Effects of Glucagonlike Peptide I-(7-36) on Release of Insulin, Glucagon, and Somatostatin by Rat Pancreatic Islet Cell Monolayer Cultures

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Glucagonlike peptide I (GLP-I-(7-36)) is cleaved from proglucagon in ileal epithelial cells and increases in human plasma after nutrient ingestion. This peptide has been shown to stimulate insulin secretion in vitro and in vivo and thus potentially acts as an incretin. To characterize its action on islet cells, the release of insulin, glucagon, and somatostatin by rat pancreatic islet monolayer cultures at varying concentrations of GLP-I-(7-36) was measured. The interaction of GLP-I-(7-36) with nutrient substrates was assessed by adding amino acids and differing glucose concentrations to the cultures. Islet cell cultures (n = 5) were incubated for 1 h in medium containing 1.67 or 16.7 mM glucose or 1.67 mM glucose supplemented with amino acids and GLP-I-(7-36) at 10^{-13}–10^{-7} M. Hormone release was compared with control cultures containing no GLP-I-(7-36); 1.67–16.7 mM glucose with and without GLP-I-(7-36) at 10^{-11} M; and 1.67, 3.3, 8.3, or 11.1 mM glucose alone or supplemented with amino acids, GLP-I-(7-36) 10^{-11} M, or both amino acids and GLP-I-(7-36). In medium with 1.67 or 16.7 mM glucose or 1.67 mM glucose and amino acids, GLP-I-(7-36) increased insulin secretion two- to threefold over control at concentrations of 10^{-9}, 10^{-11}, and 10^{-12} M, respectively. In medium with increasing concentrations of glucose, GLP-I-(7-36) at 10^{-11} M significantly increased insulin secretion at glucose concentrations ≥3.34 mM. Amino acids and GLP-I-(7-36) combined caused insulin secretion by the monolayer cultures at low glucose concentrations, whereas neither GLP-I-(7-36) nor amino acids alone were stimulatory. At higher glucose concentrations, amino acids and GLP-I-(7-36) together evoked greater insulin release than amino acids or GLP-I-(7-36) alone. With 16.7 mM glucose and GLP-I-(7-36) at 10^{-9} M, somatostatin release was increased by 40%. Glucagon release was not affected by any concentration of GLP-I-(7-36). Thus, the effect of GLP-I-(7-36) is enhanced in the presence of amino acids and glucose and, at putative physiological levels, is specific for pancreatic β-cells. We conclude that GLP-I-(7-36) is a potent insulinotropin that may be a physiological humoral modulator of nutrient-stimulated insulin secretion. *Diabetes* 38:1534–38, 1989

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Glucagonlike peptide I (GLP-I-(7-36)) has attracted increasing attention as a potential humoral stimulus of insulin secretion. In humans, this 29–amino acid peptide, cleaved from proglucagon by cells of the intestinal mucosa, is released into the circulation after nutrient intake (1–3). It has been shown to be a potent insulin secretagogue in several experimental models and when infused into humans (1,4–6). Thus, GLP-I-(7-36) is a candidate for the role of an incretin. Studies have focused on its augmentary effects on glucose-mediated insulin release. To our knowledge, no evaluation of its interactions with amino acids or its possible modulating effects on pancreatic α- and δ-cells has been undertaken.

We previously studied the effects of other putative incretins, such as secretin, gastric inhibitory polypeptide (GIP), and cholecystokinin (CCK), in rat pancreatic islet cell monolayer cultures (7,8). Because this system provides a simple means of measuring islet cell response to various stimuli, we used it to study the effects of GLP-I-(7-36) combined with amino acids and glucose on insulin, glucagon, and somatostatin release.

RESEARCH DESIGN AND METHODS

Peptides. GLP-I-(7-36) was custom synthesized at the Chemical Synthesis Facility Laboratory, Howard Hughes Medical Institute, University of Washington. The COOH-terminal arginine residue was amidated, and the synthetic GLP-I-(7-36) was purified to a single peak by high-performance liquid chromatography.

Pancreatic cell cultures. Monolayer cultures were established from the pancreases of 2- to 4-day-old Sprague-Daw-

ley rat neonates as described previously (9,10). For each set of experiments, pancreases were pooled for trypsin/collagenase digestion and the resulting cell suspensions divided into aliquots in equal amounts and put in culture dishes. Four of the five sets of experiments used aliquots of 0.5 pancreas equivalents/35-mm culture dish, whereas in the fifth set, aliquots of 1.5 pancreas equivalents/60-mm plate were used. Cultures were grown in 45% NCTC 135/45% medium 199 (vol/vol), 10% fetal bovine serum supplemented with 16.7 mM glucose, and 50 μg/ml gentamicin. Studies were performed 4 days after pancreatic harvest. Cultures were preincubated for 2 h in Krebs-Ringer bicarbonate buffer containing 0.1% bovine serum albumin (KRBB-BSA) supplemented with 1.67 mM glucose, then incubated for 1 h with a test medium. Test medium was KRBB-BSA supplemented with *1*) 1.67 mM glucose and GLP-I-(7-36) at concentrations of 10^{-13}–10^{-7} M; *2*) 16.7 mM glucose and GLP-I-(7-36) at 10^{-13}–10^{-7} M; *3*) 1.67 mM glucose, essential and nonessential amino acids (concentrations equivalent to those contained in Eagle's minimal essential medium), and GLP-I-(7-36) at 10^{-13}–10^{-7} M; *4*) 1.67–16.7 mM glucose alone or with GLP-I-(7-36) at 10^{-11} M; and *5*) 1.67, 3.3, 8.3, and 11.1 mM glucose alone or with amino acids, GLP-I-(7-36) 10^{-11} M, or both amino acids and GLP-I-(7-36) 10^{-11} M. Test medium was removed after 1 h of incubation and stored at −20°C for subsequent hormone measurements.

Hormone measurements. Insulin and glucagon concentrations were measured by radioimmunoassay as reported previously (11,12). Somatostatin was measured by radioimmunoassay with antiserum AS-77 specific for somatostatin-14 (S14) (13). Because of the relatively smaller amounts of S14 produced by the monolayer cultures, this assay required larger samples of medium; thus, 1.5 pancreas equivalents/60-mm plate were used in the S14 experiments. Assays were performed in 130 mM borate buffer with synthetic S14 (Peninsula Laboratories, San Carlos, CA) as standard and ^{125}I-Tyr^{11}-labeled S14 as tracer. Charcoal was used to separate bound from free peptide.

Data analysis. Insulin, glucagon, and S14 concentrations were expressed as means ± SE of five cultures for each experimental condition. The results of each set of experiments, derived from a common batch of pancreases, were compared with analysis of variance and the Newman-Keuls multiple-comparison test.

RESULTS

GLP-I-(7-36) stimulated insulin secretion from pancreatic monolayer cultures in the presence of both high (16.7 mM) and low (1.67 mM) glucose concentrations, but the sensitivity of the islet cells to GLP-I-(7-36) depended on the glucose concentration (Fig. 1, *A* and *B*). At 16.7 mM glucose, GLP-I-(7-36) at a concentration of 10^{-11} M stimulated insulin secretion (234% increase). There was a dose-dependent increase (707%) as GLP-I-(7-36) concentrations were raised to 10^{-7} M. With 1.67 mM glucose, GLP-I-(7-36) stimulated insulin release at 10^{-9} M (335%) and approached near maximum at 10^{-7} M (419%).

The effects on insulin secretion of 10^{-11} M GLP-I-(7-36) (a minimal stimulatory concentration) with increasing concentrations of glucose are shown in Fig. 2. GLP-I-(7-36) at 10^{-11} M increased insulin release at glucose concentrations of

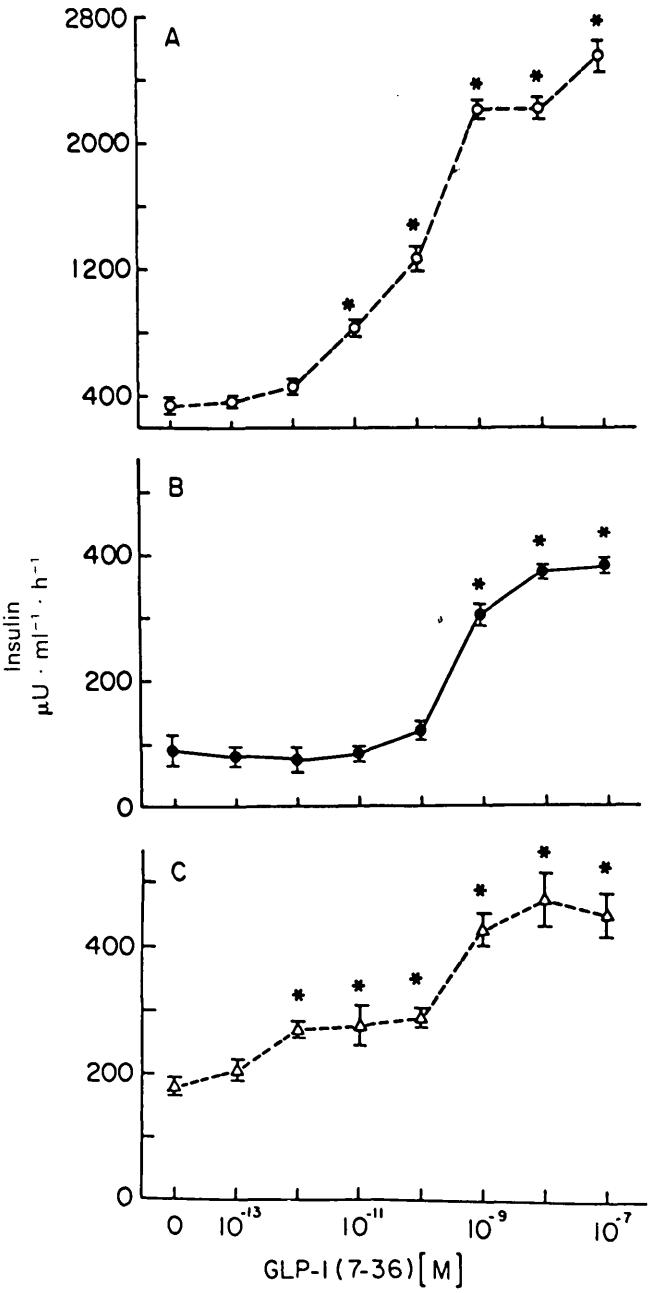

FIG. 1. Insulin release by rat pancreatic islet cell monolayer cultures in 16.7 mM glucose (*A*), 1.67 mM glucose (*B*), and 1.67 mM glucose and amino acids (C) with and without glucagonlike peptide I (GLP-I-(7-36)) at 10^{-13}–10^{-7} M. Each data point represents mean ± SE of 5 monolayer cultures. Cultures for each of these medium substrate conditions were derived from separate batches of rat pancreases. Cultures incubated in 16.7 mM glucose are from aliquots of 1.5 pancreas equivalents; other 2 experimental conditions used aliquots of 0.5 pancreas equivalents. *Insulin release greater than observed in control medium without GLP-I-(7-36) ($P < .05$).

≥3.35 mM. The magnitude of this rise (120–145%) was similar over the increasing glucose levels. Thus, at physiological concentrations of glucose, the magnitude of the insulinotropic effect of GLP-I-(7-36) was proportional to glucose-enhanced insulin secretion.

The insulinotropic effects of GLP-I-(7-36) were potentiated by amino acids (Fig. 1C). Amino acids added to the 1.67-mM glucose medium alone did not increase insulin secretion (data not shown). However, in the presence of amino acids,

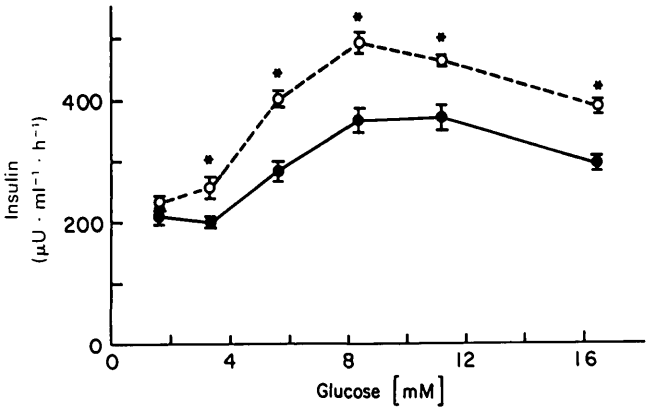

FIG. 2. Insulin release from groups of 5 rat pancreatic islet cell monolayer cultures incubated in 1.67–16.7 mM glucose with (○) and without (●) glucagonlike peptide I (GLP-1-(7-36)). *Insulin release greater than observed in control medium at equal concentration of glucose ($P < .05$).

augmentation of insulin release by GLP-I-(7-36) occurred at 10^{-12} M, a 1000-fold lower concentration than that necessary to increase insulin release in medium without amino acids (Fig. 1*B*). Amino acids and GLP-I-(7-36) at 10^{-11} M caused a rise in insulin levels at 1.67 and 3.3 mM glucose, whereas neither amino acids nor GLP-I-(7-36) 10^{-11} M alone was stimulatory at these low-glucose concentrations (Fig. 3). At 8.3 and 11.1 mM glucose, the combination of amino acids and GLP-I-(7-36) caused a greater rise in insulin release than either secretagogue alone (Fig. 3).

GLP-I-(7-36) did not stimulate glucagon release from monolayer cultures in the presence of either high glucose (16.7 mM), low glucose (1.67 mM), or low glucose and amino acids (Fig. 4). S14 release was increased 40% by GLP-I-(7-36) at 10^{-9} M in the presence of 16.7 mM glucose (Fig. 5).

DISCUSSION

Kreymann et al. (6) reported that GLP-I-(7-36) immunoreactivity in human plasma rises to concentrations of 3–5 × 10^{-11} M after oral glucose or a mixed meal. We demonstrated an insulinotropic effect of GLP-I-(7-36) on rat pancreatic islet monolayer cultures at concentrations of 10^{-11} M in the presence of physiological concentrations of glucose. Our findings agree with those in other studies, in which similar concentrations of GLP-I-(7-36) infused into the isolated rat pancreas (4) or humans (6) have stimulated the release of insulin. Thus, GLP-I-(7-36) is insulinotropic in the rat pancreatic islet monolayer culture at concentrations achieved in human plasma after food intake.

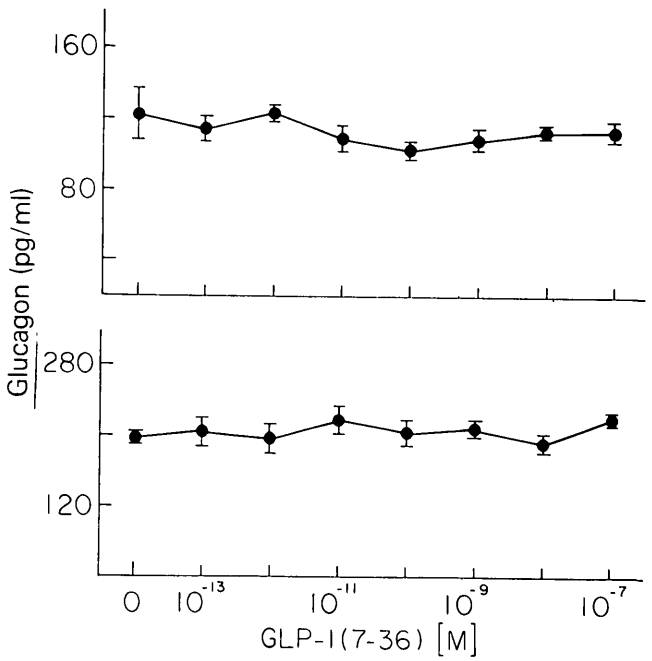

FIG. 4. Glucagon release from rat pancreatic islet cell monolayer cultures incubated with 1.67 mM glucose (*top*) or 1.67 mM glucose and amino acids (*bottom*) and increasing concentrations of glucagonlike peptide I (GLP-I-(7-36)). For both medium conditions, 5 monolayer cultures were tested at each concentration of GLP-I-(7-36).

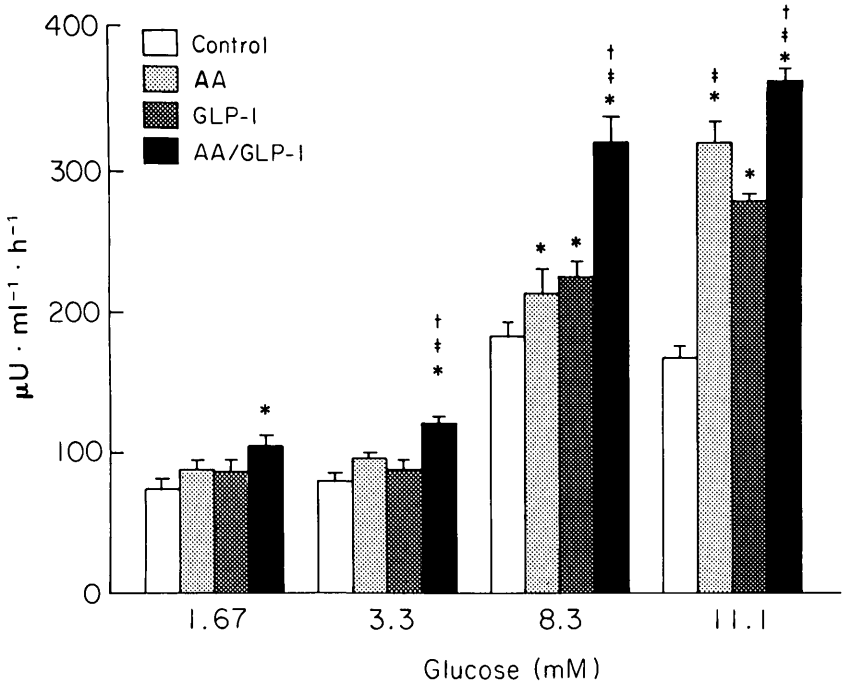

FIG. 3. Insulin release from groups of 5 rat pancreatic islet cell monolayer cultures incubated in 1.67, 3.3, 8.3, or 11.1 mM glucose alone or with amino acids, 10^{-11} M glucagonlike peptide I (GLP-I-(7-36)), or both amino acids and 10^{-11} M GLP-I-(7-36). *Insulin release greater than glucose alone. †Insulin release greater than glucose with GLP-I-(7-36). ‡Insulin release greater than glucose with amino acids ($P < .05$).

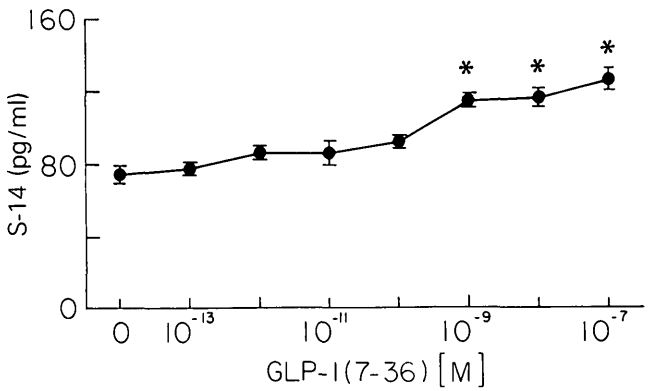

FIG. 5. Somatostatin-14 (S14) release from rat pancreatic islet cell monolayer cultures incubated with 16.7 mM glucose and increasing concentrations of glucagonlike peptide I (GLP-I-(7-36)). *S14 release greater than in control medium with equal concentration of glucose ($P < .05$). Five monolayer cultures were tested at each concentration of GLP-I-(7-36).

At physiological glucose concentrations, GLP-I-(7-36) caused greater insulin release as medium glucose concentrations increased. This glucose responsiveness is similar to that observed with GIP (14). However, the insulinotropic effect of GLP-I-(7-36) was not strictly glucose dependent in the rat pancreatic islet monolayer culture, because physiological concentrations of glucose were not necessary for GLP-I-(7-36) to stimulate β-cell secretion. At subphysiological concentrations of medium glucose, pharmacological concentrations of GLP-I-(7-36) or physiological concentrations of GLP-I-(7-36) and amino acids caused an increase in insulin release. Thus, in the rat pancreatic islet monolayer culture, the stimulation of β-cells by GLP-I-(7-36) is independently potentiated by both glucose and amino acids.

We previously studied the effects of GIP, CCK-8, and secretin on insulin release by rat pancreatic islet monolayer cultures under similar conditions (7,8). In the presence of 16.7 mM glucose, GIP and CCK-8 were stimulatory at concentrations of 5×10^{-10} M, whereas secretin had no effect at concentrations as high as 10^{-7} M. At 1.67 mM glucose supplemented with amino acids, GIP stimulated insulin secretion at a concentration of 10^{-8} M. Although quantitative comparisons among different studies are difficult because of variation in experimental conditions, it appears that GLP-I-(7-36) is a more potent insulin secretagogue than either GIP or CCK-8 in this system.

Glucagon release was not affected by GLP-I-(7-36) at any of the concentrations tested. There are previous reports that infusions of GLP-I-(7-36) caused a fall in glucagon in the human circulation (6) and in isolated perfused pig pancreas (4). Although an explanation for the disagreement of our results with these is not entirely clear, it may reflect differences between the function of dispersed and intact islets. In the perfused pig pancreas and in the rat pancreatic islet cell monolayer culture, GLP-I-(7-36) caused an increase in somatostatin release (15). Somatostatin has been proposed as a paracrine inhibitor of glucagon secretion and may account for the fall in glucagon output from the intact pig pancreas (16,17). Insulin can also inhibit α-cell function, and recent studies of islet microvasculature showing blood flow outward from β- to α-cells suggest that this is a physiologically relevant process (18). Thus, the paracrine effects of insulin and somatostatin may inhibit glucagon release in the perfused pancreas and in humans given GLP-I-(7-36). The islet cells of the rat pancreatic islet cell monolayer culture may not retain sufficiently ordered architecture necessary for paracrine modulation. We speculate that GLP-I-(7-36) does not affect pancreatic α-cells primarily but acts secondarily through its stimulation of insulin and somatostatin.

The rise in S14 release seen with GLP-I-(7-36) at 10^{-9} M is probably not physiologically significant. Circulating GLP-I-(7-36) does not reach these levels during nutrient stimulation (6) and S14 levels do not change postprandially (19).

In summary, it appears that GLP-I-(7-36) has a direct insulinotropic effect on islet β-cells rather than modulating changes in other islet hormones that indirectly affect insulin release. Specific receptors for GLP-I-(7-36) have been demonstrated on a rat insulinoma cell line (20,21), and it is likely that the effect of GLP-I-(7-36) on normal β-cells is also receptor mediated. In the rat pancreatic islet cell monolayer culture, stimulation of insulin release occurred at physiological concentrations of GLP-I-(7-36). This effect was potentiated by both glucose and amino acids. These results strengthen the proposed role of GLP-I-(7-36) as a nutrient-stimulated modulator of insulin secretion.

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REFERENCES

1. Holst J, Orskov C, Vagn Nielson O, Schwartz T: Truncated glucagon-like peptide 1, an insulin-releasing hormone from the distal gut. *FEBS Lett* 211:169–74, 1987
2. Orskov C, Holst J, Poulsen S, Kirkegaard P: Pancreatic and intestinal processing of pro-glucagon in man. *Diabetologia* 30:874–81, 1987
3. Conlon J: Pro-glucagon-derived peptides: nomenclature, biosynthetic relationships and physiological roles. *Diabetologia* 31:563–66, 1988
4. Mojsov S, Weir G, Habener J: Insulinotropin: glucagon-like peptide 1 (7-37) co-encoded in the pro-glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 79:616–19, 1988
5. Schmidt W, Siegel E, Creutzfeldt W: Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. *Diabetologia* 28:704–707, 1985
6. Kreymann B, Ghatei M, Williams G, Bloom S: Glucagon-like peptide 1 (7-36): a physiologic incretin in man. *Lancet* 2:1300–303, 1987
7. Fujimoto W, Ensinck J, Merchant F, Williams R, Smith P, Johnson D: Stimulation by gastric inhibitory polypeptide of insulin and glucagon secretion by rat islet cultures. *Proc Soc Exp Biol Med* 157:89–93, 1978
8. Fujimoto W, Williams R, Ensinck J: Gastric inhibitory polypeptide, cholecystokinin, and secretin effects on insulin and glucagon secretion by islet cultures. *Proc Soc Exp Biol Med* 160:349–53, 1979
9. Fujimoto W, Ensinck J, Williams R: Somatostatin inhibits insulin and glucagon release by monolayer cell cultures of rat endocrine pancreas. *Life Sci* 15:1999–2004, 1974
10. Fujimoto WY, Metz SA: Phasic glucose-stimulated insulin secretion by neonatal rat pancreatic islet cells: enhancement by sodium salicylate. *Diabetes* 33:872–78, 1984
11. Zaharko D, Beck LV: Studies of a simplified plasma insulin immunoassay using cellulose powder. *Diabetes* 17:444–57, 1968
12. Ensinck J, Shepard C, Dudl R, Williams R: Use of benzamidine as a

proteolytic inhibitor in the radioimmunoassay of glucagon in plasma. *J Clin Endocrinol Metab* 35:463–67, 1971
13. Baskin D, Ensinck J: Somatostatin in intestinal mucosal cells is present primarily as somatostatin-28. *Peptides* 5:615–21, 1983
14. Crockett S, Cataland S, Falko J, Mazzaferri E: The insulinotropic effect of endogenous gastric inhibitory polypeptide in normal subjects. *J Clin Endocrinol Metab* 42:1098–103, 1976
15. Orskov C, Holst J, Nielsen O: Effect of truncated glucagon-like peptide-1 (proglucagon-[78-107] amide) on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology* 123:2009–13, 1988
16. Klaff LJ, Taborsky GJ Jr: Pancreatic somatostatin is a mediator of glucagon inhibition by hyperglycemia. *Diabetes* 36:592–96, 1987
17. Klaff L, Taborsky G: Role of pancreatic somatostatin in determining glucagon response to arginine and morphine. *Am J Physiol* 252:E751–55, 1987
18. Samols E, Stagner J, Ewart R, Marks V: The order of islet microvascular cellular perfusion is B → A → D in the perfused rat pancreas. *J Clin Invest* 82:350–53, 1988
19. Ensinck J, Laschansky E, Vogel R, Francis B: Circulating pro-somatostatin-derived peptides: differential responses to food ingestion. *J Clin Invest* 83:1580–89, 1989
20. Orskov C, Nielsen J: Truncated glucagon-like peptide-1 (pro-glucagon 78-107 amide), an intestinal insulin-releasing peptide, has specific receptors on rat insulinoma cells (R1N 5AH). *FEBS Lett* 229:175–78, 1988
21. Goke R, Conlon J: Receptors for glucagon-like peptide-1 (7-36) amide on rat insulinoma-derived cells. *J Endocrinol* 116:357–62, 1988