Perspectives in Diabetes

Glucagon-Like Peptides

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Proglucagon contains the sequence of two glucagon-like peptides, GLP-1 and GLP-2, secreted from enteroendocrine cells of the small and large intestine. GLP-1 lowers blood glucose in both NIDDM and IDDM patients and may be therapeutically useful for treatment of patients with diabetes. GLP-1 regulates blood glucose via stimulation of glucose-dependent insulin secretion, inhibition of gastric emptying, and inhibition of glucagon secretion. GLP-1 may also regulate glycogen synthesis in adipose tissue and muscle; however, the mechanism for these peripheral effects remains unclear. GLP-1 is produced in the brain, and intracerebroventricular GLP-1 in rodents is a potent inhibitor of food and water intake. The short duration of action of GLP-1 may be accounted for in part by the enzyme dipeptidyl peptidase 4 (DPP-IV), which cleaves GLP-1 at the NH_2-terminus; hence GLP-1 analogs or the lizard peptide exendin-4 that are resistant to DPP-IV cleavage may be more potent GLP-1 molecules in vivo. GLP-2 has recently been shown to display intestinal growth factor activity in rodents, raising the possibility that GLP-2 may be therapeutically useful for enhancement of mucosal regeneration in patients with intestinal disease. This review discusses recent advances in our understanding of the biological activity of the glucagon-like peptides. *Diabetes* 47:159–169, 1998

THE INCRETIN CONCEPT AND β-CELL FUNCTION

Enhancement of insulin secretion from the islet β cell is a principal goal for treatment of patients with NIDDM. The observation that sulfonylureas stimulate insulin secretion has provided the rationale for the therapeutic use of these agents in the treatment of NIDDM. Nevertheless, the sulfonylurea stimulation of insulin secretion is not strictly glucose dependent, and hence hypoglycemia is an undesirable side effect of sulfonylurea treatment, particularly in elderly patients.

The observation that glucose administered via the gastrointestinal tract is associated with a greater stimulation of insulin release compared with a comparable glucose challenge given intravenously (1,2) prompted a search for the responsible "incretins," gut-derived factors that increase glucose-stimulated insulin secretion (3). The concept of the enteroinsular axis suggested that insulin secretagogues were synthesized in and released from the intestinal endocrine system after nutrient ingestion. The isolation and characterization of glucose-dependent insulinotropic peptide (GIP) represented an important advance in the identification of intestinal incretin hormones. GIP is released from enteroendocrine cells in the duodenum and proximal jejunum after nutrient intake and stimulates insulin secretion in a glucose-dependent manner (3,4). Nevertheless, immunoneutralization of GIP or removal of GIP from intestinal extracts does not result in complete elimination of incretin activity, consistent with the presence of additional gut-derived factors with insulinotropic activity (4).

After the isolation of the cDNAs and genes encoding proglucagon approximately 15 years ago (5–7), two novel glucagon-like peptides, GLP-1 and GLP-2, were identified COOH-terminal to the glucagon sequence in mammalian proglucagon (Figs. 1 and 2). Initial characterization of GLP-1 bioactivity using NH_2-terminally extended GLP-1(1-37) failed to demonstrate effects on blood glucose or insulin secretion; however, subsequent experiments using the NH_2-terminally truncated GLP-1(7-36) amide or GLP-1(7-37) peptides demonstrated potent effects on glucose-dependent insulin secretion, islet cell cAMP formation, and insulin gene expression (8–12). The principal aim of this review is to highlight recent advances in our understanding of the biology of the GLPs. Previous reviews of the biology of GLP-1 and GLP-2 offer a detailed introduction to the subject (4,13–15).

GLP-1 BIOSYNTHESIS AND SECRETION

A single proglucagon gene in mammals gives rise to an identical proglucagon RNA transcript that is translated and processed differently in brain, pancreatic islets, and intestine (Fig. 1) (16). In contrast, vertebrates such as the chicken, fish, frog, and lizard may contain two proglucagon genes, and use alternative RNA splicing for the generation of proglucagon mRNA transcripts that encode for GLP-1 but not GLP-2 in the pancreas and both GLP-1 and GLP-2 in the intestine (17,18). Although considerable progress has been made in elucidating the factors that control proglucagon gene expression in islet cells, much less is known about the regulation of proglucagon gene expression and, hence, the control of GLP-1 biosynthesis, in enteroendocrine cells. Transgenic experiments have demonstrated that different tissue-specific enhancers specify islet versus intestinal proglucagon gene expression (19,20); however, the intestine-specific proglucagon gene enhancer remains poorly defined (21). The pancreatic A-cell and enteroendocrine L-cell both express the transcription factor cdx-2/3, which regulates proglucagon gene expression in pancreas and intestine (22,23); however, transcription factors important for regulation of the proglucagon promoter specifically in the enteroendocrine

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Received for publication 9 September 1997 and accepted in revised form 8 October 1997.

CNS, central nervous system; DPP-IV, dipeptidyl peptidase 4; GIP, glucose-dependent insulinotropic peptide; GLP, glucagon-like peptide; GLP-1R, GLP-1 receptor; GRP, gastrin-releasing peptide; ICV, intracerebroventricular; PC, prohormone convertase; PGDP, proglucagon-derived peptide; RT-PCR, reverse transcription–polymerase chain reaction.

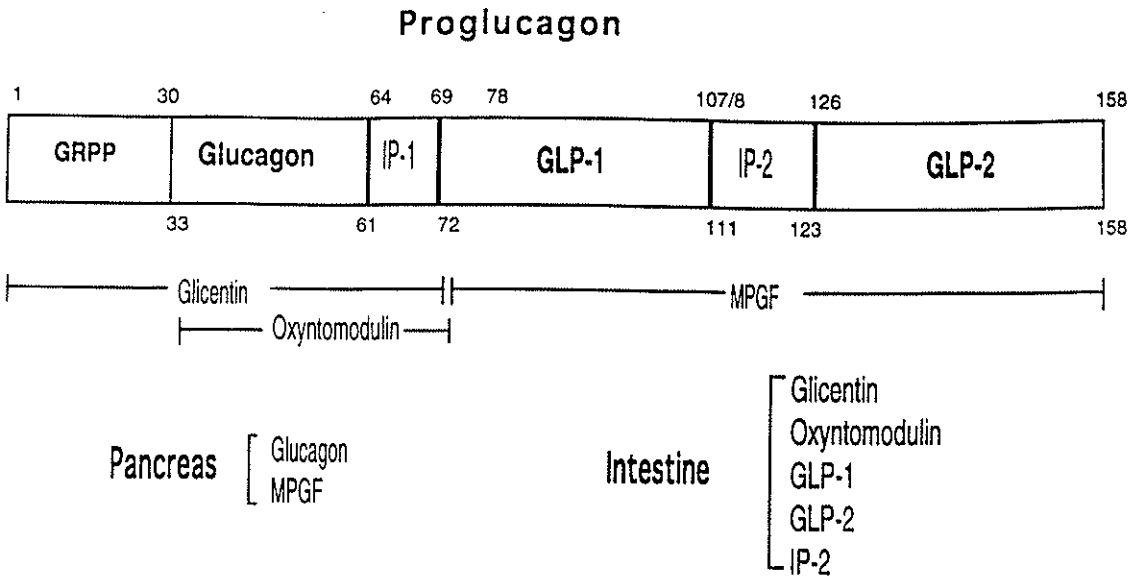

FIG. 1. Structural organization of mammalian proglucagon. The numbers refer to amino acid sequences in proglucagon. The peptides released by posttranslational processing in the pancreas and intestine are shown. GRPP, glicentin-related pancreatic polypeptide; IP, intervening peptide; MPGF, major proglucagon fragment.

cell have not been extensively characterized. Furthermore, despite interest in potential new strategies for increasing GLP-1 synthesis and secretion in diabetic patients, the factors important for the regulation of human intestinal proglucagon biosynthesis remain unknown.

The liberation of GLP-1 in the intestine but not the pancreas appears to be due to the tissue-specific expression of prohormone convertases (PCs) in the enteroendocrine cells of the small and large bowel. Whereas both PC1 and PC2 cleave proglucagon to generate the major proglucagon fragment and glicentin and oxyntomodulin (24,25), PC1 expressed in enteroendocrine cells appears to be the enzyme responsible for the liberation of both GLP-1 and GLP-2 (25). Although multiple immunoreactive forms of GLP-1 are liberated in vivo, including GLP-1(7-36) amide and GLP-1(7-37), the majority of circulating GLP-1 in humans appears to be GLP-1(7-36) amide (26). Nevertheless, in vivo studies have shown that both molecular forms of NH_2-terminally truncated GLP-1 are equipotent with regard to their insulin-stimulating properties; in addition, both appear to exhibit similar half-lives in vivo (27). Whether control of PC activity in the intestine is an important regulator of GLP-1 synthesis remains to be determined.

Because the enteroendocrine cell is exposed to both circulating humoral factors and luminal intestinal contents. intestinal proglucagon-derived peptide (PGDP) biosynthesis and secretion are subject to regulation by both hormonal and nutritional factors. Nutrient intake stimulates the synthesis and secretion of PGDPs from the enteroendocrine cell in rodents (28). In one study, proglucagon mRNA abundance decreased with fasting and increased with refeeding in rat jejunum, and the profile of circulating enteroglucagon and GLP-1 paralleled changes observed in jejunal proglucagon mRNA (29). The rapid rise in plasma GLP-1 levels after nutrient ingestion and the distal location of the majority of GLP-1–containing enteroendocrine cells in the ileum and colon has led to the suggestion that one component of the nutrient-induced secretory signal may be indirect, perhaps via GIP or gastrin-releasing peptide (GRP) release from the proximal jejunum. GLP-1 secretion from the distal ileum was abolished when intervening segments of intestine were resected.

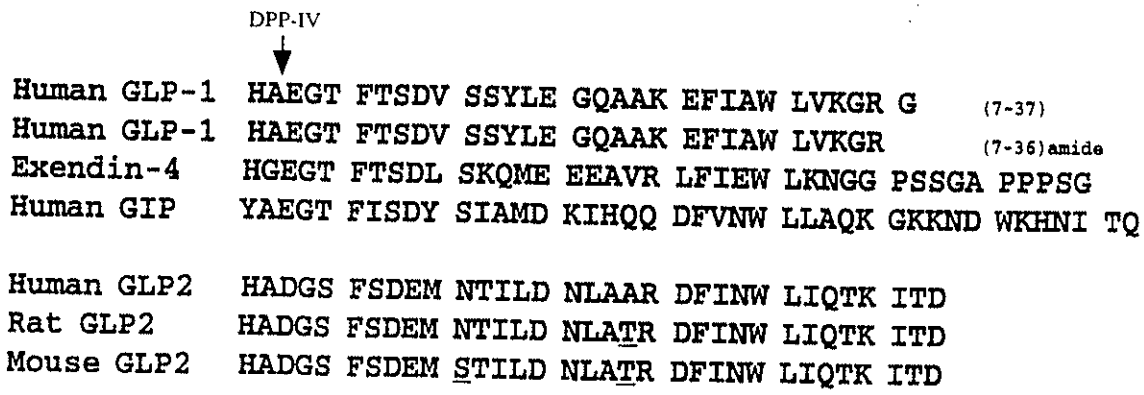

FIG. 2. Amino acid sequences of GLP-1, exendin-4, GIP, and GLP-2. The arrow designates the recognition site for DPP-IV enzymatic cleavage. Residues in rat or mouse GLP-2 that differ in sequence from human GLP-2 are underlined.

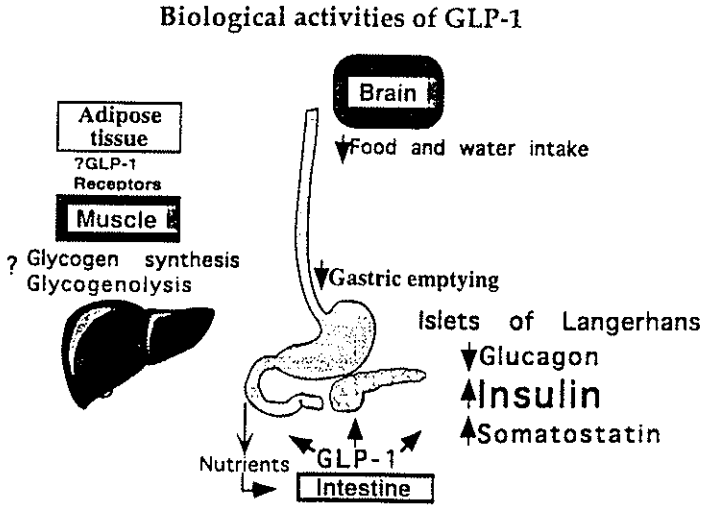

FIG. 3. Schematic representation of GLP-1 action.

and infusion of a GRP antagonist inhibited the L-cell response to nutrient ingestion, observations consistent with GRP having an important role in the humoral regulation of GLP-1 secretion (30).

In isolated perfused rat ileum preparations, GLP-1 secretion was stimulated by cholinergic agonists, bombesin, calcitonin-gene–related peptide, and GIP (31,32), and in the isolated perfused rat colon, it was stimulated by β-adrenergic and cholinergic agonists, bombesin, and calcitonin-gene–related peptide, and GIP (33). Somatostatin directly inhibits L-cell and GLP-1 secretion, and galanin antagonizes the stimulatory effect of GIP on GLP-1 release in rats (34). Luminal perfusion with glucose, pectin, or the bile acid hodeoxycholate stimulated GLP-1 secretion from the rat colon, suggesting that enteroendocrine cells in the large bowel are also sensitive to luminal contents in vivo (35).

GLP-1 secretion is also stimulated by nutrient ingestion in humans. Basal circulating levels of human GLP-1(7-36) amide range from 0.4 to 7.0 pmol/l, depending on the assay, and are stimulated after oral but not intravenous glucose administration (26,36). Secretion of GLP-1 throughout the day increases after meal ingestion, in parallel with meal-related increases in insulin secretion (37,38). GLP-1 release was also stimulated after oral administration of galactose, amino acids, and corn oil (36). GLP-1 is secreted in a pulsatile manner in humans; glucose ingestion increases the amplitude, but not the frequency, of GLP-1 secretion (39). The integrated GLP-1 pulse amplitude was reduced by atropine, consistent with the importance of cholinergic mechanisms in the control of GLP-1 secretion.

GLP-1 DEGRADATION

An important determinant of the circulating levels of bioactive GLP-1 appears to be the NH_2-terminal degradation of the peptide by the enzyme DPP-IV (40). Cleavage of GLP-1 at the penultimate alanine residue to generate GLP-1(9-36)amide occurs rapidly in plasma (40), and the half-life of intact GLP-1 in vivo appears to be less than 2 min (41). GLP-1(9-36)amide constitutes 53.5% of the concentration of intact GLP-1(7-36) amide in the fasted state; however, after nutrient ingestion, human GLP-1(9-36)amide is relatively more abundant than the intact 7-36(amide) molecule (42). GLP-1(9-36)amide also binds to the GLP-1 receptor, albeit with lower affinity than the (7-36)amide form, and may function as a competitive antagonist of the GLP-1 receptor in vivo (43). Radioimmunoassays that do not distinguish between intact GLP-1(7-36) amide and the NH_2-terminally deleted GLP-1(9-36)amide may overestimate the actual concentration of circulating bioactive GLP-1 (42); experiments that measure total immunoreactive circulating GLP-1 need to be interpreted with caution in light of this new information. DPP-IV activity is inhibited by low temperature and diprotin A (41,42), hence the importance of collecting blood samples for measurements of GLP-1 immunoreactivity on ice in the presence of appropriate protease inhibitors. Intact GLP-1 appears to be cleared predominantly through renal extraction; the contribution of extrarenal tissues to clearance of GLP-1 under normal physiological conditions remains to be determined (44).

GLP-1 ACTION

The GLP-1 receptor. GLP-1 exerts its actions via binding to a G-protein–linked receptor expressed on islet β-cells (45). The human GLP-1 receptor (46,47) is 90% homologous to the rat receptor, and the gene has been localized to 6p21 (48). No GLP-1 receptor mutations have been reported in NIDDM patients, and genetic analysis has failed to demonstrate linkage between the GLP-1 receptor gene and populations with maturity-onset diabetes of the young or NIDDM (49). GLP-1 receptor mRNA transcripts have been detected by Northern blotting in rodent tissues such as the islets, lung, kidney, stomach, and brain (45,50). GLP-1 receptor mRNA transcripts have been more difficult to detect by Northern blotting in human tissues (46,47), but have been identified in human pancreas, lung, kidney, stomach, heart, and brain by RNAse protection analyses (51).

Some controversy remains with regard to the expression of GLP-1 receptor mRNA transcripts in peripheral tissues. Although low levels of GLP-1 receptor mRNA transcripts and GLP-1 binding have been reported in rat muscle and liver (50), these findings have not been universally confirmed (51,52). Furthermore, discrepancies among results obtained using ligand binding, in situ hybridization, RNAse protection, and reverse transcription–polymerase chain reaction (RT-PCR) for characterization of GLP-1 receptor expression have led to the suggestion that structural variants of the GLP-1 receptor, or a second closely related receptor, may be expressed in different tissues (52–54); however, cDNAs encoding variant GLP-1 receptors have not yet been identified. Experiments using primary islet cultures, β-cell lines, and cells transfected with the GLP-1 receptor cDNA have shown that GLP-1 signaling is coupled to both activation of adenylate cyclase and phospholipase C pathways (12,45,50,55,56). GLP-1 binding is associated with an increase in cytosolic-free calcium (50,56,57). GLP-1 may increase intracellular [Ca^{2+}] via activation of a prolonged cAMP-sensitive inward current leading to membrane depolarization and increases in intracellular calcium (58). GLP-1 receptor responsivity may be desensitized in vitro after exposure to agonist or activation of protein kinase C (59,60), and receptor desensitization appears to correlate with receptor phosphorylation (59,61).

A number of distinct yet complementary actions contribute to the glucose-lowering properties of GLP-1 (Fig. 3). Binding of GLP-1 to its β-cell receptor stimulates insulin

secretion in a glucose-dependent manner, and GLP-1 increases insulin mRNA (12), likely via induction of insulin gene transcription through a cAMP-dependent mechanism (62,63). GLP-1 also confers glucose sensitivity to glucose-resistant β-cells (55), thereby enhancing the ability of β-cells to secrete insulin in a glucose-dependent manner. Consistent with this hypothesis, GLP-1 increased the insulinotropic effect of glibenclamide in the perfused rat pancreas (64), and GLP-1 administration to glucose-intolerant aging Wistar rats lowered plasma glucose and increased circulating insulin and insulin RNA, in keeping with a role for GLP-1 in the restoration of normal islet function and control of insulin biosynthesis (65).

GLP-1 also lowers blood glucose via inhibition of glucagon secretion (66,67). GLP-1 likely acts directly on the pancreatic A-cell and via indirect mechanisms through stimulation of somatostatin and insulin secretion. GLP-1 infusion in C-peptide–negative diabetic dogs lowered circulating plasma glucagon, suggesting that the glucagonostatic effects of GLP-1 are at least partially independent of circulating insulin (68). Consistent with a direct effect of GLP-1 on glucagon and somatostatin secretion, GLP-1 receptors have been localized to the α- and δ-cells of the islets (69). Paradoxically, GLP-1 stimulated glucagon secretion from isolated rat α cells, and this stimulatory effect was inhibited by somatostatin, raising the possibility that the glucagonostatic effects of GLP-1 are partly indirect through a paracrine effect on somatostatin secretion (70). In contrast, GLP-1 directly inhibited glucagon secretion and intracellular cAMP in the glucagon-producing InR1-G9 cell line in the absence of somatostatin (71); the relative contributions of different mechanism(s) underlying the inhibitory effect of GLP-1 on the A-cell remain unclear.

Extrapancreatic effects of GLP-1. GLP-1 attenuates meal-associated glucose excursion by directly inhibiting gastric emptying (72). GLP-1 also inhibits postprandial acid secretion, and GLP-1 receptors have been demonstrated in the stomach (45,73). The GLP-1 receptor expressed in heart is structurally identical to the pancreatic islet receptor (51), and GLP-1 increased systolic and diastolic pressure and heart rate in rats (74). The highest levels of GLP-1 receptor mRNA transcripts are found in lung (45), consistent with identification of GLP-1 binding sites in lung membrane preparations (75,76). Although GLP-1 stimulated macromolecule secretion from tracheal ring preparations (77), a physiological role, if any, for GLP-1 in pulmonary physiology in vivo remains to be determined.

The mechanism of action of GLP-1 in peripheral tissues such as liver, muscle, and adipose tissue remains unclear (Fig. 3). GLP-1–stimulated glycogen synthesis in isolated hepatocytes from normal and diabetic rats (78) and GLP-1 binding in rat hepatocyte and liver membrane preparations (79) has been demonstrated. Both fish and human GLP-1 stimulated glycogenolysis in fish hepatocyte preparations (80). In contrast, other investigators failed to demonstrate an effect of GLP-1 on hepatic glycogenolysis or glycogen synthesis in rat liver (81). Similarly, GLP-1 binding activity has been observed in rat skeletal muscle membranes (54), and GLP-1 effects on glucose incorporation into glycogen have been demonstrated by some investigators (82,83), but not by others (84). GLP-1 binding has also been demonstrated in rat and human adipose tissue membranes (53,85), and GLP-1–enhanced insulin-stimulated glucose uptake in 3T3-L1 adipocytes (86) and isolated rat adipocytes (87). Intriguingly, GLP-1 decreased intracellular cAMP in 3T3-L1 adipocytes, thereby providing indirect evidence for the presence of a second receptor with signaling properties distinct from those described for the pancreatic GLP-1 receptor (88). Although GLP-1 receptor expression has been demonstrated by RT-PCR in RNA from rat muscle and fat pad (86), other investigators, using a combination of RT-PCR, RNAse-protection, and in situ hybridization experiments, failed to detect GLP-1 receptor mRNA transcripts in adipose tissue, liver, and muscle (52). Given the lack of conclusive evidence for the expression of the pancreatic GLP-1 receptor in muscle, liver, and adipose tissue, the mechanisms and receptor(s) mediating these peripheral effects of GLP-1 remain unclear.

PGDPs and GLP-1 are synthesized in the central nervous system (CNS), and GLP-1 receptors have been localized through a combination of in situ autoradiography and hybridization studies to different regions of the CNS (89,90). Although GLP-1 immunoreactivity is widely distributed in many regions of the brain, GLP-1 mRNA transcripts are localized predominantly to the brain stem and, to a lesser extent, the hypothalamus, thereby supporting a role for peptidergic transport from brain stem neurons in the regulation of GLP-1 CNS distribution (91–93). Consistent with these findings, GLP-1 binding sites and GLP-1 receptor RNA transcripts have been identified throughout the CNS (89,90,94,95) and in the pituitary (96,97). GLP-1 may also play a role in the peripheral nervous system, as intraportal GLP-1 activates vagal nerve activity in rats (98).

A potential role for GLP-1 in the central control of feeding behavior was suggested by studies demonstrating that intracerebroventricular (ICV) administration of GLP-1 in rats inhibited food intake and induced c-*fos* immunoreactivity in the paraventricular nucleus and amygdala (99). Although ICV GLP-1 and leptin both inhibit food intake, leptin activated c-*fos*–like immunoreactivity in regions of the rat brain different from those activated by GLP-1 (100). Furthermore, the inhibitory effects of leptin were of comparatively longer duration, and GLP-1, but not leptin, produced conditioned taste aversion, implying distinct roles for these peptides in the central regulation of feeding (101). ICV GLP-1 inhibited basal water intake (102) and stimulated urinary excretion of water and sodium, and both ICV and intraperitoneal GLP-1 inhibited basal and ANG II–induced drinking behavior (103) and reduced body temperature in rats. Whether the GLP-1 effects on water regulation are related to or distinct from the peripheral effects of GLP-1 on heart rate and blood pressure (74) remains uncertain.

Studies using GLP-1 receptor antagonists. The observation that a truncated lizard GLP-1–related peptide, exendin(9-39), binds to the mammalian GLP-1 receptor and functions as a GLP-1 antagonist (46,104) has provided the opportunity to carry out studies examining the transient reduction or loss of GLP-1 action both in vitro and in vivo. Exendin(9-39) administered to rats reduced postprandial insulin levels (105), reduced insulin secretory response, and increased blood glucose after intraduodenal glucose infusion (106), thereby providing important evidence that GLP-1 is a physiologically relevant incretin in vivo. Infusion of exendin(9-39) in baboons increased fasting levels of glucose and glucagon and increased postprandial glycemic excur-

sion with reduction of postprandial insulin secretion (107). The postprandial glycemic excursion was also increased after infusion of a GLP-1–specific monoclonal antibody (107). Exendin(9-39) blocks the extrapancreatic effects of GLP-1 in the cardiovascular system, liver, and muscle (108,109), and functions as an antagonist of the brain GLP-1 receptor, inhibiting the effects of ICV GLP-1 on food and water intake (99,103), and potentiating the stimulatory actions of neuropeptide Y on food intake (99).

Despite experimental evidence that exendin(9-39) may be a relatively specific GLP-1 receptor antagonist, experiments with the cloned rat and human GIP receptors have demonstrated that relatively high concentrations of exendin(9-39) may also function as a GIP receptor antagonist (110,111). The results of recent experiments suggest that truncation of the first 3-7 amino acids of exendin-4 may produce exendin analogs that are up to 10-fold more potent as GLP-1 antagonists than exendin(9-39) (112); however, the relative specificity of these analogs for the GLP-1 versus the GIP receptor has not yet been reported.

The GLP-1 receptor –/– mouse. Targeted disruption of the gene encoding the pancreatic islet GLP-1 receptor (GLP-1R) in embryonic stem cells followed by the derivation of transgenic GLP-1R –/– mice has permitted the analysis of the role of GLP-1 in both glucose control and appetite regulation in vivo. GLP-1R –/– mice exhibit mild fasting hyperglycemia and glucose intolerance after oral glucose challenge (113). The abnormal glycemic excursion after oral glucose loading was associated with a reduction in glucose-stimulated insulin secretion, consistent with an essential role for GLP-1 signaling in the regulation of glucose-dependent insulin secretion (113). Remarkably, GLP-1R –/– mice also exhibit abnormal glycemic excursion after intraperitoneal glucose challenge, suggesting that intact GLP-1 signaling is important for the handling of a glucose load, independent of the site of glucose entry.

Despite evidence that ICV GLP-1 is a potent inhibitor of food intake, analysis of body weight in GLP-1R –/– mice (up to age 18 months; D.J.D., unpublished observations) did not demonstrate any significant changes in body mass compared with age- and sex-matched control mice (113). Disruption of GLP-1 signaling in the brain does not appear to be associated with chronically increased food intake, and GLP-1 receptor –/– mice do not eat more than control mice in short-term feeding studies (113). Furthermore, despite evidence for significantly increased leptin sensitivity in the GLP-1R –/– islet, the inhibition of food intake after ICV leptin appears relatively normal in the GLP-1R –/– mouse (114). No GLP-1 binding sites are detectable in the CNS of GLP-1R –/– mice, consistent with the presence of a single brain GLP-1 receptor. Taken together, studies with the GLP-1R –/– mouse support an essential role for GLP-1 in the regulation of glycemia and glucose-stimulated insulin secretion; however, the available data suggest that GLP-1 signaling may not be essential for regulation of satiety or body weight. The CNS results may be explained by genetic redundancy, in that multiple compensatory mechanisms likely exist for central regulation of food intake and body weight (115). Furthermore, the possibility that disruption of GLP-1 signaling from birth may be associated with subtle developmental abnormalities in the CNS that may influence the regulation of feeding and body weight cannot be excluded.

GLP-1 IN HUMAN STUDIES

Normal human subjects. GLP-1, either as the (7-36)amide or in the 7-37 forms (27), stimulates insulin secretion, inhibits glucagon secretion, and lowers blood glucose in humans in the fasting or postprandial state (116,117). Infusion of GLP-1 in normal human volunteers delays gastric emptying (72), although nausea and vomiting, likely due to inhibition of gastric emptying, have been observed with higher dosages of GLP-1 (117). GLP-1 may also regulate glycemia by modulating hepatic glucose production, predominantly through its effects on levels of circulating insulin and glucagon (118,119). The mechanisms underlying the insulin-independent effects of GLP-1 facilitating glucose disposal remain unclear. GLP-1 infusion increased glucose disposal and glucose effectiveness in short-term (4-h) studies in normal subjects (120,121), but had no effect on glucose disposal (independent from the effect of insulin) after intravenous glucose loading (119). Furthermore, no effect of GLP-1 on insulin sensitivity was observed during a 3-h hyperinsulinemic, euglycemic clamp (122) or after oral fat ingestion or intravenous glucose loading (121). Although the majority of human studies deliver GLP-1 by intravenous or subcutaneous injection, a recent promising study demonstrated that formulation of GLP-1 as a buccal tablet promotes transmucosal absorption, resulting in increased levels of insulin and decreased glucagon and glucose in healthy human volunteers (123).

NIDDM patients. The demonstration that GLP-1 exhibited considerably greater potency compared with that of GIP as a glucose-dependent stimulator of insulin secretion in diabetic subjects has stimulated considerable interest in the use of GLP-1 for the treatment of NIDDM (124). Although both GLP-1 and GIP stimulate insulin secretion, GLP-1, but not GIP, inhibits gastric emptying and lowers circulating glucagon in NIDDM patients (124,125). GLP-1 infusion normalized fasting hyperglycemia in NIDDM patients with poor glycemic control (126) and improved basal and glucose- and arginine-stimulated insulin secretion in NIDDM subjects (127). Several short-term studies in NIDDM patients have demonstrated that GLP-1, whether administered by intravenous infusion or subcutaneous injection, normalizes both fasting and postprandial glycemia (128), predominantly by enhancing β-cell function and inhibiting both gastric emptying and glucagon secretion (129–131). Additional evidence for a beneficial effect of GLP-1 on islet function in NIDDM patients derives from studies demonstrating that the glucose-lowering effect of GLP-1 is enhanced by the sulfonylurea glibenclamide in patients previously resistant to glibenclamide alone (64).

A recent study has examined the effect of more prolonged GLP-1 treatment on glucose control in NIDDM patients receiving intensive insulin therapy for 1 week followed by either an additional 7 days on insulin alone or insulin plus GLP-1 at meals. The GLP-1–treated group required less exogenous insulin and exhibited a reduction in postprandial hyperglycemia but increased preprandial glycemia, possibly due to the short duration of action of GLP-1 (132). GLP-1 treatment also increased LDL particle diameter and reduced both lipoprotein lipase and hepatic lipase activity. A second study reported the results of a 3-week, double-blind crossover trial of GLP-1 or saline three times a day before meals in five NIDDM patients with poor glycemic control. GLP-1 treatment lowered postprandial glucagon levels and improved

postprandial glycemic control, despite no significant increase in postprandial insulin levels in these patients (133).

IDDM patients. GLP-1 lowered postprandial blood glucose and the meal-related insulin requirement in IDDM patients in association with a reduction in circulating glucagon and somatostatin (116). These observations emphasized the potential importance of GLP-1 for lowering blood glucose independent of its actions on the pancreatic β-cell. The glucose-lowering properties of GLP-1 in IDDM patients after meal ingestion are likely due in large part to a delay of gastric emptying and inhibition of glucagon secretion (134). Administration of lower dosages of GLP-1 to IDDM patients decreased postprandial glycemic excursion but not glucagon levels, suggesting that delayed gastric emptying is a major contributor to the decreased blood glucose observed in these studies (135). In contrast, inhibition of glucagon secretion is the primary determinant of the reduction in fasting glycemia observed in IDDM patients infused with GLP-1 (136).

Novel glucagon-like peptides. Peptides originally isolated from the venom of the Gila monster lizard *H. suspectum* or *H. horridum* increased cyclic AMP and amylase secretion from dispersed pancreatic acini in vitro. Screening of lizard venom for the presence of peptides with amino terminal histidine residues culminated in the isolation of two peptides, designated exendin-3 (137) and exendin-4 (138). Both peptides exhibit approximately 50% amino acid identity to mammalian GLP-1 (Fig. 2), but are encoded by unique exendin genes with different patterns of tissue-specific expression in the lizard (18). Both exendin peptides increase cAMP in dispersed pancreatic acini, but only exendin-3 increases amylase secretion; exendin-4 does not most likely because exendin-4 does not bind to the pancreatic vasoactive intestinal peptide (VIP) receptor (138). Exendin-4 binds to the GLP-1 receptor and stimulates glucose-dependent insulin secretion in islet cells in vitro (46,104) and in animal studies in vivo (106). Exendin-4 also mimics the majority of peripheral actions of GLP-1 in the cardiovascular system, stomach, and brain (139). Intriguingly, GLP-1 also binds to the putative exendin receptor and increases acinar cAMP; however, the identity of the exendin receptor expressed on pancreatic acinar cells remains unclear (140).

The first three amino acids at the NH_2-terminus of exendin-3 are His1-Ser2-Asp3; exendin-4 differs by two amino acid substitutions, Gly2 and Glu3. The presence of a penultimate glycine, instead of alanine, at position 2 in exendin-4 raises the possibility that exendin-4 will be comparatively more resistant to degradation by the enzyme DPP-IV than mammalian GLP-1s with a position 2 alanine. Furthermore, exendin-4 is a less favorable substrate than GLP-1 for the human neutral endopeptidase 24.11 (141). Preliminary studies have suggested that exendin-4 may be more potent than native GLP-1 in studies examining insulin secretion in vivo (139), perhaps due in part to its increased stability in vivo. These data suggest that more detailed analysis may be warranted of the potential role of exendin-4 and structurally related GLP-1 analogs in the treatment of diabetes.

The molecular cloning of proglucagon cDNAs from *Xenopus laevis* revealed the structure of three unique GLP-1–related molecules, designated xenGLP-1A–C (142). All three *Xenopus* GLP-1 molecules bound and activated the human GLP-1 receptor and stimulated insulin secretion from the perfused rat pancreas. Remarkably, xenGLP-1B exhibited a higher affinity for the GLP-1 receptor than human GLP-1 and was equipotent to human GLP-1 in cAMP stimulation assays, despite eight amino acid substitutions in the *Xenopus* compared with the human molecule. In independent structure-function studies of the mammalian GLP-1 molecule, important amino acid residues and peptide domains critical for GLP-1 receptor binding and signal transduction have been defined (143–147). Furthermore, the demonstration that circulating GLP-1 has a very short half-life in part due to cleavage by the enzyme DPP-IV has stimulated considerable interest in the design and testing of more stable GLP-1 analogs that would be more resistant to enzymatic degradation in vivo. Complementary approaches for enhancing and simplifying GLP-1 delivery include the development of GLP-1–containing tablets for buccal absorption. Preliminary studies have demonstrated transmucosal absorption of bioactive GLP-1 in fasting human subjects associated with increased levels of insulin and decreased glucagon and blood glucose (123), suggesting that this approach may be promising for the future treatment of NIDDM patients.

GLUCAGON-LIKE PEPTIDE 2

After the isolation of the cDNAs and genes encoding proglucagon, the sequence of a second GLP, GLP-2, was identified COOH-terminal to the GLP-1–like sequence in mammalian proglucagons. GLP-2 is predicted to represent a 33–amino acid peptide, with a sequence that is highly conserved in mammalian proglucagons (Fig. 2), there being only a single amino acid difference between rat and human GLP-2 (7,148). The observation that angler fish islet proglucagon cDNAs did not encode a GLP-2–like sequence (149) raised the possibility that GLP-2 may not be biologically important due to its lack of conservation in various species. Subsequent experiments have shown that a GLP-2 sequence is indeed present in the fish genome; isolation of trout intestinal proglucagon cDNAs demonstrated that fish intestinal proglucagon mRNAs encoded a GLP-2 sequence that was not present in the pancreatic proglucagon mRNA due to alternative mRNA splicing (17).

A biological role for GLP-2 was deduced after studies examining the link between proglucagon gene expression and intestinal growth. Two case reports describing human patients with glucagonomas and small bowel growth stimulated analyses of the link between proglucagon-derived peptides and intestinal growth (150,151). Experimental damage to the intestine or surgical resection of the bowel is generally associated with increased secretion of the intestinal PGDPs (152,153). Furthermore, proglucagon gene expression is increased in the intestinal remnant after small bowel resection (154,155). The study of transplantable glucagonomas in rodents (156) facilitated the identification of GLP-2 as the PGDP with significant intestinal growth factor activity. GLP-2 promotes the rapid stimulation of small bowel growth via a direct effect on crypt cell proliferation and by inhibiting enterocyte apoptosis (157), with increased small bowel epithelium detected within 4 days of GLP-2 administration (158).

GLP-2 is intestinotrophic in rodents across a wide spectrum of ages; maintaining the increased mass of the mucosal epithelium appears to be dependent on ongoing GLP-2 stimulation (157). Long-term studies have shown that mice treated with GLP-2 daily for up to 3 months maintained increased bowel mass for the entire treatment period, with no evidence for tachyphylaxis or downregulation of biological

activity (157). GLP-2 is also trophic to the large bowel epithelium, consistent with its distribution in enteroendocrine cells of both the small and large intestine (157,159). The small bowel hyperplasia commonly observed in animal models of diabetes is associated with increased GLP-2 synthesis and secretion (160). Furthermore, treatment of diabetic rats with insulin normalizes GLP-2 and reverses the increased mucosal proliferation in vivo (160).

Analysis of the regulation of GLP-2 secretion suggests that GLP-2 is co-secreted from the enteroendocrine cell with GLP-1, oxyntomodulin, and glicentin (161–163). Although a predominant molecular form of tissue and circulating GLP-2 appears to be the 33–amino acid peptide, GLP-2 is also degraded at the NH_2-terminus by the enzyme dipeptidyl peptidase IV (DPP-IV) to yield GLP-2(3-33) (163,164). Consistent with this observation, DPP-IV–resistant GLP-2 analogs exhibit greater intestinotrophic properties (compared with wild-type GLP-2) in vivo (164). GLP-2 infusion in rats stimulated basolateral glucose transporter translocation, suggesting a possible role for GLP-2 in control of intestinal glucose transport (165). Although GLP-2 is synthesized along with GLP-1 in selected regions of the CNS, a biological role for GLP-2 in the brain has not yet been elucidated.

Nutrient absorption after oral nutrient tolerance testing in GLP-2–treated mice is normal, with no detectable abnormalities in absorption of simple carbohydrates, fats, or amino acids (166). Furthermore, duodenal perfusion of nutrients in GLP-2–treated mice resulted in normal to enhanced absorption in vivo (166). Taken together with the normal profile of gene products and enzymatic activities detected in GLP-2–induced small bowel, the available data suggest that the small bowel growth induced by GLP-2 appears to represent physiologically normal intestine. Furthermore, recent experiments have suggested that the combination of GLP-2 with other peptide growth factors may lead to enhancement of the proliferative capacity of the intestinal mucosa in vivo (159). Infusion of GLP-2 into fasted rats maintained on total parenteral nutrition (TPN) prevented TPN-associated gut hypoplasia (167), suggesting that GLP-2 alone may circumvent the requirement for luminal nutrition in ongoing maintenance of the mucosal epithelium. These observations raise the possibility that GLP-2 may be useful as a therapeutic growth factor in situations marked by intestinal failure in vivo.

SUMMARY

The past decade has witnessed a tremendous increase in our understanding of the factors that control the synthesis, secretion, and biological activities of the GLPs. Nevertheless, significant questions remain unanswered. For example, what are the mechanisms responsible for the actions of GLP-1 in peripheral tissues such as fat, liver, and muscle? The available evidence suggests that some of these effects may be indirect or possibly mediated by a GLP-1 receptor that uses signaling mechanisms distinct from those characterized for the pancreatic islet GLP-1 receptor. Are the CNS effects of GLP-1 physiologically important for control of body weight and satiety? The absence of obesity in GLP-1R –/– mice demonstrates that GLP-1 signaling is not essential for control of body weight in mice, but this does not rule out the possibility that pharmacological activation of the CNS GLP-1 receptor may be therapeutically useful for reduction of food intake. Can well-tolerated peptide analogs of GLP-1 be developed that exhibit improved pharmacokinetics while remaining both safe and efficacious, and will these analogs have a role in the treatment of diabetic patients? Will alternative delivery systems for delivery of GLP-1, perhaps via the gastrointestinal tract, oral mucosa, or lung, be developed for optimized and more convenient delivery of GLP-1 in human studies? Can nonpeptide analogs of GLP-1 be identified that will be more suitable candidates for pharmaceutical development?

The intestinotrophic activities of GLP-2 strongly suggest that GLP-2 acts directly on the intestinal crypt cell to stimulate proliferation; however, the molecular mechanism(s) used by GLP-2 for its biological effects in the intestine remains unknown. The homology of GLP-2 with GLP-1, glucagon, and GIP suggests that GLP-2 exerts its effects through a new receptor, likely a novel yet related member of the G-protein–linked receptor superfamily. Given that the study of GLP-2 and its actions is only beginning, it is anticipated that additional new biological activities of GLP-2 will be elucidated. It will be important to determine whether GLP-2 shows sufficient therapeutic potential in animal models of disease to warrant its development as a pharmaceutical agent for the treatment of specific human intestinal diseases. The increasing interest in the potential therapeutic applications of the GLPs suggests that answers to many of the above questions will soon be forthcoming.

ACKNOWLEDGMENTS

D.J.D. is supported by a Scientist Award from the Medical Research Council of Canada, and is a consultant to Amylin Pharmaceuticals and Allelix Biopharmaceuticals.

The author thanks P. Brubaker, D. Irwin, and G. Lewis for critical review of the manuscript.

REFERENCES

1. Elrick H, Stimmler L, Hlad CJ Jr, Arai Y: Plasma insulin response to oral and intravenous glucose administration. *J Clin Invest* 24:1076–1082, 1964
2. Perley MJ, Kipnis DM: Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. *J Clin Invest* 46:1954–1962, 1967
3. Creutzfeldt W, Ebert R: New developments in the incretin concept today. *Diabetologia* 28:565–573, 1985
4. Fehmann H-C, Goke R, Goke B: Cell and molecular biology of the incretin hormones glucagon-like peptide 1 and glucose-dependent releasing polypeptide. *Endocrine Rev* 16:390–410, 1995
5. Bell GI, Santerre RF, Mullenbach GT: Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* 302:716–718, 1983
6. Lopez LC, Frazier ML, Su CJ, Kumar A, Saunders GF: Mammalian pancreatic preproglucagon contains three glucagon-related peptides. *Proc Natl Acad Sci USA* 80:5485–5489, 1983
7. Heinrich G, Gros P, Lund PK, Bentley RC, Habener JF: Pre-proglucagon messenger ribonucleic acid: nucleotide and encoded amino acid sequences of the rat pancreatic complementary deoxyribonucleic acid. *Endocrinology* 115:2176–2181, 1984
8. Schmidt WE, Siegel EG, 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
9. Mojsov S, Weir GC, Habener JF: 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. *J Clin Invest* 79:616–619, 1987
10. Holst JJ, Orskov C, Nielsen OV, Schwartz TW: Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett* 211:169–174, 1987
11. Kreymann B, Ghatei MA, Williams G, Bloom SR: Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* ii:1300–1304, 1987
12. Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF: Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci USA* 84:3434–3438, 1987
13. Thorens B, Waeber G: Glucagon-like peptide-I and the control of insulin secretion in the normal state and in NIDDM. *Diabetes* 42:1219–1225, 1993

14. Holst JJ: Glucagonlike peptide 1: a newly discovered gastrointestinal hormone. *Gastroenterology* 107:1848–1855, 1994
15. Drucker DJ: Intestinal growth factors. *Am J Physiol* 36:G3–G6, 1997
16. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF: Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem* 261:11880–11889, 1986
17. Irwin DM, Wong J: Trout and chicken proglucagon: Alternative splicing generates mRNA transcripts encoding glucagon-like peptide 2. *Mol Endocrinol* 9:267–277, 1995
18. Chen YE, Drucker DJ: Tissue-specific expression of unique mRNAs that encode proglucagon-derived peptides or exendin-4 in the lizard. *J Biol Chem* 272:4108–4115, 1997
19. Efrat S, Teitelman G, Anwar M, Ruggiero D, Hanahan D: Glucagon gene regulatory region directs oncoprotein expression to neurons and pancreatic alpha cells. *Neuron* 1:605–613, 1988
20. Lee YC, Asa SL, Drucker DJ: Glucagon gene 5′-flanking sequences direct expression of SV40 large T antigen to the intestine producing carcinoma of the large bowel in transgenic mice. *J Biol Chem* 267:10705–10708, 1992
21. Jin T, Drucker DJ: The proglucagon gene upstream enhancer contains positive and negative domains important for tissue-specific proglucagon gene transcription. *Mol Endocrinol* 9:1306–1320, 1995
22. Jin T, Drucker DJ: Activation of proglucagon gene transcription through a novel promoter element by the caudal-related homeodomain protein cdx-2/3. *Mol Cell Biol* 16:19–28, 1996
23. Laser B, Meda P, Constant I, Philippe J: The caudal-related homeodomain protein cdx-2/3 regulates glucagon gene expression in islet cells. *J Biol Chem* 271:28984–28994, 1996
24. Rothenberg ME, Eilertson CD, Klein K, Zhou Y, Lindberg I, McDonald JK, Mackin RB, Noe BD: Processing of mouse proglucagon by recombinant prohormone convertase 1 and immunopurified prohormone convertase 2 in vitro. *J Biol Chem* 270:10136–10146, 1995
25. Dhanvantari S, Seidah NG, Brubaker PL: Role of prohormone convertases in the tissue-specific processing of proglucagon. *Mol Endocrinol* 10:342–355, 1996
26. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ: Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes* 43:535–539, 1994
27. Orskov C, Wettergren A, Holst JJ: Biological effects and metabolic rates of glucagonlike peptide-1 7-36 amide and glucagonlike peptide-1 7-37 in healthy subjects are indistinguishable. *Diabetes* 42:658–661, 1993
28. Reimer RA, McBurney MI: Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1 and insulin in rats. *Endocrinology* 137:3948–3956, 1996
29. Hoyt EC, Lund PK, Winesett DE, Fuller CR, Ghatei MA, Bloom SR, Ulshen MR: Effects of fasting, refeeding and intraluminal triglyceride on proglucagon expression in jejunum and ileum. *Diabetes* 45:434–439, 1996
30. Roberge JN, Gronau KA, Brubaker PL: Gastrin-releasing peptide is a novel mediator of proximal nutrient-induced proglucagon-derived peptide secretion from the distal gut. *Endocrinology* 137:2383–2388, 1996
31. Herrmann-Rinke C, Voge A, Hess M, Goke B: Regulation of glucagon-like peptide-1 secretion from rat ileum by neurotransmitters and peptides. *J Endocrinol* 147:25–31, 1995
32. Dumoulin V, Dakka T, Plaisancie P, Chayvialle J-A, Cuber J-C: Regulation of glucagon-like peptide-1(7-36) amide, peptide YY, and neurotensin secretion by neurotransmitters and gut hormones in the isolated vascularly perfused rat ileum. *Endocrinology* 136:5182–5188, 1995
33. Plaisancie P, Bernard C, Chayvialle J-A, Cuber J-C: Regulation of glucagon-like peptide-1 (7-36)amide, peptide YY, and neurotensin secretion by intestinal neurotransmitters and hormones in the isolated vascularly perfused rat colon. *Endocrinology* 135:2398–2403, 1994
34. Herrmann-Rinke C, Horsch D, McGregor GP, Goke B: Galanin is a potent inhibitor of glucagon-like peptide-1 secretion from rat ileum. *Peptides* 17:571–576, 1996
35. Plaisancié P, Dumoulin V, Chayvialle J-A, Cuber J-C: Luminal glucagon-like peptide-1(7-36) amide-releasing factors in the isolated vascularly perfused rat colon. *J Endocrinol* 145:521–526, 1995
36. Herrmann C, Goke R, Richter G, Fehmann H-C, Arnold R, Goke B: Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digest* 56:117–126, 1995
37. Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V: Glucagon-like peptide-1(7-36) amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol* 138:159–166, 1993
38. Orskov C, Wettergren A, Holst JJ: Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day. *Scand J Gastroenterol* 31:665–670, 1996
39. Balks HJ, Holst JJ, von zur Muhlen A, Brabant G: Rapid oscillations in plasma glucagon-like peptide-1 (GLP-1) in humans: cholinergic control of GLP-1 secretion via muscarinic receptors. *J Clin Endocrinol Metab* 82:786–790, 1997
40. Mentlein R, Gallwitz B, Schmidt WE: Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36) amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 214:829–835, 1993
41. Kieffer TJ, McIntosh CHS, Pederson RA: Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 136:3585–3596, 1995
42. Deacon CF, Johnsen AH, Holst JJ: Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab* 80:952–957, 1995
43. Knudsen LB, Pridal L: Glucagon-like peptide-1-(9-36) amide is a major metabolite of glucagon-like peptide-1-(7-36)amide after in vivo administration to dogs and it acts as an antagonist on the pancreatic receptor. *Eur J Pharmacol* 318:429–435, 1996
44. Ruiz-Grande C, Alarcón C, Alcántara A, Castilla C, López Novoa JM, Villanueva-Peñacarrillo ML, Valverde I: Renal catabolism of truncated glucagon-like peptide 1. *Horm Metab Res* 25:612–616, 1993
45. Thorens B: Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci USA* 89:8641–8645, 1992
46. Thorens B, Porret A, Bühler L, Deng S-P, Morel P, Widmann C: Cloning and functional expression of the human islet GLP-1 receptor: demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. *Diabetes* 42:1678–1682, 1993
47. Dillon JS, Tanizawa Y, Wheeler MB, Leng X-H, Ligon BB, Rabin DU, Yoo-Warren H, Permutt MA, Boyd AE III: Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. *Endocrinology* 133:1907–1910, 1993
48. Stoffel M, Espinosa R III, Le Beau MM, Bell GI: Human glucagon-like peptide-1 receptor gene: localization to chromosome band 6p21 by fluorescence in situ hybridization and linkage of a highly polymorphic simple tandem repeat DNA polymorphism to other markers on chromosome 6. *Diabetes* 42:1215–1218, 1993
49. Zhang Y, Cook JTE, Hattersley AT, Firth R, Saker PJ, Warren-Perry M, Stoffel M, Turner RC: Non-linkage of the glucagon-like peptide-1 receptor gene with maturity onset diabetes of the young. *Diabetologia* 37:721–724, 1994
50. Wheeler MB, Lu M, Dillon JS, Leng X-H, Chen C, Boyd AE III: Functional expression of the rat glucagon-like peptide-I receptor, evidence for coupling to both adenyl cyclase and phospholipase-C. *Endocrinology* 133:57–62, 1993
51. Wei Y, Mojsov S: Tissue-specific expression of the human receptor for glucagon-like peptide 1: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett* 358:219–224, 1995
52. Bullock BP, Heller RS, Habener JF: Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide 1 receptor. *Endocrinology* 137:2968–2978, 1996
53. Mérida E, Delgado E, Molina LM, Villanueva-Peñacarrillo ML, Valverde I: Presence of glucagon and glucagon-like peptide-1-(7-36)amide receptors in solubilized membranes of human adipose tissue. *J Clin Endocrinol Metab* 77:1654–1657, 1993
54. Delgado E, Luque MA, Alcántara A, Trapote MA, Clemente F, Galera C, Valverde I, Villanueva-Peñacarrillo ML: Glucagon-like peptide-1 binding to rat skeletal muscle. *Peptides* 16:225–229, 1995
55. Holz GG, Kühtreiber WM, Habener JF: Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature* 361:362–365, 1993
56. Lu M, Wheeler MB, Leng X-H, Boyd AE III: The role of the free cytoplasmic calcium level in β-cell signal transduction by gastric inhibitory polypeptide and glucagon-like peptide I(7-37). *Endocrinology* 132:94–100, 1993
57. Gromada J, Dissing S, Bokvist K, Renström E, Frokjær-Jensen J, Wulff BS, Rorsman P: Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting βTC3-cells by enhancement of intracellular calcium mobilization. *Diabetes* 44:767–774, 1995
58. Holz GG, Leech CA, Habener JF: Activation of a cAMP-regulated Ca^{2+}-signaling pathway in pancreatic β-cells by the insulinotropic hormone glucagon-like peptide-1. *J Biol Chem* 270:17749–17757, 1995
59. Widmann C, Dolci W, Thorens B: Desensitization and phosphorylation of the glucagon-like peptide-1 (GLP-1) receptor by GLP-1 and 4-phorbol 12-myristate 13-acetate. *Mol Endocrinol* 10:62–75, 1996
60. Gromada J, Dissing S, Rorsman P: Desensitization of the glucagon-like peptide 1 receptors in insulin-secreting βTC3 cells: role of PKA-independent mechanisms. *Br J Pharmacol* 118:769–775, 1996

61. Widmann C, Dolci W, Thorens B: Heterologous desensitization of the glucagon-like peptide-1 receptor by phorbol esters requires phosphorylation of the cytoplasmic tail at four different sites. *J Biol Chem* 271:19957–19963, 1996
62. Fehmann H-C, Habener JF: Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma βTC-1 cells. *Endocrinology* 130:159–166, 1992
63. Wang YH, Egan JM, Raygada M, Nadiv O, Roth J, Montrose-Rafizadeh C: Glucagon-like peptide-1 affects gene transcription and messenger ribonucleic acid stability of components of the insulin secretory system in RIN 1046-38 cells. *Endocrinology* 136:4910–4917, 1995
64. Gutniak MK, Juntti-Berggren L, Hellstrom PM, Guenifi A, Holst JJ, Efendic S: Glucagon-like peptide I enhances the insulinotropic effect of glibenclamide in NIDDM patients and in the perfused rat pancreas. *Diabetes Care* 19:857–863, 1996
65. Wang Y, Perfetti R, Greig NH, Holloway HW, DeOre KA, Montrose-Rafizadeh C, Elahi D, Egan JM: Glucagon-like peptide-1 can reverse the age-related decline in glucose tolerance in rats. *J Clin Invest* 99:2883–2889, 1997
66. Kawai K, Suzuki S, Ohashi S, Mukai H, Ohmori H, Murayama Y, Yamashita K: Comparison of the effects of glucagon-like peptide-1-(1-37) and -(7-37) and glucagon on islet hormone release from isolated perfused canine and rat pancreases. *Endocrinology* 124:1768–1773, 1989
67. Komatsu R, Matsuyama T, Namba M, Watanabe N, Itoh H, Kono N, Tarui S: Glucagonostatic and insulinotropic action of glucagon-like peptide I-(7-36)-amide. *Diabetes* 38:902–905, 1989
68. Freyse E-J, Becher T, El-Hag O, Knospe S, Goke B, Fischer U: Blood glucose lowering and glucagonostatic effects of glucagon-like peptide I in insulin-deprived diabetic dogs. *Diabetes* 46:824–828, 1997
69. Heller RS, Kieffer TJ, Habener JF: Insulinotropic glucagon-like peptide I receptor expression in glucagon-producing alpha-cells of the rat endocrine pancreas. *Diabetes* 46:785–791, 1997
70. D'alessio DA, Fujimoto WY, Ensinck JW: Effects of glucagonlike peptide I(7-36) on release of insulin, glucagon, and somatostatin by rat pancreatic islet cell monolayer cultures. *Diabetes* 38:1534–1538, 1989
71. Matsumura T, Itoh H, Watanabe N, Oda Y, Tanaka M, Namba M, Kono N, Matsuyama T, Komatsu R, Matsuzawa Y: Glucagonlike peptide-1(7-36) amide suppresses glucagon secretion and decreases cyclic AMP concentration in cultured In-R1-G9 cells. *Biochem Biophys Res Commun* 186:503–508, 1992
72. Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ: Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Dig Dis Sci* 38:665–673, 1993
73. Schmidtler J, Dehne K, Allescher H-D, Schusdziarra V, Classen M, Holst JJ, Polack A, Schepp W: Rat parietal cell receptors for GLP-1-(7-36) amide: Northern blot, cross-linking, and radioligand binding. *Am J Physiol* 267:G423–G432, 1994
74. Barragan JM, Rodriguez RE, Blazquez E: Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7-36 amide) in rats. *Am J Physiol* 266:E459–E466, 1994
75. Kanse SM, Kreymann B, Ghatei MA, Bloom SR: Identification and characterization of glucagon-like peptide-1 7-36 amide-binding sites in the rat brain and lung. *FEBS Lett* 241:209–212, 1988
76. Richter G, Göke R, Göke B, Schmidt H, Arnold R: Characterization of glucagon-like peptide-I(7-36)amide receptors of rat lung membranes by covalent cross-linking. *FEBS Lett* 280:247–250, 1991
77. Richter G, Feddersen O, Wagner U, Barth P, Göke R, Göke B: GLP-1 stimulates secretion of macromolecules from airways and relaxes pulmonary artery. *Am J Physiol* 265:L374–L381, 1993
78. Valverde I, Morales M, Clemente F, Lopez-Delgado MI, Delgado E, Perea A, Villanueva-Penacarillo ML: Glucagon-like peptide 1: a potent glycogenic hormone. *FEBS Lett* 349:313–316, 1994
79. Villanueva-Peñacarrillo ML, Delgado E, Trapote MA, Alcántara A, Clemente F, Luque MA, Perea A, Valverde I: Glucagon-like peptide-1 binding to rat hepatic membranes. *J Endocrinol* 146:183–189, 1995
80. Conlon JM, Youson JH, Mommsen TP: Structure and biological activity of glucagon and glucagon-like peptide from a primitive bony fish, the bowfin (*Amia calva*). *Biochem J* 295:857–861, 1993
81. Nakagawa Y, Kawai K, Suzuki H, Ohashi S, Yamashita K: Glucagon-like peptide-1(7-36) amide and glycogen synthesis in the liver. *Diabetologia* 39:1241–1242, 1996
82. Villanueva-Peñacarrillo ML, Alcántara AI, Clemente F, Delgado E, Valverde I: Potent glycogenic effect of GLP-1(7-36) amide in rat skeletal muscle. *Diabetologia* 37:1163–1166, 1994
83. Morales M, Lopez-Delgado MI, Alcantara A, Luque MA, Clemente F, Marquez L, Puente J, Vinambres C, Malaisse WJ, Villanueva-Penacarrillo ML, Valverde I: Preserved GLP-1 effects on glycogen synthase a activity and glucose metabolism in isolated hepatocytes and skeletal muscle from diabetic rats. *Diabetes* 46:1264–1269, 1997
84. Furnsinn C, Ebner K, Waldhausl W: Failure of GLP-1 (7-36) amide to affect glycogenesis in rat skeletal muscle. *Diabetologia* 38:864–867, 1995
85. Valverde I, Merida E, Delgado E, Trapote MA, Villanueva-Penacarillo ML: Presence and characterization of glucagon-like peptide-1(7-36) amide receptors in solubilized membranes of rat adipose tissue. *Endocrinology* 132:75–79, 1993
86. Egan JM, Montrose-Rafizadeh C, Wang Y, Bernier M, Roth J: Glucagon-like peptide-1(7-36) amide (GLP-1) enhances insulin-stimulated glucose metabolism in 3T3-L1 adipocytes: one of several potential extrapancreatic sites of GLP-1 action. *Endocrinology* 135:2070–2075, 1994
87. Miki H, Namba M, Nishimura T, Mineo I, Matsumura T, Miyagawa J, Nakajima H, Kuwajima M, Hanafusa T, Matsuzawa Y: Glucagon-like peptide-1 (7-36) amide enhances insulin-stimulated glucose uptake and decreases intracellular cAMP content in isolated rat adipocytes. *Biochim Biophys Acta* 1312:132–136, 1996
88. Montrose-Rafizadeh C, Yang H, Wang Y, Roth J, Montrose MH, Adams LG: Novel signal transduction and peptide specificity of glucagon-like peptide receptor in 3T3-L1 adipocytes. *J Cell Physiol* 172:275–283, 1997
89. Shimizu I, Hirota M, Ohboshi C, Shima K: Identification and localization of glucagon-like peptide-1 and its receptor in rat brain. *Endocrinology* 121:1076–1082, 1987
90. Uttenthal LO, Toledano A, Blazquez E: Autoradiographic localization of receptors for glucagon-like peptide-1(7-36) amide in rat brain. *Neuropeptides* 21:143–146, 1992
91. Kreymann B, Ghatei MA, Burnet P, Williams G, Kanse S, Diani AR, Bloom SR: Characterization of glucagon-like peptide-1-(7-36)amide in the hypothalamus. *Brain Res* 502:325–331, 1989
92. Drucker DJ, Asa S: Glucagon gene expression in vertebrate brain. *J Biol Chem* 263:13475–13478, 1988
93. Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C: Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience* 77:257–270, 1997
94. Alvarez E, Roncero I, Chowen JA, Thorens B, Blazquez E: Expression of the glucagon-like peptide-1 receptor gene in rat brain. *J Neurochem* 66:920–927, 1996
95. Campos RV, Lee YC, Drucker DJ: Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology* 134:2156–2164, 1994
96. Beak SA, Small CJ, Ilovaiskaia I, Hurley JD, Ghatei MA, Bloom SR, Smith DM: Glucagon-like peptide-1 (GLP-1) releases thyrotropin (TSH): characterization of binding sites for GLP-1 on alpha-TSH cells. *Endocrinology* 137:4130–4135, 1996
97. Göke R, Larsen PJ, Mikkelsen JD, Sheikh SP: Identification of specific binding sites for glucagon-like peptide-1 on the posterior lobe of the rat pituitary. *Neuroendocrinology* 62:130–134, 1995
98. Nakabayashi H, Nishizawa M, Nakagawa A, Takeda R, Niijima A: Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. *Am J Physiol* 271:E808–E813, 1996
99. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JPH, Smith DM, Ghatei MA, Herbert J, Bloom SR: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379:69–72, 1996
100. Van Dijk G, Thiele TE, Donahey JCK, Campfield LA, Smith FJ, Burn P, Bernstein IL, Woods SC, Seeley RJ: Central infusions of leptin and GLP-1-(7-36) amide differentially stimulate c-FLI in the rat brain. *Am J Physiol* 271:R1096–R1100, 1996
101. Thiele TE, Van Dijk G, Campfield LA, Smith FJ, Burn P, Woods SC, Bernstein H, Seeley RJ: Central infusion of GLP-1, but not leptin, produces conditioned taste aversion in rats. *Am J Physiol* 272:R726–R730, 1997
102. Navarro M, Rodriguez de Fonseca F, Alvarez E, Chowen JA, Zueco JA, Gomez R, Eng J, Blazquez E: Colocalization of glucagon-like peptide-1(GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. *J Neurochem* 67:1982–1991, 1996
103. Tang-Christensen M, Larsen PJ, Goke R, Fink-Jensen A, Jessop DS, Moller M, Sheikh SP: Central administration of GLP-1(7-36) amide inhibits food and water intake in rats. *Am J Physiol* 271:R848–R856, 1996
104. Göke R, Fehmann H-C, Linn T, Schmidt H, Krause M, Eng J, Göke B: Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting β-cells. *J Biol Chem* 268:19650–19655, 1993
105. Wang Z, Wang RM, Owji AA, Smith DM, Ghatei MA, Bloom SR: Glucagon-like peptide 1 is a physiological incretin in rat. *J Clin Invest* 95:417–421, 1995

106. Kolligs F, Fehmann H-C, Göke R, Göke B: Reduction of the incretin effect in rats by the glucagon-like peptide 1 receptor antagonist exendin (9-39) amide. *Diabetes* 44:16–19, 1995
107. D'alessio DA, Vogel R, Prigeon R, Laschansky E, Koerker D, Eng J, Ensinck JW: Elimination of the action of glucagon-like peptide 1 causes an impairment of glucose tolerance after nutrient ingestion by healthy baboons. *J Clin Invest* 97:133–138, 1996
108. Barragan JM, Rodriguez RE, Eng J, Blazquez E, Defize LHK: Interactions of exendin-(9-39) with the effects of glucagon-like peptide-1-(7-36) amide and of exendin-4 on arterial blood pressure and heart rate in rats. *Regul Pept* 67:63–68, 1996
109. Alcantara AI, Morales M, Delgado E, Lopez-Delgado MI, Clemente F, Luque MA, Malaisse WJ, Valverde I: Exendin-4 agonist and exendin(9-39)amide antagonist of the GLP-1(7-36) amide effects in liver and muscle. *Arch Biochem Biophys* 341:1–7, 1997
110. Wheeler MB, Gelling RW, McIntosh CHS, Georgiou J, Brown JC, Pederson RA: Functional expression of the rat pancreatic islet glucose-dependent insulinotropic polypeptide receptor: ligand binding and intracellular signalling properties. *Endocrinology* 136:4629–4639, 1995
111. Gremlich S, Porret A, Hani EH, Cherif D, Vionnet D, Froguel P, Thorens B: Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. *Diabetes* 44:1202–1208, 1995
112. Montrose-Rafizadeh C, Yang H, Rodgers BD, Beday A, Pritchette LA, Eng J: High potency antagonists of the pancreatic glucagon-like peptide-1 receptor. *J Biol Chem* 272:21201–21207, 1997
113. Scrocchi LA, Brown TJ, MacLusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ: Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide receptor gene. *Nat Med* 2:1254–1258, 1996
114. Scrocchi LA, Brown TJ, Drucker DJ: Leptin sensitivity in nonobese glucagon-like peptide I receptor -/- mice. *Diabetes* 46:2029–2034, 1997
115. Erickson JC, Clegg KE, Palmiter RD: Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 381:415–418, 1996
116. Gutniak M, Orskov C, Holst JJ, Ahrén B, Efendic S: Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 326:1316–1322, 1992
117. Ritzel R, Orskov C, Holst JJ, Nauck MA: Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7-36 amide] after subcutaneous injection in healthy volunteers: dose-response relationships. *Diabetologia* 38:720–725, 1995
118. Hvidberg A, Nielsen MT, Hilsted J, Orskov C, Holst JJ: Effect of glucagon-like peptide-1 (proglucagon 78-107amide) on hepatic glucose production in healthy man. *Metabolism* 43:104–108, 1994
119. Toft-Nielsen M, Madsbad S, Holst JJ: The effect of glucagon-like peptide 1 (GLP-1) on glucose elimination in healthy subjects depends on the pancreatic glucoregulatory hormones. *Diabetes* 45:552–556, 1996
120. D'alessio DA, Kahn SE, Leusner CR, Ensinck JW: Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. *J Clin Invest* 93:2263–2266, 1994
121. D'alessio DA, Prigeon RL, Ensinck JW: Enteral enhancement of glucose disposition by both insulin-dependent and insulin-independent processes: a physiological role of glucagon-like peptide I. *Diabetes* 44:1433–1437, 1995
122. Orskov L, Holst JJ, Moller J, Orskov C, Moller N, Alberti KG, Schmitz O: GLP-1 does not acutely affect insulin sensitivity in healthy man. *Diabetologia* 39:1227–1232, 1996
123. Gutniak MK, Larsson H, Heiber SJ, Juneskans OT, Holst JJ, Ahren B: Potential therapeutic levels of glucagon-like peptide I achieved in humans by a buccal tablet. *Diabetes Care* 19:843–848, 1996
124. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W: Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 91:301–307, 1993
125. Willms B, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Nauck MA: Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36)amide in type 2 (non-insulin dependent) diabetic patients. *J Clin Endocrinol Metab* 81:327–332, 1996
126. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W: Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:741–744, 1993
127. Rachman J, Gribble FM, Barrow BA, Levy JC, Buchanan KD, Turner RC: Normalization of insulin responses to glucose by overnight infusion of glucagon-like peptide 1(7-36) amide in patients with NIDDM. *Diabetes* 45:1524–1530, 1996
128. Gutniak MK, Linde B, Holst JJ, Efendic S: Subcutaneous injection of the incretin hormone glucagon-like peptide 1 abolishes postprandial glycemia in NIDDM. *Diabetes Care* 17:1039–1044, 1994
129. Ahren B, Larsson H, Holst JJ: Effects of glucagon-like peptide-1 on islet function and insulin sensitivity in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82:473–478, 1997
130. Rachman J, Barrow BA, Levy JC, Turner RC: Near normalization of diurnal glucose concentrations by continuous administration of glucagon-like peptide 1 (GLP-1) in subjects with NIDDM. *Diabetologia* 40:205–211, 1997
131. Nauck MA, Wollschlager D, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Willms B: Effects of subcutaneous glucagon-like peptide 1 (GLP-1[7-36 amide]) in patients with NIDDM. *Diabetologia* 39:1546–1553, 1996
132. Juntti-Berggren L, Pigon J, Karpe F, Hamsten A, Gutniak M, Vignati L, Efendic S: The antidiabetogenic effect of GLP-1 is maintained during a 7-day treatment period and improves diabetic dyslipoproteinemia in NIDDM patients. *Diabetes Care* 19:1200–1206, 1996
133. Todd JF, Wilding JP, Edwards CM, Ghatei MA, Bloom SR: Glucagon-like peptide-1 (GLP-1): a trial of treatment in non-insulin-dependent diabetes mellitus. *Eur J Clin Invest* 27:533–536, 1997
134. Dupre J, Behme MT, Hramiak IM, McFarlane P, Williamson MP, Zabel P, McDonald TJ: Glucagon-like peptide I reduces postprandial glycemic excursions in IDDM. *Diabetes* 44:626–630, 1995
135. Dupre J, Behme MT, Hramiak IM, McDonald TJ: Subcutaneous glucagon-like peptide I combined with insulin normalizes postcibal glycemic excursions in IDDM. *Diabetes Care* 20:381–384, 1997
136. Creutzfeld WO, Kleine N, Willms B, Orskov C, Holst JJ, Nauck MA: Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7-36) amide in type 1 diabetic patients. *Diabetes Care* 19:580–586, 1996
137. Eng J, Andrews PC, Kleinman WA, Singh L, Raufman JP: Purification and structure of exendin-3, a new pancreatic secretagogue isolated from *Heloderma horridum* venom. *J Biol Chem* 265:20259–20262, 1990
138. Eng J, Kleinman WA, Singh L, Singh G, Raufman JP: Isolation and characterization of exendin-4, an exendin 3 analogue from *Heloderma suspectum* venom. *J Biol Chem* 267:7402–7405, 1992
139. Raufman J-P: Bioactive peptides from lizard venoms. *Regul Pept* 61:1–18, 1996
140. Raufman J-P, Singh L, Singh G, Eng J: Truncated glucagon-like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas: identification of a mammalian homologue of the reptilian peptide exendin-4. *J Biol Chem* 267:21432–21437, 1992
141. Hupe-Sodmann K, McGregor GP, Bridenbaugh R, Rüdiger G, Burkhard G, Thole H, Zimmermann B, Voigt K: Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1(7-36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Regul Pept* 58:149–156, 1995
142. Irwin DM, Satkunarajah M, Wen Y, Brubaker PL, Pederson RA, Wheeler MB: The *Xenopus* proglucagon gene encodes novel GLP-1-like peptides with insulinotropic properties. *Proc Natl Acad Sci USA* 94:7915–7920, 1997
143. Gefel D, Hendrick GK, Mojsov S, Habener J, Weir GC: Glucagon-like peptide-I analogs: effects on insulin secretion and adenosine 3',5'-monophosphate formation. *Endocrinology* 126:2164–2168, 1990
144. Adelhorst K, Hedegaard BB, Knudsen LB, Kirk O: Structure-activity studies of glucagon-like peptide-1. *J Biol Chem* 269:6275–6278, 1994
145. Hjorth SA, Adelhorst K, Pedersen BB, Kirk O, Schwartz TW: Glucagon and glucagon-like peptide 1: selective receptor recognition via distinct peptide epitopes. *J Biol Chem* 269:30121–30124, 1994
146. Gallwitz B, Witt M, Morys-Wortmann C, Folsch UR, Schmidt WE: GLP-1/GIP chimeric peptides define the structural requirements for specific ligand-receptor interaction of GLP-1. *Regul Pept* 63:17–22, 1996
147. Gallwitz B, Witt M, Paetzold G, Morys-Wortmann C, Zimmermann B, Eckart K, Fölsch UR, Schmidt WE: Structure/activity characterization of glucagon-like peptide-1. *Eur J Biochem* 225:1151–1156, 1994
148. Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC: Exon duplication and divergence in the human preproglucagon gene. *Nature* 304:368–371, 1983
149. Lund PK, Goodman RH, Dee PC, Habener JF: Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. *Proc Natl Acad Sci USA* 79:345–349, 1982
150. Gleeson MH, Bloom SR, Polak JM, Henry K, Dowling RH: Endocrine tumour in kidney affecting small bowel structure, motility, and absorptive function. *Gut* 12:773–782, 1971
151. Stevens FM, Flanagan RW, O'Gorman D, Buchanan KD: Glucagonoma syndrome demonstrating giant duodenal villi. *Gut* 25:784–791, 1984
152. Bloom SR, Polak JM: The hormonal pattern of intestinal adaptation. *Scand J Gastroenterol* 17:93–103, 1988
153. Sagor GR, Ghatei MA, Al-Mukhtar MYT, Wright NA, Bloom SR: Evidence for a humoral mechanism after small intestinal resection. *Gastroenterology* 84:902–906, 1983

154. Taylor RG, Verity K, Fuller PJ: Ileal glucagon gene expression: ontogeny and response to massive small bowel resection. *Gastroenterology* 99:724–729, 1990
155. Fuller PJ, Beveridge DJ, Taylor RG: Ileal proglucagon gene expression in the rat: characterization in intestinal adaptation using in situ hybridization. *Gastroenterology* 104:459–466, 1993
156. Ehrlich P, Tucker D, Asa SL, Brubaker PL, Drucker DJ: Inhibition of pancreatic proglucagon gene expression in mice bearing subcutaneous endocrine tumors. *Am J Physiol* 267:E662–E671, 1994
157. Tsai C-H, Hill M, Asa SL, Brubaker PL, Drucker DJ: Intestinal growth-promoting properties of glucagon-like peptide 2 in mice. *Am J Physiol* 273:E77–E84, 1997
158. Drucker DJ, Ehrlich P, Asa SL, Brubaker PL: Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proc Natl Acad Sci USA* 93:7911–7916, 1996
159. Drucker DJ, Deforest L, Brubaker PL: Intestinal response to growth factors administered alone or in combination with h[Gly2]-glucagon-like peptide 2. *Am J Physiol* 273:G1252–G1262, 1997
160. Fischer KD, Dhanvantari S, Drucker DJ, Brubaker PL: Intestinal growth is associated with elevated levels of glucagon-like peptide-2 in diabetic rats. *Am J Physiol* 273:E815–E820, 1997
161. Orskov C, Holst JJ, Knuhtsen S, Baldissera FGA, Poulsen SS, Nielsen OV: Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. *Endocrinology* 119:1467–1475, 1986
162. Orskov C, Holst JJ: Radio-immunoassays for glucagon-like peptides 1 and 2 (GLP-1 and GLP-2). *Scand J Clin Lab Invest* 47:165–174, 1987
163. Brubaker PL, Crivici A, Izzo A, Ehrlich P, Tsai C-H, Drucker DJ: Circulating and tissue forms of the intestinal growth factor, glucagon-like peptide 2. *Endocrinology* 138:4837–4843, 1997
164. Drucker DJ, Shi Q, Crivici A, Sumner-Smith M, Tavares W, Hill M, Deforest L, Cooper S, Brubaker PL: Regulation of the biological activity of glucagon-like peptide 2 by dipeptidyl peptidase IV. *Nat Biotechnol* 15:673–677, 1997
165. Cheeseman CI, Tsang R: The effect of gastric inhibitory polypeptide and glucagon-like peptides on intestinal hexose transport. *Am J Physiol* 271:G477–G482, 1996
166. Brubaker PL, Izzo A, Hill M, Drucker DJ: Intestinal function in mice with small bowel growth induced by glucagon-like peptide-2. *Am J Physiol* 272:E1050–E1058, 1997
167. Chance WT, Foley-Nelson T, Thomas I, Balasubramaniam A: Prevention of parenteral nutrition-induced gut hypoplasia by coinfusion of glucagon-like peptide-2. *Am J Physiol* 273:G559–G563, 1997