GLP-I(1-37) at two concentrations, 5×10^{-9} and 5×10^{-7} M. Details of the experiment and explanation of symbols are described in legend to Fig. 1.

cagon-containing A cells of the mantle appear to be downstream from the B cells of the core, and therefore glucagon may not reach the B cells in high enough concentration to exert a significant influence (20). The mantle of A and D cells are, however, adjacent and this makes the possibility of paracrine interaction more feasible, although experimental support for such an interaction is not available. The finding that GLP-I(7-37) is a more potent insulin secretagogue than glucagon raises important questions about its potential intra-islet role.

Amino and carboxyl-termini of glucagon, GLP-I(7-37) and GLP-II are closely related to each other in their amino acid sequences and to vasointestinal peptide that possibly exerts a neuronal stimulation of insulin secretion (21). A most striking similarity among them is the conservation of a histidine residue at position 1. It is noteworthy that gastric inhibitory peptide, also closely related in its structure to the GLPs, has a tyrosine residue at position 1 instead of histidine (22). Inasmuch as a histidine residue at this position is essential for adenylate cyclase stimulation in various systems, the greater insulinotropic potency of GLP-I(7-37) compared with GIP may in part be accounted for by the histidine substitution for tyrosine (23).

Additional evidence in support of the concept that GLP-I(7-37) is a potent insulinotropic peptide is provided by our recent observation that GLP-I(7-37), and not GLP-I(1-37) or GLP-II, is a potent activator of adenylate cyclase at concentrations as low as 5×10^{-11} M and also stimulates cellular levels of insulin mRNA and insulin release in a rat insulinoma cell line (RIN-38) (Drucker, D. J., J. Philippe, S. Mojsov, W. L. Chick, and J. F. Habener, manuscript in preparation). Further, studies by Schmidt and co-workers showed that in isolated precultured islets, 10^{-9} to 10^{-8} M concentrations of the peptide GLP-I(1-36 des Gly-Arg amide) were required to release insulin (24).

Determining whether GLP-I(7-37) is the hormone whose primary function is to stimulate insulin secretion in response to feeding, or is one of a complex group of hormones involved in maintaining glucose homeostasis, will require further investigation.

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Note added in proof. In studies of isolated perfused pig ileum and pancreas, Orskov et al. (25) recently reported finding secretion of a GLP-1 peptide from ileum, but the pancreas secreted a large peptide with both GLP-1 and GLP-2 immunoreactivity.

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