

Biology of Incretins: GLP-1 and GIP



Daniel J. Drucker, MD

LAURIE L. BAGGIO and DANIEL J. DRUCKER

Department of Medicine, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Banting and Best Diabetes Centre, University of Toronto, Toronto, Ontario, Canada

This review focuses on the mechanisms regulating the synthesis, secretion, biological actions, and therapeutic relevance of the incretin peptides glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). The published literature was reviewed, with emphasis on recent advances in our understanding of the biology of GIP and GLP-1. GIP and GLP-1 are both secreted within minutes of nutrient ingestion and facilitate the rapid disposal of ingested nutrients. Both peptides share common actions on islet β -cells acting through structurally distinct yet related receptors. Incretin-receptor activation leads to glucose-dependent insulin secretion, induction of β -cell proliferation, and enhanced resistance to apoptosis. GIP also promotes energy storage via direct actions on adipose tissue, and enhances bone formation via stimulation of osteoblast proliferation and inhibition of apoptosis. In contrast, GLP-1 exerts glucoregulatory actions via slowing of gastric emptying and glucose-dependent inhibition of glucagon secretion. GLP-1 also promotes satiety and sustained GLP-1-receptor activation is associated with weight loss in both preclinical and clinical studies. The rapid degradation of both GIP and GLP-1 by the enzyme dipeptidyl peptidase-4 has led to the development of degradation-resistant GLP-1-receptor agonists and dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes. These agents decrease hemoglobin A1c (HbA1c) safely without weight gain in subjects with type 2 diabetes. GLP-1 and GIP integrate nutrient-derived signals to control food intake, energy absorption, and assimilation. Recently approved therapeutic agents based on potentiation of incretin action provide new physiologically based approaches for the treatment of type 2 diabetes.

The concept that certain factors produced by the intestinal mucosa in response to nutrient ingestion are capable of stimulating the release of substances from the endocrine pancreas and thereby reducing blood glu-

cose levels was first introduced in the early 1900s.^{1,2} The term *incretin* subsequently was used to denote these glucose-lowering, intestinal-derived factors.³ With the development of the radioimmunoassay, this communication between the intestine and the endocrine pancreas was confirmed when it was shown that oral glucose administration is associated with a much greater increase in plasma insulin levels when compared with the same amount of glucose given intravenously.^{4,5} This phenomenon has been dubbed the *incretin effect*, and is estimated to account for approximately 50%–70% of the total insulin secreted after oral glucose administration. Thus, incretins are hormones that are secreted from the gastrointestinal tract into the circulation in response to nutrient ingestion that enhances glucose-stimulated insulin secretion.

The first incretin hormone to be identified was isolated from crude extracts of porcine small intestine and initially were named *gastric inhibitory polypeptide* (GIP), based on its ability to inhibit gastric acid secretion in dogs.⁶ However, subsequent studies using more purified preparations of GIP revealed that GIP could also stimulate insulin secretion in animals and humans. Because the inhibitory effect of GIP on gastric acid secretion was seen only at pharmacologic doses, whereas its incretin action occurred at physiologic levels, GIP was renamed *glucose-dependent insulinotropic polypeptide*, to reflect its physiologic action yet retain the acronym. In accordance with its role as an incretin hormone, GIP is released from K-cells of

Abbreviations used in this paper: DPP-4, dipeptidyl peptidase-4; ER, endoplasmic reticulum; GIP, glucose-dependent insulinotropic polypeptide; GIPR, glucose-dependent insulinotropic polypeptide receptor; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; HbA1c, hemoglobin A1c; K_{ATP}, adenosine triphosphate-sensitive potassium channel; Kv, voltage-dependent K⁺; LAR, long-acting release; MAPK, mitogen-activated protein kinase; PC, prohormone convertase; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; Pdx-1, pancreas duodenum homeobox-1; T2DM, type 2 diabetes mellitus.

© 2007 by the AGA Institute

0016-5085/07/\$32.00

doi:10.1053/j.gastro.2007.03.054

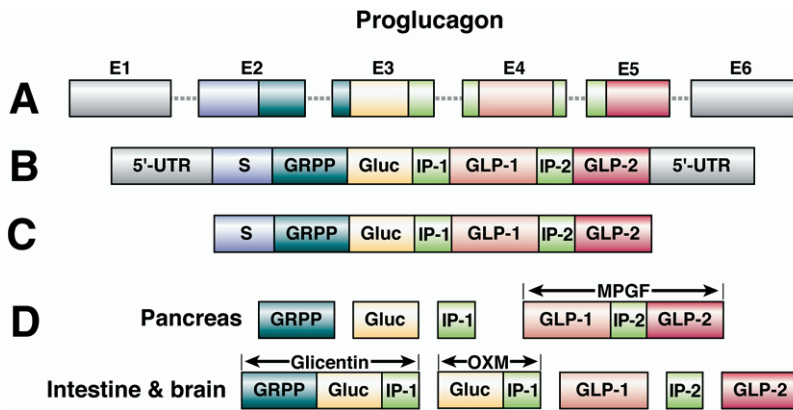


Figure 1. Structures of (A) the proglucagon gene, (B) mRNA, and (C) protein. (D) Tissue-specific posttranslational processing of proglucagon in the pancreas leads to the generation of Gliocentin-related polypeptide (GRPP), glucagon (GLUC), intervening peptide-1 (IP-1), and major proglucagon fragment (MPGF), whereas gliocentin, oxyntomodulin (oxm), intervening peptide-2 (IP-2), and GLP-1 and GLP-2 are liberated after proglucagon processing in the intestine and brain.

the small intestine, primarily in response to glucose or fat ingestion, and potentiates glucose-stimulated insulin secretion. It was recognized, however, that GIP alone could not fully account for the incretin effect *in vivo*. This was based on the observations that immunoneutralization of endogenous GIP activity attenuates but does not abolish the incretin effect in rodents and in humans surgical resection of the ileum is associated with diminished incretin activity, despite preservation of normal plasma GIP levels.⁷

The discovery of a second incretin hormone, glucagon-like peptide-1 (GLP-1), followed the cloning and sequencing of mammalian proglucagon genes and complementary DNAs (cDNAs). In addition to glucagon, the proglucagon gene also encoded 2 peptides that were approximately 50% homologous to glucagon and thus aptly were named *glucagon-like peptide-1* and *glucagon-like peptide-2*. Based on their homology to glucagon, both peptides were tested for insulinotropic activity, but only GLP-1 was capable of stimulating insulin secretion. GLP-1 is a tissue-specific posttranslational proteolytic product of the proglucagon gene that is released from intestinal L-cells in response to nutrient ingestion and enhances glucose-stimulated insulin secretion.^{8,9}

To date, only GIP and GLP-1 fulfill the definition of an incretin hormone in humans. Furthermore, studies have shown that these 2 peptides potentiate glucose-stimulated insulin secretion in an additive manner, likely contribute equally to the incretin effect, and together can fully account for the incretin effect in humans. The following sections provide an overview of GLP-1 and GIP structure, regulation, biological actions, and therapeutic potential for the treatment of type 2 diabetes (T2DM).

Proglucagon Gene Structure and Tissue-Specific Regulation of Proglucagon Gene Expression

The proglucagon gene is located on the long arm of human chromosome 2 and comprises 6 exons and 5 introns, with the entire coding sequence for GLP-1 con-

tained within exon 4 (Figure 1A).¹⁰ The proglucagon gene is expressed in the α -cells of the endocrine pancreas, the L-cells of the intestine, and neurons located in the caudal brainstem and hypothalamus; mammalian proglucagon gene transcription generates a single messenger RNA (mRNA) transcript that is structurally identical in all 3 cell types (Figure 1B).^{11,12}

Proglucagon Gene Expression in the Pancreas

In the pancreas, proglucagon gene expression is up-regulated by fasting and hypoglycemia and is inhibited by insulin. Activation of the protein kinase C (PKC) signaling pathway increases islet proglucagon mRNA levels and activators of the cAMP/protein kinase A (PKA) pathway stimulate pancreatic proglucagon gene transcription via a cAMP response element located within the proglucagon gene promoter. Membrane depolarization and calcium influx also stimulate proglucagon gene transcription in islet cells. Gastrin stimulates proglucagon gene expression in glucagon-producing pancreatic cells that stably express the cholecystokinin-2 (CCK-2) receptor in an Egr-1-dependent manner; however, levels of proglucagon mRNA transcripts are normal in gastrin-/- mice. Transgenic mouse studies have indicated that approximately 1.3 kb of rat proglucagon gene 5'-flanking sequences are sufficient to direct pancreatic α -cell- and brain-specific rat proglucagon gene expression.¹³ Specific sequences located within the proximal promoter region of the rodent proglucagon gene bind the transcription factors Pax-6, Foxa1, Cdx-2/3, Isl-1, Brn4, and c-Maf to mediate pancreatic α -cell-specific proglucagon gene expression. Pax-6 and Cdx-2/3 associate with p300, a co-activator protein, to synergistically regulate islet-specific proglucagon gene transcription. Genetic inactivation of the murine Pax-6 gene results in defective formation of islet cell lineages,¹⁴ and mice that are homozygous for a dominant-negative version of Pax-6 (small eye [SEY^{Neu}]) have significant reductions in pancreatic proglucagon mRNA transcript levels.¹⁵ Mice with genetic inactivation of the Foxa1 (hepatocyte nuclear factor 3 α [HNF-3 α]) gene are hypoglycemic and exhibit reduced pancreatic

proglucagon mRNA transcript and plasma glucagon levels, implicating a key role for Foxa1 in pancreatic proglucagon gene expression. However, in contrast, the importance of Brn4 for proglucagon gene regulation is unclear because Brn4 potentially activates pancreatic α -cell-specific proglucagon gene expression in vitro, but mice with targeted inactivation of the *Brn4* gene have normal α -cell development and pancreatic proglucagon mRNA levels.¹⁶ Sequences within the 5'-flanking region immediately upstream of the proglucagon gene promoter contain islet cell-specific enhancer-like elements and bind the transcription factor Beta2/NeuroD as well as members of the Foxa, HNF, and Ets families of transcription factors to enhance or repress proglucagon gene expression in a tissue-specific manner.

Insulin-mediated inhibition of pancreatic α -cell-specific proglucagon gene expression is regulated via an insulin-responsive element located within the gene promoter, as well as through synergistic interactions between proximal promoter elements and more distal enhancer-like elements. RNA silencing and overexpression studies have shown that insulin inhibits proglucagon gene expression in α -cells via nuclear exclusion of the transcription factor FoxO1.¹⁷

Proglucagon Gene Expression in the Intestine

Studies using primary intestinal cell cultures or transformed enteroendocrine tumor cell lines have shown that, similar to proglucagon gene expression in the pancreas, the level of intracellular cAMP and activation of cAMP/PKA signaling are major determinants of intestinal proglucagon gene expression.^{18–20} The Wnt signaling pathway is a potential mediator of PKA-dependent proglucagon gene transcription in the intestine and Wnt signaling mediates proglucagon gene expression in L-cells via expression of the transcription factor TCF-4.²¹ Increased levels of cAMP may up-regulate intestinal proglucagon gene transcription by activation of PKA or via the cAMP-regulated guanine nucleotide exchange factor II exchange protein directly activated by cAMP (Epac2)/mitogen-activated protein kinase (MAPK) pathway. A primary regulator of intestinal proglucagon gene expression in vivo is nutrient ingestion.²² Fasting reduces whereas refeeding stimulates proglucagon gene expression in the rat intestine,²³ and diets that are high in fiber²⁴ or short-chain fatty acids²⁵ increase intestinal proglucagon mRNA levels. Gastrin-releasing peptide (GRP) and GIP increase intestinal proglucagon mRNA levels in mouse enteroendocrine cells and primary fetal rat intestinal cultures, respectively. Surgical removal of portions of the small bowel is associated with increased proglucagon mRNA levels in the remnant intestine. Adenoviral-mediated overexpression of the transcription factor Pax-6 in primary intestinal cultures or rat colonic epithelium is associated with enhanced endogenous proglucagon gene expression,²⁶ whereas homozygous

mice that express the dominant-negative (SEY^{Neu}) form of Pax-6 exhibit significant reductions in proglucagon mRNA levels in the small and large intestines.²⁷ Hence, Pax-6 is essential for proglucagon gene expression in the intestine and pancreas. In contrast to proglucagon gene expression in the endocrine pancreas and brain, transgenic mouse studies suggest that a much larger region (≈ 2.3 kb) of rat proglucagon 5'-flanking sequences is required for proglucagon gene expression in the pancreas, brain, and intestine,²⁸ indicating that DNA sequences located between -2.3 and -1.3 kb in the rat proglucagon promoter are important for specifying intestinal proglucagon gene expression. The sequences situated between -2.3 and -1.3 kb have been designated the proglucagon *gene upstream enhancer element*, and cell transfection and electrophoretic mobility shift assay (EMSA) studies using enteroendocrine cell lines have indicated that the proglucagon gene upstream enhancer element is composed of multiple positive and negative *cis*-acting DNA regulatory subdomains and plays an integrative role in regulating tissue-specific proglucagon gene transcription.²⁹

Regulation of Human Proglucagon Gene Expression

Although the majority of studies to date have focused primarily on the regulation of proglucagon gene expression in rodents, a limited number of studies have examined transcriptional regulation of the human proglucagon gene. In transgenic mice, approximately 1.6 kb of human proglucagon gene 5'-flanking sequences can direct proglucagon gene transcription to the brain and intestine, but not pancreatic islets,³⁰ whereas transfection of rodent islet cell lines with human proglucagon promoter-reporter plasmids indicates that sequences within the first 6 kb of the human proglucagon gene 5'-flanking region are required for pancreas-specific gene expression.³⁰ A combination of cell transfection and transgenic reporter studies have identified a conserved region within intron 1, designated *ECR3*, as critical for expression of the human proglucagon gene in islet α -cells. These studies suggest that the human proglucagon gene likely uses a distinct set of transcription factors and DNA sequences to specify tissue-specific proglucagon gene transcription.

Posttranslational Processing of Proglucagon

The proglucagon mRNA is translated into a single 180 amino acid precursor protein that undergoes tissue-specific posttranslational processing to yield specific peptide profiles in the pancreas, intestine, and brain (Figure 1C and D).¹¹ Although several prohormone convertase (PC) enzymes have been identified, only PC1/3 and PC2 appear to be important for proglucagon processing.³¹

In pancreatic α -cells, the predominant proglucagon posttranslational processing products are glicentin-related polypeptide, glucagon, intervening peptide-1, and the major proglucagon fragment (Figure 1D). Glucagon, the major counterregulatory hormone to insulin, regulates hepatic glucose production via activation of glycogenolysis and gluconeogenesis and inhibition of glycolysis, and is essential for maintaining glucose homeostasis in the fasting state. The physiologic importance of glucagon for blood glucose regulation is exemplified by the hypoglycemic phenotype of mice that harbor a targeted inactivation of the glucagon receptor gene.^{32,33} To date, no physiologic actions have been identified for glicentin-related polypeptide, intervening peptide-1, or major proglucagon fragment. Islet α -cell-specific posttranslational processing of proglucagon to glucagon is mediated, at least in part, by PC2, and mice that lack active PC2 exhibit mild hypoglycemia and deficient processing of proglucagon to mature glucagon.³⁴

Posttranslational processing of proglucagon in enteroendocrine L-cells and the central nervous system (CNS) liberates glicentin, oxyntomodulin, GLP-1, intervening peptide-2, and GLP-2 (Figure 1D). The physiologic actions of glicentin are not well defined but it exerts trophic effects in the rodent small intestine.³⁵ Oxyntomodulin inhibits gastrointestinal secretion and motility and stimulates pancreatic enzyme secretion and intestinal glucose uptake.³⁶ More recent studies in rodents and humans have identified roles for oxyntomodulin in promoting satiety and regulating intrinsic heart rate.³⁷⁻³⁹ GLP-1 exerts a number of actions that are important for regulating glucose homeostasis (described in detail later). To date, no physiologic actions have been identified for intervening peptide-2. GLP-2 stimulates cell proliferation and inhibits apoptosis in the intestinal crypt compartment.⁴⁰ GLP-2 also upregulates intestinal glucose transport, improves intestinal barrier function, and inhibits food intake,^{41,42} gastric emptying, and acid secretion. GLP-2 also reduces bone resorption and promotes neuronal proliferation and survival.⁴³ The prohormone convertase PC1/3 has been localized to intestinal L-cells and shown to be both necessary and sufficient for posttranslational processing of proglucagon in the intestine.^{44,45} PC1/3 null mice exhibit increased levels of intestinal proglucagon accompanied by marked decreases in proglucagon processing to glicentin, oxyntomodulin, GLP-1, and GLP-2.⁴⁶

The prohormone convertase enzymes responsible for the posttranslational processing of proglucagon in the CNS are not well established; however, high levels of PC1/3 and PC2 are present throughout the CNS, including the hypothalamus, where neurons that express proglucagon also can be found.

GLP-1 Secretion, Metabolism, and Clearance

GLP-1 is secreted from intestinal endocrine L-cells, which are located mainly in the distal ileum and colon. In contrast, GIP is released from intestinal K-cells that are localized to more proximal regions (duodenum and jejunum) of the small intestine. However, endocrine cells that produce GLP-1 or GIP, as well as cells that produce both peptides, can be found throughout all regions of the porcine, rat, and human small intestine.^{47,48} The L-cell is an open-type intestinal epithelial endocrine cell that directly contacts luminal nutrients through its apical surface and neural and vascular tissue through its basolateral surface. Accordingly, GLP-1 secretion from intestinal L-cells is stimulated by a variety of nutrient, neural, and endocrine factors.

Meal ingestion, particularly one rich in fats and carbohydrates, is the primary physiologic stimulus for GLP-1 secretion.⁴⁹ GLP-1 release can be stimulated by mixed meals or individual nutrients including glucose and other sugars, fatty acids, essential amino acids, and dietary fiber. Oral, but not intravenous, glucose administration stimulates GLP-1 secretion in humans.^{50,51} In rodents and humans, GLP-1 is released rapidly into the circulation after oral nutrient ingestion, and its secretion occurs in a biphasic pattern starting with an early (within 10–15 min) phase that is followed by a longer (30–60 min) second phase.⁵¹ Because the majority of GLP-1-secreting L-cells are located in the distal small intestine, it is unlikely that the early phase of GLP-1 secretion can be mediated by direct nutrient contact with the L-cell. Indeed, several studies have shown that the autonomic nervous system, the neurotransmitters GRP and acetylcholine, and the peptide hormone GIP all can contribute to the rapid release of GLP-1 after nutrient ingestion. The role of the vagus nerve as an important mediator of nutrient-induced GLP-1 secretion has been established by studies in rats in which it was shown that bilateral subdiaphragmatic vagotomy completely blocks fat-induced GLP-1 secretion, whereas direct electrical stimulation of the celiac branches of the vagus (that innervate the jejunum, ileum, and colon) increases GLP-1 secretion.⁵² In humans, administration of atropine, a nonspecific muscarinic-receptor antagonist, diminishes oral glucose-stimulated first-phase GLP-1 secretion independently of gastric emptying.⁵³ In similar studies, either atropine or the M1 muscarinic-receptor antagonist pirenzepine could completely inhibit fat-induced GLP-1 secretion in rats.⁵⁴ In primary fetal rat intestinal cultures and human endocrine L-cells, M1 and M2 muscarinic-receptor agonists stimulate GLP-1 secretion, whereas muscarinic-receptor antagonists inhibit GLP-1 release.^{54,55} GRP stimulates GLP-1 secretion in rodents and humans, and GRP receptor null mice have a decreased GLP-1 secretory response to gastric glucose. GIP-induced GLP-1 secretion has been demonstrated in vitro in canine L-cells and in vivo in

rodents, and may be mediated by GRP. However, GIP has no effect on GLP-1 secretion in humans.^{9,56} The neuropeptide calcitonin gene-related peptide also has been proposed to play a role in the regulation of GLP-1 secretion. In contrast to the indirect mechanisms that mediate early GLP-1 release, the second or late phase of GLP-1 secretion likely is caused by direct stimulation of intestinal L-cells by digested nutrients.⁵⁷ Therefore, nutrient-generated stimulatory signals can be transmitted to L-cells either indirectly, through neural or endocrine mediators, or via direct contact, to produce the early and late phases of GLP-1 secretion, respectively. However, because L-cells seem to be present throughout the entire length of the small intestine, it is possible that early GLP-1 secretion also can occur by direct association of nutrients with L-cells located in more proximal regions of the small intestine.^{47,48}

Leptin receptors are expressed in rodent and human intestinal L-cells, and leptin stimulates GLP-1 secretion from fetal rat, mouse, and human endocrine L-cell cultures in vitro, as well as in vivo in rats and leptin-deficient *ob/ob* mice.⁵⁸ Moreover, high-fat diet-induced obesity in mice is associated with leptin resistance and decreased basal and oral glucose-stimulated GLP-1 levels.⁵⁸

GLP-1 secretion is stimulated by activation of a number of intracellular signals including PKA, PKC, calcium, and MAPK. Studies using a mouse intestinal L-cell line that expresses the adenosine triphosphate (ATP)-sensitive potassium channel (K_{ATP}) subunits sulfonylurea receptor 1 and inward rectifying potassium channel 6.2, as well as glucokinase, and sodium-glucose cotransporters 1 and 3 suggest that glucose stimulates GLP-1 secretion via glucose metabolism and K_{ATP} channel closure,⁵⁹ whereas nonmetabolizable sugars promote GLP-1 release via a sodium-glucose cotransporter-dependent mechanism.⁶⁰ Unsaturated long-chain free fatty acids stimulate GLP-1 secretion via GPR120, a G-protein-coupled receptor that is expressed abundantly in the intestine.

In comparison with the stimulation of GLP-1 secretion, relatively few studies have examined the factors responsible for inhibition of GLP-1 release. However, limited studies have shown that insulin, somatostatin, and the neuropeptide galanin can inhibit GLP-1 secretion from intestinal L-cells in vitro and in vivo.

Multiple forms of GLP-1 are secreted in vivo, including GLP-1(1-37) and GLP-1(1-36)NH₂, which are thought to be inactive, and GLP-1(7-37) and GLP-1(7-36)NH₂, which are biologically active (Figure 2). GLP-1(7-37) and GLP-1(7-36)NH₂ are produced from their full-length precursors by the action of PC1/3⁶¹ and appear to be equipotent in their ability to stimulate insulin secretion.⁶² The addition of an amide group to GLP-1(1-36)NH₂ and GLP-1(7-36)NH₂ likely is mediated by the enzyme peptidylglycine α -amidating monooxygenase and may enhance the survival of GLP-1 in plasma.⁶³ In humans, the majority of GLP-1 in the circulation is GLP-1(7-36)NH₂.⁶⁴

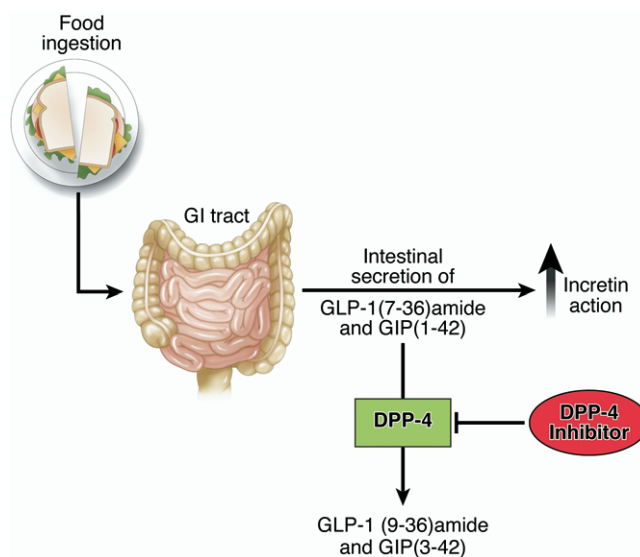


Figure 2. Bioactive GLP-1(7-36)amide and GIP (1-42) are released from the small intestine after meal ingestion and enhance glucose-stimulated insulin secretion (incretin action). DPP-4 rapidly converts GLP-1 and GIP to their inactive metabolites GLP-1 (9-36) and GIP (3-42) in vivo. Inhibition of DPP-4 activity prevents GLP-1 and GIP degradation, thereby enhancing incretin action.

The half-life of bioactive GLP-1 in the circulation is less than 2 minutes owing to rapid inactivation by the ubiquitous proteolytic enzyme dipeptidyl peptidase-4 (DPP-4).⁶⁵ DPP-4, also known as CD26, is a serine protease that specifically cleaves dipeptides from the amino terminus of oligopeptides or proteins that contain an alanine or proline residue in position 2, thereby modifying or inhibiting their activity. GLP-1, which contains a penultimate alanine residue and thus is a substrate for DPP-4, is metabolized rapidly to GLP-1 (9-37) or GLP-1 (9-36)NH₂ (Figure 2).^{66–68} DPP-4 also binds collagen and adenosine deaminase, and plays a role in T-cell costimulation and proliferation. DPP-4 is widely expressed and can be found in multiple tissues and cell types including the kidney, lung, adrenal gland, liver, intestine, spleen, testis, pancreas, and CNS, as well as on the surface of lymphocytes and macrophages. Notably, DPP-4 also is found on the surface of endothelial cells, including those lining blood vessels that drain the intestinal mucosa, which are positioned directly adjacent to the sites of GLP-1 secretion.⁶⁹ Consequently, more than half of the GLP-1 that enters the portal circulation already has been inactivated by DPP-4 before entry into the systemic circulation.⁶⁹ In addition to a cell-surface membrane-bound form, DPP-4 also exists as a soluble protein in the circulation.⁷⁰

In rats, more than 50% of an intravenous bolus of GLP-1 is converted to its N-terminal metabolite by DPP-4 within 2 minutes of peptide administration. Conversely, GLP-1 remains intact when infused into DPP-4-deficient rodents.^{67,71} In healthy or diabetic humans, intravenous

or subcutaneous GLP-1 is metabolized rapidly (within 30 min) to GLP-1 (9-36)NH₂, which accounts for more than 75% of the immunodetectable circulating GLP-1 in these individuals (Figure 2).⁶⁵ Numerous studies in both animals and humans have demonstrated that inhibition of DPP-4 activity prolongs the half-life of intact, biologically active GLP-1.

Neutral endopeptidase 24.11 (NEP-24.11), a membrane-bound zinc metallopeptidase,⁷² has been shown to have endoproteolytic activity on GLP-1 in vitro and up to 50% of GLP-1 entering the circulation may undergo C-terminal cleavage by NEP-24.11.^{73,74}

The plasma half-life of intact GLP-1 is approximately 2 minutes, whereas that of its metabolite has been estimated to be approximately 5 minutes as a result of renal clearance. The major route of GLP-1 elimination is through the kidney and involves mechanisms that include glomerular filtration and tubular uptake and catabolism.⁷⁵ In rats, bilateral nephrectomy or ureter ligation is associated with an increased circulating half-life of GLP-1⁷⁵ and GLP-1 levels are increased in patients with renal failure⁷⁶ or chronic renal insufficiency.⁷⁷ By using specific N- and C-terminal assays, it was determined that concentrations of GLP-1 metabolites are increased in patients with renal failure, whereas levels of intact bioactive GLP-1 are similar to those of healthy individuals.⁷⁷ These studies indicate that the kidneys are important for elimination of GLP-1 and its metabolites.⁷⁷

Fasting plasma levels of bioactive GLP-1 typically range between 5 and 10 pmol/L in humans and increase approximately 2- to 3-fold after a meal,^{64,78,79} with the absolute peak values being dependent on both the size and nutrient composition of the meal.^{79,80} Postprandial levels of intact, biologically active GLP-1 are reduced in obese and type 2 diabetic individuals.^{79,81-83} Because the elimination rates of GLP-1 are similar in healthy, obese, and type 2 diabetic individuals,⁸⁴ the decrease in GLP-1 levels observed in obese and type 2 diabetic humans likely is caused by reductions in GLP-1 secretion. Although leptin can stimulate GLP-1 secretion, obese individuals often exhibit leptin resistance. Hence, it has been proposed that leptin resistance may be responsible for the decreased GLP-1 levels in obese humans.⁵⁸ The factors responsible for decreased meal-stimulated GLP-1 secretion in type 2 diabetic patients are not known.

The GLP-1 Receptor

The GLP-1 receptor (GLP-1R) belongs to the class B family of 7-transmembrane-spanning, heterotrimeric G-protein-coupled receptors, which also includes receptors for glucagon, GLP-2, and GIP.⁸⁵ The rat and human GLP-1R cDNAs were cloned and sequenced in the early 1990s from their respective pancreatic islet cDNA libraries. Both receptors are 463 amino acids in length and exhibit 90% amino acid sequence identity. The human GLP-1R gene spans 40 kb, consists of at least 7 exons,

and has been mapped to chromosome 6, band p21.1. In rodents and humans, a single structurally identical GLP-1R has been identified and is expressed in a wide range of tissues including α -, β -, and δ -cells of the pancreatic islets, lung, heart, kidney, stomach, intestine, pituitary, skin, nodose ganglion neurons of the vagus nerve, and several regions of the CNS including the hypothalamus and brainstem. Although GLP-1R expression is detected in canine muscle and adipose tissues,⁸⁶ evidence for the presence of GLP-1Rs in human or rodent adipose tissue, liver, or muscle is equivocal. GLP-1R expression in islets is down-regulated in response to dexamethasone, high glucose, activation of PKC, and GLP-1. Conversely, GLP-1R expression is up-regulated in diabetic rats after 7 days of treatment with a DPP-4 inhibitor.

The N-terminal extracellular region of the GLP-1R is essential for GLP-1 binding, whereas distinct domains within the third intracellular loop are critical for efficient coupling of the receptor to specific G-proteins. The GLP-1R can couple to $G\alpha_s$, $G\alpha_q$, $G\alpha_i$, and $G\alpha_o$,^{87,88} leading to increases in intracellular Ca²⁺, adenylate cyclase, and phospholipase C, and activation of PKA, PKC, phosphatidylinositol-3 kinase (PI-3K), Epac2, and MAPK signal transduction pathways.^{87,89-92} In islet cell lines, the GLP-1R undergoes rapid homologous and heterologous desensitization and internalization, with both processes being dependent on phosphorylation of specific residues in the GLP-1R C-terminal tail. The GLP-1R localizes in lipid rafts and interacts with caveolin-1 to regulate receptor subcellular localization, trafficking, and signaling. However, GLP-1R desensitization has not been observed in vivo, even after long-term GLP-1R agonist administration.⁹³

Exendin (9-39), an N-terminally truncated peptide derivative of the lizard GLP-1R agonist exendin-4, binds the GLP-1R and functions as a specific GLP-1R antagonist.⁹⁴ Exendin (9-39) or mice with a targeted disruption of the GLP-1R gene (GLP-1R^{-/-})⁹⁵ often are used to examine the physiologic consequences of transient and sustained loss of GLP-1R signaling, respectively.

Biological Actions of GLP-1

Pancreas

GLP-1R agonists produce several biological actions in the pancreas (Figure 3) including stimulation of glucose-dependent insulin secretion.^{8,9,96} The binding of GLP-1 to its specific receptor on pancreatic β -cells leads to activation of adenylate cyclase activity and production of cAMP (Figure 4). Subsequently, GLP-1 stimulates insulin secretion via mechanisms that include the following: (1) direct inhibition of K_{ATP} channels, which leads to β -cell membrane depolarization; (2) increases in intracellular Ca²⁺ levels resulting from GLP-1-dependent influx of extracellular Ca²⁺ through voltage-dependent Ca²⁺

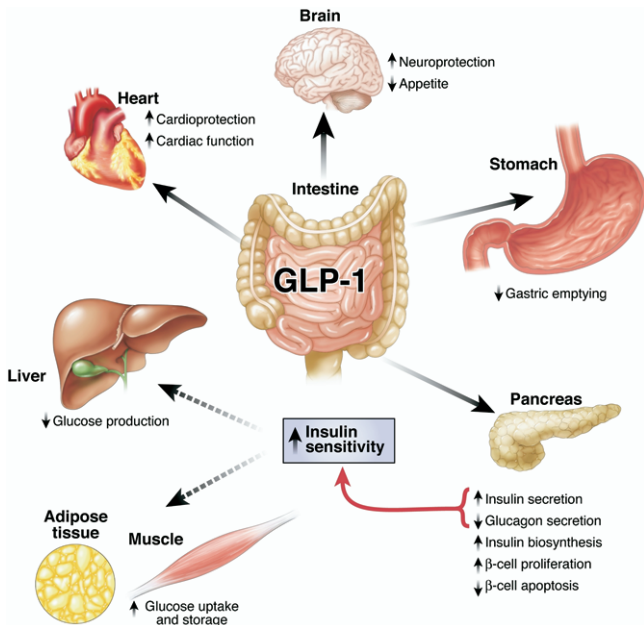


Figure 3. GLP-1 actions in peripheral tissues. The majority of the effects of GLP-1 are mediated by direct interaction with GLP-1Rs on specific tissues. However, the actions of GLP-1 in liver, fat, and muscle most likely occur through indirect mechanisms.

channels, activation of nonselective cation channels, and mobilization of intracellular Ca^{2+} stores; (3) increases in mitochondrial ATP synthesis, which lead to further membrane depolarization; (4) closure of voltage-dependent K^+ (K_v) channels and consequent reductions in K_v currents, thereby preventing β -cell repolarization; and (5) direct effects on β -cell insulin storage granule exocytosis that occur distal to increases in ATP and intracellular Ca^{2+} . The primary effector of GLP-1-induced insulin secretion is cAMP, and cAMP mediates its stimulatory effect on insulin secretion via 2 distinct mechanisms: (1) PKA-dependent phosphorylation of downstream targets and (2) PKA-independent activation of Epac2. Both cAMP/PKA and PI-3K/PKC ζ signaling pathways mediate the antagonistic effect of GLP-1 on K_v currents. GLP-1 can enhance K_{ATP} channel-independent glucose-induced insulin secretion by PKA- and PKC-dependent signaling pathways. The glucose-dependent actions of GLP-1 are not understood completely, but may converge on the K_{ATP} and K_v channels, and potentially at the level of insulin granule exocytosis.

GLP-1 also acts synergistically with glucose to promote insulin gene transcription, mRNA stability, and biosynthesis, and thus has the potential to replenish β -cell insulin stores and prevent exhaustion of β -cell reserves.^{90,97,98} GLP-1R agonists maintain β -cell insulin stores and secretory capacity by increasing glucose-induced insulin biosynthesis at the translational level.⁹⁹ The mechanisms whereby GLP-1R agonists increase insulin gene transcription and biosynthesis include activation of cAMP/PKA-dependent and -independent signal-

ing pathways, and increases in intracellular Ca^{2+} levels.^{90,100} Nuclear factor of activated T (NFAT) cells may also be an important mediator of GLP-1-induced insulin gene transcription. Pancreas duodenum homeobox 1 (Pdx-1), a transcription factor essential for pancreatic development and β -cell function, also plays a central role in mediating the actions of GLP-1 on insulin gene transcription and secretion. GLP-1 increases Pdx-1 gene transcription and the binding of Pdx-1 to the insulin gene promoter.¹⁰¹ In addition, β -cell-specific inactivation of the Pdx-1 gene in mice and dominant-negative suppression of Pdx-1 in insulinoma cells are associated with loss of GLP-1R agonist-dependent effects on pancreatic β -cells.^{98,102} GLP-1 also increases mRNA levels of the β -cell K_{ATP} channel subunits sulfonylurea receptor 1 and inward rectifying potassium channel 6.2, and regulates K_{ATP} channel function.

GLP-1 confers glucose sensitivity to glucose-resistant β -cells, thereby improving the capacity of β -cells to sense and respond to glucose.¹⁰³ GLP-1 up-regulates the expression of glucose transporters and glucokinases, molecular components of β -cell glucose sensors, and thus provides a possible mechanism whereby GLP-1 is able to restore glucose responsiveness to previously resistant β -cells.

GLP-1 also inhibits glucagon and stimulates somatostatin secretion. The stimulatory effect of GLP-1 on somatostatin secretion likely is caused by direct interaction with GLP-1Rs on somatostatin-secreting pancreatic δ -cells.¹⁰⁴ The mechanism(s) whereby GLP-1 inhibits glucagon secretion from pancreatic α -cells is less clear, and

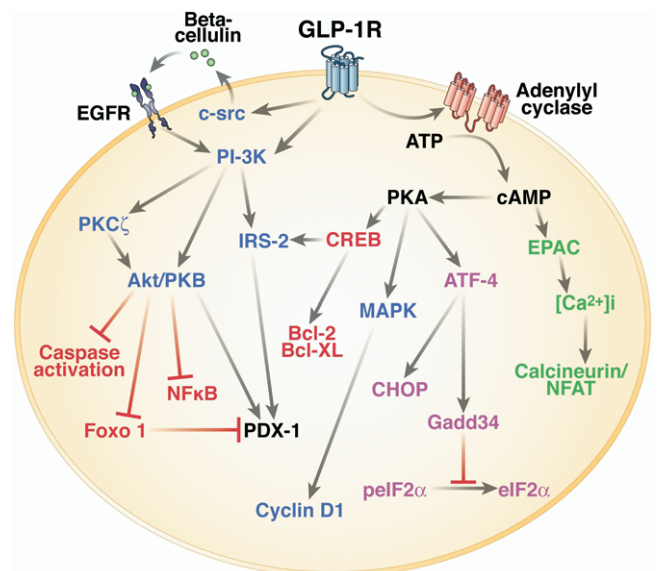


Figure 4. GLP-1R-dependent intracellular signal transduction pathways in the pancreatic β -cell. Although there is considerable overlap between pathways, the major effectors that couple GLP-1R activation to insulin secretion and biosynthesis (green), β -cell proliferation and neogenesis (blue), inhibition of apoptosis (red), and ER stress reduction (purple) are highlighted.

may involve direct binding to pancreatic α -cell GLP-1Rs.¹⁰⁵ Alternatively, the inhibitory effect of GLP-1 on glucagon secretion may be mediated indirectly via stimulation of insulin and/or somatostatin secretion. In mice with β -cell-specific inactivation of the Pdx-1 gene, GLP-1R-dependent insulin secretion is defective and the ability of GLP-1R agonist administration to inhibit glucagon secretion is lost, suggesting that insulin or another β -cell-derived mediator could contribute significantly to the inhibition of glucagon secretion.⁹⁸ However, the suppressive effect of GLP-1 on glucagon secretion also has been observed in fasting type 1 diabetic patients with no residual β -cell secretory capacity,¹⁰⁶ indicating that the glucagonostatic effect of GLP-1 can be mediated independently of endogenous insulin levels. The glucagonostatic action of GLP-1 also is glucose-dependent,¹⁰⁷ thereby reducing the potential for developing hypoglycemia. The cellular mechanisms that mediate the inhibitory actions of GLP-1 on glucagon secretion are less well characterized but are believed to involve (1) cAMP/PKA-dependent closure of α -cell K_{ATP} channels, which leads to membrane depolarization and subsequent inactivation of T-type Ca^{2+} and Na^{+} channels; (2) inhibition of A-type K^{+} channels, which prevents α -cell membrane repolarization; and (3) reductions in intracellular Ca^{2+} levels.

GLP-1R agonists stimulate β -cell proliferation and neogenesis and inhibit β -cell apoptosis, thereby increasing β -cell mass (Figure 3). GLP-1 activates the expression of immediate early genes encoding transcription factors that regulate islet cell proliferation and differentiation. Treatment of pancreatic exocrine or epithelial cells or rat and human pancreatic ductal cell lines with GLP-1R agonists promotes their conversion to islet-like cells that produce and secrete insulin in a glucose-dependent manner, and GLP-1 accelerates the differentiation and maturation of human and porcine fetal islet cells. GLP-1R agonists inhibit apoptosis in primary rodent islets, purified β -cells, and islet cell lines after exposure to cytotoxic agents or dexamethasone.¹⁰⁸⁻¹¹⁰ GLP-1 also preserves morphology, improves glucose-stimulated insulin secretion, and inhibits apoptosis in freshly isolated human islets.^{110,111} In vivo, GLP-1R agonists improve glucose tolerance, enhance β -cell proliferation and neogenesis, and inhibit β -cell apoptosis in experimental rodent models of diabetes, leading to increased β -cell mass. Furthermore, administration of exendin-4 during the prediabetic neonatal period prevents the development of adult-onset diabetes in rats after experimentally-induced intrauterine growth retardation. Similarly, exendin-4 increases β -cell mass and delays diabetes onset in Goto-Kakizaki rats and db/db mice, and increases insulin secretion but fails to expand β -cell mass in insulin-receptor substrate-2 (IRS-2) knockout mice. GLP-1R agonists also improve β -cell function and survival during endoplasmic reticulum (ER) stress.¹¹² Obese diabetic db/db mice develop ER stress and exendin-4 reduces levels of the ER stress markers

CHOP-10 and spliced Xpb-1 in db/db islet β -cells.¹¹² Exendin-4 attenuates translational down-regulation of insulin and improves survival of purified rat β -cells and islet cell lines after ER stress induction in vitro via mechanisms that include enhancement of ATF-4 translation, increased expression of GADD34, and dephosphorylation of eIF2 α .¹¹²

The molecular mechanisms that couple GLP-1R activation to β -cell mass expansion and cytoprotection include multiple signal transduction pathways downstream of the GLP-1R (Figure 4),^{113,114} however, activation of the transcription factor Pdx-1 appears to be a shared component in all GLP-1R-dependent molecular pathways. The importance of Pdx-1 is illustrated by the inability of exendin-4 to stimulate proliferation or inhibit apoptosis in β -cell-specific Pdx-1 $^{-/-}$ mice.⁹⁸ The proliferative effects of GLP-1R agonists in vitro are mediated via transactivation of the epidermal growth factor receptor (EGFR), which leads to increases in PI-3K and activation of PKC ζ ¹¹⁵ and/or Akt-protein kinase B (PKB). GLP-1R agonists also stimulate β -cell replication via IRS-2 signaling,¹¹⁶ as well as activation of cAMP/PKA, PI-3K, and MAPK signaling pathways, and up-regulation of expression of the cell-cycle regulator cyclin D1. The specific mechanisms involved in GLP-1-dependent β -cell differentiation/neogenesis are not well defined but likely involve activation of PKC and MAPK, as well as synergistic interaction with transforming growth factor- β and regulation of smad transcription factor activity. The transcription factor FoxO1, a key negative regulator of β -cell growth, also plays an important role in mediating the proliferative, neogenic, and cytoprotective effects of GLP-1 on the β -cell. Studies in mice treated with the β -cell toxin streptozotocin indicate that exendin-4 mediates β -cell regeneration by mechanisms that involve up-regulation of IRS-2 expression and promotion of FoxO1 nuclear exclusion, resulting in increased Pdx-1 expression.^{117,118} Similarly, in vitro studies show that GLP-1 mediates its proliferative and anti-apoptotic effects in β -cells via EGFR- and PI-3K-dependent inhibition of FoxO1 activity and consequent up-regulation of expression of the transcription factors Pdx-1 and Foxa2.^{117,118} GLP-1R-dependent inhibition of β -cell apoptosis is associated with diminished levels of pro-apoptotic proteins including active caspase 3, poly-ADP-ribose polymerase cleavage, and thioredoxin interacting protein, and up-regulation of pro-survival factors including Bcl-2, Bcl-xL, and inhibitor of apoptosis protein-2. The cumulative experimental evidence indicates that the cytoprotective effect of GLP-1R agonists is coupled to the following: (1) activation of cAMP/PKA with subsequent phosphorylation and activation of cAMP response element binding protein (CREB), leading to activation of IRS-2 and induction of the Akt-PKB growth and survival pathway,^{119,120} and (2) activation of Akt-PKB and enhancement of the DNA binding activity of its downstream target the transcription factor

nuclear factor- κ B (NF κ B), an important cellular regulator of apoptosis.¹¹⁰ In addition, GLP-1R agonists improve metabolic control via enhancing glucose-stimulated insulin secretion, reducing fasting and postprandial blood glucose levels, promotion of satiety and weight loss, and lowering of HbA_{1c} and plasma free fatty acid levels. Hence, GLP-1R agonists may preserve β -cell mass by (1) directly interacting with GLP-1Rs on pancreatic β -cells or islet precursors and activating signal transduction pathways that modify β -cell proliferation, neogenesis, and apoptosis, and/or (2) reducing increased circulating glucose and free fatty acids and thereby indirectly protecting β -cells from a deleterious metabolic environment.

The physiologic importance of GLP-1R agonist-mediated actions in the pancreas is illustrated by studies using the GLP-1R antagonist exendin (9-39), GLP-1 immunoneutralizing antisera, and GLP-1R $^{-/-}$ mice. Elimination of GLP-1 action with specific antisera or exendin (9-39) impairs glucose tolerance and reduces glucose-stimulated insulin levels in both animals and humans. Similarly GLP-1R $^{-/-}$ mice are characterized by a mild fasting hyperglycemia and a modest glucose intolerance in response to an oral or peripheral glucose challenge in association with defective glucose-stimulated insulin secretion.⁹⁵ Human studies with exendin (9-39) demonstrate that even the low basal levels of GLP-1 in the fasting state are important for glucoregulation because administration of exendin (9-39) to healthy humans significantly increases fasting levels of both glucose and glucagon, suggesting that low basal GLP-1 levels exert a tonic inhibitory effect on pancreatic α -cells.¹²¹ Exendin (9-39) also blocks GLP-1R agonist-mediated differentiation of human pancreatic ductal cells, and inhibits the anti-apoptotic effect of GLP-1 in β -cells in vitro. GLP-1R $^{-/-}$ mice have normal β -cell mass but abnormal islet architecture with fewer large β -cell clusters and altered α -cell topography,¹²² indicating that endogenous GLP-1R signaling is important for normal islet development. In addition, GLP-1R $^{-/-}$ mice exhibit defective regeneration of β -cell mass and deterioration of glucose tolerance after partial pancreatectomy and are more susceptible to streptozotocin-induced β -cell apoptosis.¹⁰⁹

Central and Peripheral Nervous Systems

GLP-1Rs and GLP-1-containing nerve fibers are present in regions of the CNS that regulate a diverse array of homeostatic functions including feeding behavior, gastric motility, glucoregulation, and cardiovascular function (Figure 3). GLP-1Rs are located on the nodose ganglion of abdominal vagal afferent nerve fibers whose central branches terminate in the nucleus of the solitary tract of the brainstem. Information from the nucleus of the solitary tract is relayed to the hypothalamus and other forebrain regions by way of ascending second-order neurons. Rodent studies demonstrate that central or pe-

ripheral administration of GLP-1R agonists reduces short-term food and water intake and decreases body weight.^{123–126} Similarly, peripheral administration of GLP-1R agonists promotes satiety, decreases energy intake, and leads to weight loss in healthy, diabetic, and obese humans. GLP-1 and exendin-4 are relatively small molecules that diffuse readily across the blood-brain barrier to directly access the CNS. Thus, GLP-1R agonists could reduce food intake by direct interaction with GLP-1Rs localized to hypothalamic CNS centers that regulate ingestive behavior. Conversely, GLP-1R agonists could modify food intake indirectly by virtue of their ability to inhibit gastric emptying (see later), thereby producing gastric distension and an associated sensation of satiety.

Experimental evidence points to a neural mechanism for GLP-1R agonist-dependent inhibition of food intake. In rats, subdiaphragmatic bilateral vagotomy or surgical transection of the brainstem-hypothalamic pathway precludes peripheral GLP-1-induced anorexia and blocks neuronal activation of hypothalamic feeding circuits.¹²⁷ Likewise, systemic treatment with capsaicin, to selectively ablate nodose ganglionic neurons and the vagus nerve, completely blocks the anorectic effect of peripherally administered exendin-4 in mice.¹²⁸ In addition, peripheral administration of a much larger GLP-1-albumin recombinant fusion protein, which is unable to cross the blood-brain barrier, activates neurons in the CNS that are coupled to feeding and inhibits food intake in mice.¹²⁹ Alternatively, the anorectic effects of GLP-1R agonists could be secondary to activation of central signaling pathways that trigger visceral illness or anxiogenic behavior. Peripheral administration of noxious agents such as lithium chloride can activate GLP-1-producing neurons in the CNS. In addition, central infusion of GLP-1 and systemic administration of lithium chloride induce a similar pattern of neuronal activation in the CNS and elicit comparable aversive behavioral responses in rats (including inhibition of food intake) that are blocked by prior administration of the GLP-1R antagonist exendin (9-39). Although GLP-1Rs in the CNS can be activated by GLP-1 produced in either the intestine or the CNS, the relative importance of peripheral vs central GLP-1 for the regulation of feeding behavior is not known.

GLP-1R agonist-dependent insulin secretion also may be mediated indirectly, in part, via a neural mechanism. It has been estimated that more than 50% of secreted GLP-1 is inactivated by DPP-4 on its release from the intestinal L-cell, whereas a large amount of the remaining intact peptide subsequently is inactivated as it passes through the liver, with only small amounts of GLP-1 actually reaching the pancreas in the intact bioactive form.^{69,130} This led to the proposal that intestinally derived GLP-1 must trigger a local neural intermediary that relays the signal to the pancreas.¹³¹ This proposal is substantiated by the observations that the GLP-1 receptor is expressed on nodose ganglion cells of the afferent vagus and ad-

ministration of GLP-1 into the hepatic portal vein of rats activates hepatic vagal afferent fibers that then, in a reflex manner, increase the activity of pancreatic vagal efferent nerves. Furthermore, infusion of GLP-1 into the portal vein increases glucose-stimulated insulin secretion and enhances glucose disposal, effects that are abolished by administration of ganglionic blockers.¹³¹ Similarly, studies in mice have shown that an intact sensory afferent nervous system is essential for GLP-1R agonist-mediated insulin secretion because low doses of GLP-1 can increase glucose-dependent insulin secretion in control but not capsaicin-treated mice.¹³² At least part of the sensory neural component of GLP-1-dependent insulin secretion could be mediated by glucose sensors located in the hepatic portal vein that are activated by an increase in the portal vein-arterial glucose gradient. Moreover, activation of GLP-1R signaling is essential for maintaining the glucose competence of the hepatoportal glucose sensor because portal infusion of glucose significantly increases peripheral glucose clearance, whereas co-infusion of glucose and exendin (9-39) into the portal vein increases glycemia in wild-type mice. Likewise, enhanced glucose clearance after portal glucose infusion is abolished in GLP-1R^{-/-} mice.¹³³ Under hyperglycemic conditions, GLP-1R signaling in the murine CNS activates peripheral neural pathways to inhibit muscle glucose use, increase insulin secretion, and support enhanced hepatic glycogen storage, indicating a novel role for CNS GLP-1 as an important modulator of whole-body glucose homeostasis.¹³⁴

GLP-1R agonists also exert proliferative, neogenic, and anti-apoptotic actions on neuronal cells. GLP-1R agonists stimulate neurite outgrowth, enhance nerve growth factor-induced differentiation, and improve cell survival after nerve growth factor withdrawal from PC12 cells. In addition, GLP-1 and exendin-4 inhibit glutamate-induced apoptosis in cultures of rat hippocampal neurons and restore cholinergic marker activity in the basal forebrain of ibotenic acid-treated rats, a rodent model of neurodegeneration.¹³⁵ Similarly, central infusion of GLP-1R agonists reduce the levels of amyloid- β peptide (A β) in mice, decrease amounts of amyloid precursor protein in PC12 cells in vitro, and protect cultured hippocampal neurons from A β - and iron-induced oxidative injury. The neuroprotective effects of GLP-1 and exendin-4 were also demonstrated by studies showing improved behavioral and neural morphometric parameters in rats after pyridoxine-induced peripheral nerve degeneration, an experimental model of peripheral sensory neuropathy.

GLP-1R-dependent pathways also may be important for learning and memory. GLP-1R agonist administration enhances learning in rats; an effect that is blocked by co-administration of exendin (9-39).¹³⁶ Conversely, GLP-1R^{-/-} mice show deficiencies in learning behavior that are corrected by hippocampal *Glp1r* gene transfer.¹³⁶ GLP-

1R^{-/-} mice also exhibit increased susceptibility to kainate-induced seizures and hippocampal neuronal degeneration, whereas GLP-1R agonist treatment prevents kainate-induced apoptosis in wild-type animals.¹³⁶ Hence, it has been proposed that GLP-1R agonists may be of therapeutic use for the treatment of neurodegenerative diseases and other neurologic disorders, including diabetic peripheral neuropathy.

Gastrointestinal System

GLP-1R agonists exhibit potent inhibitory effects on pentagastrin- and meal-stimulated gastric acid secretion and gastric emptying (Figure 3). Deceleration of gastric emptying attenuates increases in meal-associated blood glucose levels by slowing the transit of nutrients from the stomach to the small intestine and contributes to the normalization of blood glucose levels in type 2 diabetic patients after exogenous GLP-1 administration.^{137,138} Inhibition of gastric emptying also contributes to the observed reductions in postprandial blood glucose levels after GLP-1 administration in patients with type 1 diabetes.¹³⁹ The GLP-1-mediated reduction in meal-related glycemic excursion often is associated with reduced, rather than increased, postprandial insulin levels.^{138,140,141} Moreover, in human studies, when erythromycin is used to antagonize GLP-1-dependent deceleration of gastric emptying, insulin secretory responses to a meal are increased, but the glucose-lowering effect of GLP-1 is greatly reduced.¹⁴² Thus, it has been proposed that the gastric emptying effect of GLP-1R agonists may, in fact, be more important than their incretin effect on β -cells with regard to meal-stimulated glucose control.

GLP-1R agonists inhibit gastric emptying and acid secretion through complex mechanisms. GLP-1Rs are expressed in the stomach on gastric parietal cells, suggesting that GLP-1R agonists can inhibit gastric emptying and acid secretion via direct mechanisms. However, inhibition of GLP-1R signaling with exendin (9-39) or vagal afferent denervation abolish the inhibitory effect of centrally or peripherally administered GLP-1 on gastric emptying rate and acid secretion. In addition, although GLP-1 and exendin-4 can directly access the CNS from the periphery, intraperitoneal administration of a much larger albumin-linked GLP-1R agonist activates neurons in the CNS that are coupled to gastrointestinal motility and inhibition of gastric emptying,¹²⁹ thus underscoring the importance of ascending neural pathways for GLP-1R agonist-dependent control of gastric emptying. Collectively, the experimental data indicate that the inhibitory effect of GLP-1 on gastric emptying and acid secretion is mediated by the vagus nerve and involves GLP-1Rs located in the CNS and/or on vagal afferent fibers that relay sensory information to the brainstem.

Cardiovascular System

GLP-1Rs are expressed in both the rodent and human heart, although the identity of the specific cell type(s) within the heart that express the GLP-1R is not known. GLP-1Rs also are present in the nucleus of the solitary tract and area postrema, regions of the CNS that regulate cardiovascular function. Intravenous administration of GLP-1R agonists increases systolic, diastolic, and mean arterial blood pressures and heart rate in rodents and increases heart rate in conscious calves. The stimulatory effects of GLP-1R agonists on the rat cardiovascular system occur independently of catecholamines and are blocked by central or peripheral administration of the GLP-1R antagonist exendin (9-39),¹⁴³ suggesting that GLP-1R agonists mediate their actions on the cardiovascular system by both central and peripheral mechanisms. In telemetry studies with freely moving rats, GLP-1R agonist-mediated increases in heart rate and blood pressure are coupled to activation of the following: (1) autonomic control centers in the CNS, (2) GLP-1-responsive hypothalamic and medullary catecholamine neurons that project to sympathetic preganglionic neurons, and (3) neurons in the adrenal medulla, suggesting that central GLP-1 activates the sympathetic nervous system to modify cardiovascular function.¹⁴⁴ Moreover, additional experimental evidence from rodent studies points to a role for the GLP-1R in the area postrema as a molecular link between peripheral GLP-1 and central autonomic control centers that mediate the neuroendocrine and autonomic actions of peripheral GLP-1.¹⁴⁵ In contrast to its positive effects on heart rate and blood pressure in rodents, GLP-1R agonists do not significantly affect these parameters in human beings.¹⁴⁶⁻¹⁴⁹ However, a single study in type 2 diabetic patients found that infusion of GLP-1 is associated with improved endothelial function.¹⁵⁰

GLP-1 also exhibits cardioprotective effects in experimental models of cardiac injury or heart failure. In dogs with rapid pacing-induced dilated cardiomyopathy, infusion of GLP-1 increases cardiac output and improves left ventricular and systemic hemodynamics, in association with increased myocardial insulin sensitivity and glucose uptake.¹⁵¹ GLP-1 also attenuates myocardial stunning and reduces infarct size after ischemia-reperfusion in conscious dogs and anesthetized rats, respectively. Moreover, studies using isolated heart preparations have shown that GLP-1 has direct protective effects on the heart. GLP-1 reduces infarct size and increases left ventricular function and myocardial glucose uptake after ischemia-reperfusion injury in isolated rat hearts.^{152,153} The protective effects of GLP-1 in these studies are mediated by cAMP and the prosurvival kinases PI-3K/Akt and p44/42 MAPK.¹⁵² GLP-1R signaling is essential for normal cardiac structure and function as GLP-1R^{-/-} mice exhibit increased septal and posterolateral myocardial

wall thickness and abnormal cardiac contractile responses to external stresses.¹⁵⁴ The beneficial effects of GLP-1 on cardiovascular function also pertain to human patients. A 72-hour infusion of GLP-1 in patients with acute myocardial infarction and angioplasty improved regional and global left ventricular function and was associated with a reduced in-hospital mortality rate and duration of hospitalization.¹⁵⁵ However, it is not known if the positive effects of GLP-1 on cardiovascular parameters are mediated by direct interaction with GLP-1Rs in the heart, or indirectly through GLP-1-mediated improvements in glucose, insulin, and free fatty acids levels.

Muscle, Adipose Tissue, and Liver

GLP-1 increases glucose incorporation into glycogen in isolated primary rat hepatocytes and skeletal muscle and enhances insulin-stimulated glucose metabolism in cultures of 3T3 L1 adipocytes and primary rat adipocytes. GLP-1 also inhibits hepatic glucose production and stimulates glucose uptake in fat and muscle. GLP-1 and exendin-4 increase glycogen synthase activity and glucose metabolism in rat soleus muscle and human skeletal muscle. GLP-1 and exendin-4 also augment insulin-stimulated glucose uptake in L6 myotubes, but only exendin-4 and not GLP-1 exhibits similar effects in 3T3-L1 adipocytes. In addition, GLP-1 has lipolytic effects in rat adipocytes¹⁵⁶ and displays both lipolytic and lipogenic actions in human adipocytes.¹⁵⁷ Exendin-4 improves insulin sensitivity and reverses hepatic steatosis in ob/ob mice, and GLP-1 and exendin-4 stimulate cAMP production in isolated primary rat hepatocytes.^{158,159} However, other studies do not support a direct role for GLP-1R signaling in these tissues,^{160,161} and whether or not GLP-1R agonists can influence glucose disposal and insulin sensitivity independent of changes in insulin or glucagon is not clear. Some studies have proposed that GLP-1 can enhance glucose clearance in healthy and diabetic humans independently of islet hormone action,¹⁶²⁻¹⁶⁴ whereas others have shown that GLP-1 has no direct effect on glucose disposition.¹⁶⁵⁻¹⁶⁹ Furthermore, it has been suggested that, rather than increasing glucose disposal, GLP-1 suppresses hepatic glucose production.¹⁷⁰ The means by which GLP-1 mediates these extrapancreatic effects in humans, independent of changes in the insulin:glucose ratio, is not known as there is conflicting experimental evidence for the presence of GLP-1Rs in these tissues. In addition, GLP-1R binding and signaling, as well as the effects of GLP-1R agonists and antagonists in liver, fat, and muscle, differ from those of the pancreatic GLP-1R. Hence, it has been proposed that any GLP-1-dependent actions in these tissues may be mediated by a second or related GLP-1R.

Other Tissues

The GLP-1R has been detected on rat pituitary cells and GLP-1 stimulates cAMP production and thy-

roid-stimulating hormone release from cultured mouse pituitary thyrotrophs and rat primary dispersed anterior pituitary cells. GLP-1 also stimulates the release of luteinizing hormone-releasing hormone from a rodent-derived hypothalamic cell line, and central administration of GLP-1 increases plasma thyroid-stimulating hormone, luteinizing hormone, corticosterone, and vasopressin levels and activates hypothalamic neuroendocrine cells in the rat.¹⁷¹ Although these studies implicate a modulatory role for GLP-1 in the hypothalamic-pituitary axis, GLP-1R^{-/-} mice do not exhibit any major impairments in hypothalamic-pituitary function.¹⁷² However, in healthy human beings, short-term infusion of GLP-1 produces transient increases in circulating adrenocorticotropic hormone and cortisol levels.¹⁶⁸

In both the rat and humans, GLP-1R mRNA transcripts are abundant in the lung, submucosal glands of the trachea, pulmonary artery smooth muscle cells, and type II pneumocytes, where activation of GLP-1R signaling enhances mucous secretion, pulmonary muscle relaxation, and surfactant secretion, respectively. However, the precise function of GLP-1 in the respiratory system and its relevance to normal pulmonary physiology are not known.

The GLP-1R also is expressed in the kidney and intravenous infusion of GLP-1 produces natriuretic and diuretic responses in the rat that are associated with increases in glomerular filtration rate and inhibition of sodium reabsorption in the proximal tubule. In hypertension-prone salt-sensitive rats maintained on a high-salt diet, a 14-day infusion of GLP-1 prevents hypertension development, improves endothelial function, and reduces both renal and cardiac damage. The antihypertensive effect of GLP-1 was attributed to GLP-1-dependent increases in salt and water excretion.¹⁷³ Similarly, a 3-hour intravenous infusion of GLP-1 in obese humans enhances sodium excretion and reduces H⁺ secretion and glomerular hyperfiltration, implicating a potential protective role for GLP-1 in the kidney.¹⁷⁴

Potential Actions of GLP-1 (9-36)NH₂

GLP-1 (9-36)NH₂ is the primary GLP-1 metabolite in vivo; because the levels of GLP-1 (9-36)NH₂ in the circulation are greater than those of intact bioactive GLP-1,^{65,68} there is interest in determining possible functions of this peptide in vivo. GLP-1 (9-36)NH₂ binds GLP-1Rs with low affinity and at pharmacologic doses functions as a weak competitive antagonist of β -cell and gastrointestinal tract GLP-1Rs in vivo. However, intravenous administration of GLP-1 (9-36)NH₂ in combination with glucose has no effect on insulin secretion, glucose elimination, or insulin-independent glucose disposal in either wild-type or GLP-1R^{-/-} mice.¹⁷⁵ Paradoxically, GLP-1 (9-36)NH₂ enhances insulin-independent glucose clearance in anesthetized pigs.¹⁷⁶ Similarly, infusion of GLP-1 (9-36)NH₂ into healthy fasted humans in conjunction with a test meal significantly reduces

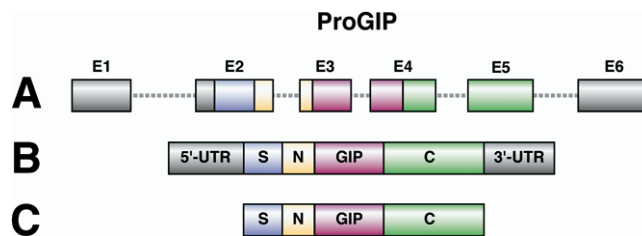


Figure 5. (A) ProGIP gene, (B) mRNA, and (C) protein. Bioactive GIP is generated from its proGIP protein precursor by posttranslational cleavage at single arginine residues that flank GIP.

postprandial glycemia, independently of changes in insulin or glucagon secretion or gastric emptying rate.¹⁷⁷ In contrast, a separate study in healthy human beings found that infusion of GLP-1 (9-36)NH₂ has no direct effect on glucose tolerance, insulin secretion or sensitivity, or GLP-1 action.¹⁷⁸ Moreover, this same study showed that simultaneous infusion of GLP-1 (9-36)NH₂ with GLP-1 (7-36)NH₂ does not alter the ability of GLP-1 (7-36)NH₂ to enhance glucose-stimulated insulin secretion.¹⁷⁸ Thus, the biological importance of GLP-1 (9-36)NH₂ with respect to glucoregulation remains obscure. Interestingly, in studies of dogs with pacing-induced dilated cardiomyopathy, 48 hours of continuous infusion of GLP-1 (9-36)NH₂ mimics the effects of native GLP-1 by significantly reducing left ventricular end-diastolic pressure, and increasing left ventricular contractility, cardiac output, and myocardial glucose uptake.¹⁷⁹ Although these studies did not determine if the effects of GLP-1 (9-36)NH₂ are mediated through the known GLP-1R, they fuel the concept that GLP-1 (9-36)NH₂ (and possibly GLP-1) could mediate its actions on the cardiovascular system via a unique receptor.

Structure and Regulation of the GIP Gene

The human GIP gene (Figure 5) is comprised of 6 exons, with the majority of GIP-encoding sequences found in exon 3, and has been localized to the long arm of chromosome 17. GIP gene expression has been detected in the stomach and intestinal K-cells in both rodents and humans, whereas submandibular salivary gland expression is found exclusively in the rat. Rat duodenal and salivary gland GIP mRNA levels are increased after glucose- or fat-rich meals and are decreased in response to prolonged fasting. Little information is available regarding the factors that are essential for mediating GIP gene expression, although studies of the human GIP gene promoter indicate that basal promoter activity is regulated by 2 cAMP-responsive elements and have identified the presence of binding sites for several transcription factors including Sp1, activator protein (AP-1), and AP-2. Sequences in the rodent GIP promoter contain functional GATA-4 and Isl-1 transcription factor binding elements that mediate cell-specific promoter activity. More recent studies have indicated that Pdx-1 also

can mediate cell-specific GIP gene expression. Pdx-1 protein is detectable in the nucleus of GIP-expressing mouse K cells, and the number of GIP-expressing cells is reduced significantly in Pdx-1^{-/-} mice.¹⁸⁰ Moreover, EMSA and chromatin immunoprecipitation (CHIP) assays demonstrate Pdx-1 binding to the GIP promoter region, and overexpression of Pdx-1 in transient transfection assays increases the activity of GIP promoter/reporter gene constructs.¹⁸⁰

GIP Biosynthesis, Secretion, Metabolism, and Clearance

The predicted amino acid sequence for both the rat and human GIP cDNAs indicate that GIP is derived from a larger proGIP prohormone precursor that encodes a signal peptide, an N-terminal peptide, GIP, and a C-terminal peptide (Figure 5). Studies using specific PC knockout mice or cell lines that overexpress PC enzymes demonstrate that the mature 42-amino acid bioactive form of GIP is released from its 153-amino acid proGIP precursor via PC1/3-dependent posttranslational cleavage at flanking single arginine residues.⁴⁶ The peptides encoded within the GIP N- or C-terminal sequences have no known function. The GIP sequence is highly conserved among species with human, mouse, rat, porcine, and bovine GIP exhibiting more than 90% amino acid sequence identity.

GIP is synthesized within and released from intestinal K-cells, the majority of which are located in the duodenum and proximal jejunum, with smaller numbers also occurring throughout the entire small intestine.^{47,181} GIP is secreted in response to nutrient ingestion, especially glucose or fat. More specifically, it is the rate of nutrient absorption rather than the mere presence of nutrients in the intestine that stimulates GIP release. Thus, GIP secretion is reduced in individuals with intestinal malabsorption or after the administration of pharmacologic agents that reduce nutrient absorption.^{182,183} There appear to be species-specific differences in the nutritional regulation of GIP release because fat is the most potent stimulator of GIP secretion in humans, whereas carbohydrates are the most effective in the rodent and pig. In vitro studies using cultured canine endocrine cells indicate that activation of adenylyl cyclase, increases in intracellular Ca²⁺ levels, K⁺-mediated depolarization, glucose, GRP, and β -adrenergic stimulation can increase GIP secretion. In humans, basal circulating GIP levels range between 0.06 and 0.1 nmol/L, depending on the assay used to measure total vs intact GIP, and increase to 0.2–0.5 nmol/L after a meal.^{79,184} GIP levels are normal or slightly increased in patients with T2DM.^{79,185}

The half-life of intact biologically active GIP is less than 2 minutes in rodents,⁶⁷ and approximately 7 and 5 minutes in healthy subjects and type 2 diabetic patients, respectively.¹⁸⁶ GIP has an alanine residue in position 2 and is also a target for DPP-4-mediated inactivation

(Figure 2). A role for DPP-4 in the cleavage of GIP (1-42) and generation of the inactive metabolite GIP (3-42) has been established clearly and studies with rodents and both healthy and diabetic humans indicate that DPP-4 is the primary enzyme responsible for inactivating GIP in vivo.^{67,186} Although pharmacologic doses of GIP (3-42) can function as weak antagonists of the GIP receptor in vitro and in rodents, physiologic levels of GIP (3-42) do not antagonize the insulinotropic effects of GIP in vivo. Interestingly, a direct comparison of intact incretin hormones levels after exogenous intravenous infusion in humans found that 40% of GIP remains intact and bioactive versus 20% for GLP-1,^{67,186} indicating that GIP may be less susceptible to DPP-4 in vivo, and this is reflected in the slightly longer plasma half-life for GIP vs GLP-1. Administration of whey protein reduces DPP-4 activity in the proximal small intestine, but not in the distal gut or plasma, and is associated with increased intact GIP levels after glucose administration. The observations that GIP levels are increased in uremic patients or individuals with chronic renal failure, together with impaired GIP clearance in nephrectomized rats, implicates the kidney as the major route of GIP clearance.⁷⁷ Measurement of arteriovenous differences in GIP levels across various organ beds in the anesthetized pig also identifies the kidney as the major site of GIP metabolism, but the liver and extremities also contribute to GIP extraction.¹⁸⁷ The elimination rates for intact GIP and its metabolite are similar in obese type 2 diabetic patients and healthy individuals.¹⁸⁸

The GIP Receptor

The GIP receptor (GIPR) initially was cloned from a rat cerebral cortex cDNA library and was followed by the cloning of the hamster and human GIPRs. The human GIPR gene comprises 14 exons that span approximately 14 kb¹⁸⁹ and is localized to chromosome 19, band q13.3. The GIPR gene is expressed in the pancreas, stomach, small intestine, adipose tissue, adrenal cortex, pituitary, heart, testis, endothelial cells, bone, trachea, spleen, thymus, lung, kidney, thyroid, and several regions in the CNS. Similar to the GLP-1R, the GIPR is a member of the 7-transmembrane-spanning, heterotrimeric G-protein-coupled receptor superfamily.¹⁹⁰

Relatively little is known about the factors responsible for regulating GIPR expression. The GIPR gene 5'-flanking region contains a cAMP response element, and binding sites for Oct-1, Sp1, and Sp3 transcription factors. In addition, *cis*-acting negative regulatory sequences that control cell-specific GIPR gene expression have been identified in more distal 5'-flanking regions. GIPR mRNA and protein levels are reduced in islets of diabetic rats, consistent with the observation of defective GIP action in diabetic animals and human beings.¹⁹¹

Activation of GIPR signaling is coupled to increases in cAMP and intracellular Ca²⁺ levels, as well as activation of PI-3K, PKA, PKB, MAPK, and phospholipase A2. In

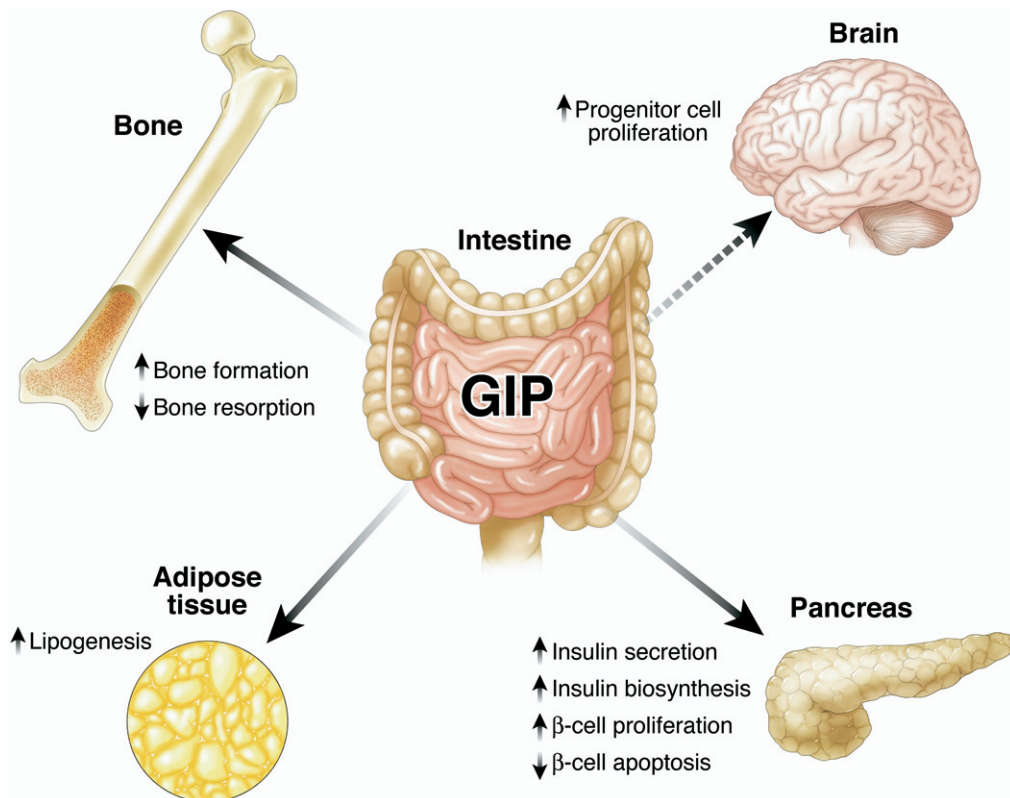


Figure 6. GIP actions in peripheral tissues.

in vitro structure/function studies indicate that the N-terminal domain and the first extracellular loop of the GIPR are essential for high-affinity GIP binding, whereas portions of the N-terminal domain and the first transmembrane domain are important for receptor activation and cAMP coupling. Although the majority of the C-terminal tail of the GIPR appears to be dispensable for intracellular signaling, a minimum receptor length of approximately 405 amino acids is required for efficient transport and plasma membrane insertion.

The GIPR undergoes very rapid and reversible homologous desensitization and site-directed mutagenesis, and C-terminal deletion analyses demonstrate the importance of particular serine residues in the C-terminal tail of the GIPR. Specifically, serines 406 and 411 are important for receptor desensitization, whereas serines 426 and 427 regulate the rate of GIPR internalization.¹⁹² In addition, regulator of G-protein signaling-2, G-protein receptor kinase 2, and β -arrestin 1 all have been implicated in GIPR desensitization.

Biological Actions of GIP

The actions of GIP on the pancreatic β -cell are analogous to those of GLP-1. However, GIP also exhibits unique physiologic actions in extrapancreatic tissues (Figure 6).

Pancreas

The primary physiologic role for GIP is that of an incretin hormone. GIP is released from intestinal K-cells

in response to nutrient ingestion, binds to its specific receptor on pancreatic β -cells, and enhances glucose-dependent insulin secretion. The molecular mechanisms whereby GIP potentiates glucose-dependent insulin secretion overlap considerably with those of GLP-1 and include increases in cAMP, inhibition of K_{ATP} channels, increases in intracellular Ca^{2+} , and stimulation of exocytosis.^{193,194} GIP stimulation of insulin secretion is mediated by activation of both cAMP/PKA and cAMP/Epac2, in addition to phospholipase A2 and specific protein kinase signaling pathways.^{195–197} Recently, a novel role for GIP in the regulation of K_v channel cell surface expression was identified as a potential mechanism whereby GIP modulates insulin secretion.¹⁹⁸ GIP also up-regulates β -cell insulin gene transcription and biosynthesis, as well as the expression of components of β -cell glucose sensors.¹⁹⁹ The physiologic importance of GIP as an incretin hormone is illustrated by disruption of GIP action in vivo. Elimination of GIP signaling using GIPR peptide antagonists, receptor-specific antisera, or by targeted inactivation of the murine GIPR gene (GIPR^{-/-}) is associated with impaired oral glucose tolerance and defective glucose-stimulated insulin secretion in rodents.^{200–204}

GIP acts synergistically with glucose to stimulate cell proliferation and improve survival of pancreatic β -cell lines after exposure to wortmannin, streptozotocin, glucolipotoxicity, or serum or glucose deprivation. Moreover, the protective effects of GIP are observed in islets from wild-type but not GIPR^{-/-} mice.²⁰⁵ The molecular

signaling pathways that mediate the proliferative and anti-apoptotic actions of GIP have been elucidated using heterologous cells transfected with the GIPR, rodent β -cell lines, or murine islets, and include activation of cAMP/PKA, PKA/CREB, MAPK, PI-3K-dependent activation of Akt-PKB, reductions in caspase 3 activity, and down-regulation of *bax* gene transcription.²⁰⁵⁻²⁰⁸ The limited information available from in vivo studies has shown that infusion of GIP into diabetic rats for 2 weeks significantly reduces β -cell apoptosis by activation of PI-3K/Akt-PKB and subsequent phosphorylation and nuclear exclusion of FoxO1, resulting in decreased expression of the pro-apoptotic *bax* gene and up-regulation of the anti-apoptotic *bcl-2* gene.²⁰⁵ Finally, GIP reduces biochemical markers associated with ER stress in islet cell lines after induction of ER stress in vitro.¹¹²

Central Nervous System

In the CNS, GIP is expressed in the hippocampus and GIPR expression is detectable in several regions of the brain including the cerebral cortex, hippocampus, and olfactory bulb. Exogenous administration of GIP induces proliferation of hippocampal progenitor cells in vivo in rats as well as in adult-derived hippocampal progenitor cells cultured in vitro.²⁰⁹ Conversely, adult GIPR^{-/-} mice have reduced numbers of new proliferating cells in the hippocampal dentate gyrus.²⁰⁹ Transgenic mice that overexpress the GIPR exhibit enhanced sensorimotor coordination and memory recognition compared with wild-type mice. Thus, GIP action in the CNS may play a role in neural progenitor cell proliferation and behavior modification.²⁰⁹

Adipose Tissue

Functional GIPRs are expressed on isolated rat adipocytes and 3T3-L1 cells²¹⁰ and GIP is implicated in the control of lipid metabolism and the development of obesity. Fat ingestion is a potent stimulator of GIP secretion in humans and GIP plasma levels are increased in some obese individuals.^{211,212} The anabolic effects of GIP in fat include stimulation of fatty acid synthesis and re-esterification, enhancement of insulin-stimulated incorporation of fatty acids into triglycerides, up-regulation of lipoprotein lipase synthesis, and reduction of glucagon-stimulated lipolysis. However, GIP also may have lipolytic effects. GIPR^{-/-} mice are resistant to diet-induced obesity and exhibit reduced adipocyte mass, despite several months of high-fat feeding.²¹³ Moreover, ob/ob:GIPR^{-/-} mice gain less weight, have reduced adiposity, and improved glucose tolerance and insulin sensitivity relative to ob/ob mice.²¹³ Although food intake is comparable in GIPR^{-/-} and wild-type mice during high-fat feeding, GIPR^{-/-} mice expend more energy and use fat as their preferred energy substrate, thereby preventing the accumulation of fat in adipocytes.²¹³ In ob/ob mice, chronic administration of the GIPR antagonist (Pro³)GIP

improves glucose tolerance, enhances insulin sensitivity, and corrects obesity-associated islet hypertrophy and β -cell hyperplasia.²¹⁴ However, GIPR^{-/-} mice maintained on a normal chow diet are glucose intolerant²⁰⁴ and administration of (Pro³)GIP impairs glucose tolerance in wild-type mice.²¹⁵ In addition, GIPR activation is associated with improvements in glucose tolerance and increased insulin secretion in animal models of diabetes. Hence, although type 2 diabetic patients are relatively resistant to the insulinotropic effects of exogenous GIP administration and there is no direct link between obesity and GIP in humans, the relative merits of inhibition versus activation of GIPR signaling need to be considered in any future therapeutic applications of GIP or its analogues.

Bone

GIPR mRNA and protein are expressed in normal bone and osteoblast-like cell lines.²¹⁶ GIP stimulates increases in cAMP and intracellular Ca²⁺ levels in cultured osteoblasts and these effects are coupled to markers of new bone formation, including increases in alkaline phosphatase activity and collagen type 1 mRNA.²¹⁶ GIP also increases bone mineral density in ovariectomized rats, a rodent model of postmenopausal osteoporosis.²¹⁷ Relative to age-matched wild-type mice, younger GIPR^{-/-} mice exhibit reduced bone size and mass, abnormal bone microarchitecture, impaired biomechanical properties, and altered bone turnover.²¹⁸ However, as the mice age, these differences become less apparent.²¹⁸ Conversely, bone mass is greater in GIP-overexpressing transgenic mice compared with wild-type controls. The presence of GIPR mRNA and protein also recently was detected in rodent osteoclast cells and GIP administration was found to have inhibitory effects on bone resorption.²¹⁹ Moreover, adult GIPR^{-/-} mice show reductions in parameters for bone formation as well as increases in plasma Ca²⁺ levels after meal ingestion, suggesting that GIP may provide a direct link between Ca²⁺ from a meal and calcium deposition in bone. These studies implicate an important and novel role for GIP in the regulation of bone remodeling. However, acute administration of GIP does not alter markers of bone turnover in human studies,²²⁰ and whether more long-term application of GIP will modulate bone turnover in humans is not known.

Other Tissues

GIP inhibits gastric acid secretion in the stomach, but only at supraphysiologic doses,²²¹ and GIP also has been shown to up-regulate intestinal hexose transport. In the liver, GIP attenuates glucagon-stimulated hepatic glucose production, likely through indirect mechanisms because GIPRs have not been detected in the liver. GIP can enhance insulin-dependent glucose disposal in animals, although this effect is not seen in humans.¹⁸⁷ GIP also stimulates glucocorticoid secretion in rats via a

cAMP/PKA-dependent signaling pathway.²²² Although GIP does not appear to regulate cortisol secretion in healthy humans, abnormal expression of the GIPR in adrenocortical adenomas is associated with the development of food-dependent Cushing's syndrome.²²³ The GIPR is present in the vascular endothelium and GIP stimulates increases in intracellular Ca^{2+} levels in endothelial cell cultures.²²⁴ Infusion of GIP into dogs induces endothelin-1 secretion and vasoconstriction or nitric oxide production and vasodilatation, depending on the vascular bed involved. The opposing effects of GIP in different vascular beds are attributed to variation in the degree of activation of signal transduction pathways in different endothelial cell types. Although GIPR mRNA also is detected in the heart, testis, lung, and several other tissues, the physiologic actions of GIP in these tissues are not known.

Incretins and Incretin Mimetics as Therapeutic Agents for the Treatment of Type 2 Diabetes

Several studies have shown that the magnitude of nutrient-stimulated insulin secretion is diminished in subjects with T2DM, prompting investigation as to whether incretin secretion and/or incretin action is diminished in diabetic subjects. Plasma levels of GIP appear normal to increased in subjects with T2DM, whereas meal-stimulated plasma levels of GLP-1 are modestly but significantly diminished in patients with impaired glucose tolerance and in subjects with T2DM.⁷⁹ Whether successful restoration of metabolic control is associated with improvement in meal-stimulated GLP-1 secretion is not known. In contrast, although the glucoregulatory actions of GLP-1 are preserved in subjects with T2DM, the acute insulinotropic response to native GIP is diminished substantially in diabetic subjects.⁵⁶ No information is available on the efficacy of chronic GIP administration and the more potent GIP analogues have not been examined in subjects with T2DM. Although the mechanism(s) underlying the reduced response to GIP remain unclear, preclinical studies suggest that hyperglycemia may be associated with down-regulation of GIPR expression in rodent islets.¹⁹¹ Hence, current therapeutic strategies have been focused on the use of GLP-1R agonists for the treatment of T2DM.

As short-term infusion of GLP-1 provides for near normalization of 24-hour glucose profiles in subjects with T2DM, the antidiabetic efficacy of native GLP-1 administration was examined in patients receiving subcutaneous injections of GLP-1 three times daily before each meal. GLP-1 significantly reduced meal-related glycemic excursion, in association with increased levels of plasma insulin and reduced levels of plasma glucagon, over a 3-week treatment period.²²⁵ Continuous subcutaneous GLP-1 administration (4.8 pmol/kg/min) via an infusion pump in 10 subjects with T2DM was associated

with inhibition of gastric emptying, reduced levels of free fatty acids, and a significant reduction in both fasting and postprandial glucose, fructosamine, and HbA1c, with a mean weight loss of 1.9 kg at the end of the 6-week study period. Little nausea was reported in this study, implying that the plasma levels of GLP-1 reached during this study (~ 282.3 pmol/L of total GLP-1 immunoreactivity after 6 weeks) were likely less than the maximally tolerated efficacious dose.¹⁴⁸ A second study examined the efficacy of native GLP-1 in elderly patients with T2DM (mean initial HbA1c of 7.2%) via subcutaneous infusion at a lower infusion rate (maximum infusion rate, 2 pmol/kg/min). GLP-1 therapy was well tolerated and improved both glucose disposal and insulin secretion, but no significant change in HbA1c was noted at the end of the 3-month treatment period.²²⁶

Because native GLP-1 is degraded rapidly, degradation-resistant GLP-1R agonists have been developed for the treatment of T2DM. Exendin-4 is a 39 amino acid peptide originally isolated from the venom of the *Heloderma suspectum* lizard that exhibits approximately 53% amino acid identity with native GLP-1 and is a potent agonist at the mammalian GLP-1 receptor.²²⁷ Because exendin-4 contains a glycine at position 2, it is not a substrate for DPP-4 and exhibits a longer circulating $t_{1/2}$, relative to native GLP-1, after subcutaneous administration. Exendin-4 significantly lowered blood glucose in preclinical studies, and appeared to be 10- to 100-fold more potent than native GLP-1 in vivo because of the much more rapid degradation and clearance of the native GLP-1 peptide.¹⁵⁸

The antidiabetic efficacy of synthetic exendin-4 (Exenatide) administered via twice-daily injection was examined over 4 weeks in subjects with T2DM not adequately controlled on metformin and/or sulphonylurea agents. Exenatide therapy significantly reduced levels of HbA1c, improved parameters of β -cell function, and decreased both fasting and postprandial glucose concentrations, with mild to moderate nausea reported as the principal treatment-related side effects.²²⁸ These findings formed the basis for a set of phase 3 clinical trials, using Exenatide at a dose of 5 or 10 μg twice daily for 30 weeks in subjects with T2DM not achieving adequate glycemic control on metformin and/or sulphonylurea. Exenatide therapy significantly reduced HbA1c in all 3 treatment groups, with no significant increase in hypoglycemia detected in subjects treated with Exenatide and metformin alone. Forty-six percent of subjects treated with metformin and Exenatide 10 μg twice a day achieved an HbA1c of less than 7%, with a mean weight loss of 2.8 kg. Nausea was reported more frequently during the initial few weeks of therapy, and declined thereafter; nausea was not a prerequisite for weight loss as subjects who never reported nausea experienced a mean weight loss of 2.2 kg.²²⁹ Forty-three percent of patients had detectable anti-Exenatide antibodies after 30 weeks of therapy; however,

the presence or absence of antibodies did not predict the therapeutic response to Exenatide.

Exenatide also produced a significant reduction in HbA1c (0.86 after 30 weeks in subjects treated with 10 µg twice daily) when added as adjunctive therapy in subjects with T2DM inadequately controlled with sulphonylureas. The mean weight loss was 1.6 kg from baseline, with 41% of subjects on Exenatide 10 µg twice daily achieving a HbA1c of less than 7% at the end of 30 weeks. The incidence of reported nausea was approximately 30% after 1 month of therapy, but decreased to approximately 10% after 28 weeks. The incidence of mild-moderate hypoglycemia was 36% in subjects treated with Exenatide 10 µg twice daily, but no cases of severe hypoglycemia were reported.²³⁰ Similar results (mean HbA1c reductions, ~0.8%) were reported in a 30-week study of patients receiving Exenatide who were previously not adequately controlled on metformin/sulphonylurea (43% glipizide, 42% glibenclamide, 14% glimepiride, 3% glibenclamide combination with metformin) therapy. Patients achieved a mean weight loss of approximately 1.6 kg, with mild to moderate hypoglycemia reported in 28% of subjects receiving 10 µg twice daily.²³¹ Forty-nine percent of subjects had anti-Exenatide antibodies at the end of the 30-week treatment period. Exenatide also has been evaluated when added on to patients receiving thiazolidinedione therapy with or without concomitant metformin over 16 weeks. Exenatide therapy was associated with a significant reduction in HbA1c of approximately 0.8%, with 62% of subjects achieving a HbA1c of less than 7% from an initial baseline of 7.9%, in association with a mean weight loss of 1.5 kg.

The effects of Exenatide 10 µg twice daily were compared with insulin glargine over 26 weeks in subjects with T2DM not adequately controlled on combination metformin/sulphonylurea therapy. The dose of insulin glargine was titrated so as to achieve a fasting glucose of less than 5.6 mmol/L. Both Exenatide and insulin glargine reduced levels of HbA1c by approximately 1.1%; Exenatide produced a greater reduction in postprandial glucose whereas therapy with insulin glargine was more effective in decreasing fasting glucose. The number of mild to moderate symptomatic hypoglycemic events was not significantly different, whereas gastrointestinal complaints (nausea, vomiting, and diarrhea) were reported more frequently in subjects receiving Exenatide. In contrast, insulin glargine therapy was associated with a mean weight gain of 1.8 kg, whereas Exenatide-treated subjects experienced a mean weight loss of 2.3 kg.²³² The withdrawal rate was 19.4% vs 9.7% for subjects treated with Exenatide versus insulin glargine, respectively. A similar open-label study compared the efficacy of twice-daily Exenatide therapy with insulin Aspart, twice daily, over 52 weeks. Exenatide therapy was associated with greater reductions in postprandial glucose levels but similar reductions in end-of-study HbA1c levels compared with

insulin treatment. Although the withdrawal rate was higher in the Exenatide-treated group (21.3% vs 10.1%), Exenatide-treated subjects experienced modest weight loss, whereas subjects treated with insulin gained weight (mean between-group difference, 5.4 kg).²³³ Exenatide was approved for the treatment of T2DM in the United States in April 2005.

Because Exenatide needs to be injected twice daily, there is considerable effort directed at the development of GLP-1R agonists that require less frequent parenteral administration. Liraglutide is a human GLP-1R agonist with 2 amino acid substitutions and a fatty acid acyl group that enables noncovalent binding to albumin, thereby extending the pharmacokinetic profile of the GLP-1 molecule. Liraglutide exhibits a prolonged pharmacokinetic profile after a single injection, and exhibits all of the actions of native GLP-1.²³⁴ Liraglutide has been examined in a series of phase 2 studies at doses of up to 2 mg/day. Liraglutide added to metformin produced a significant reduction in fasting glucose (3.9 mmol/L) and HbA1c (0.8%) levels in a 5-week study of subjects with T2DM,²³⁵ in association with a mean weight reduction of 2.9 kg relative to a control group treated with metformin and glimepiride. The dose of liraglutide was increased weekly from 0.5 to 2 mg once per day, thereby minimizing the report of nausea in Liraglutide-treated subjects. Although gastrointestinal side effects were significantly more common in subjects treated with Liraglutide and metformin compared with patients receiving glimepiride and metformin (61% vs 19.4%, respectively), only 4% of the Liraglutide-treated subjects withdrew from the study. Liraglutide is now being evaluated in phase 3 clinical trials in subjects with T2DM not achieving optimal glycemic control on conventional therapy.

Several additional long-acting GLP-1R agonists also are being developed including several that use the pharmacokinetics of albumin to obtain a long-acting pharmacokinetic profile suitable for once-weekly administration. Exenatide long-acting release (LAR) is a poly-lactide-glycolide microsphere suspension containing 3% peptide that produces stable prolonged release of Exenatide after a single subcutaneous injection. A single injection of Exenatide LAR produced significant improvements in glucose control without evidence of tachyphylaxis in diabetic Zucker diabetic fatty rats over a 28-day observation period.²³⁶ Exenatide LAR was evaluated at 2 doses, 0.8 and 2 mg administered once weekly to 15 subjects with T2DM. Both doses of Exenatide LAR were associated with significant reductions in HbA1c, and subjects receiving the 2-mg weekly dose experienced significant weight loss over the 16-week treatment period. Exenatide LAR is currently being compared with twice-daily Exenatide in a head-to-head study of patients with T2DM.

Albugon (Naliglutide) is a recombinant GLP-1-albumin protein that exhibits a reduced affinity for the GLP-1R, but displays a broad spectrum of GLP-1R-dependent

actions in preclinical studies, including inhibition of food intake and gastric emptying and reduction of glycemia excursion after meal ingestion.¹²⁹ Similarly, CJC-1134 is a preformed conjugate containing exendin-4 covalently linked *ex vivo* to recombinant human serum albumin via a chemical linker, after which the purified exendin-4–albumin conjugate is injected subcutaneously for the treatment of T2DM. Previous studies with an earlier generation GLP-1–albumin hybrid (CJC-1131) demonstrated efficacy in preclinical murine models of diabetes, and preliminary analysis of CJC-1131 and CJC-1134 in human subjects showed a pharmacokinetic profile consistent with once-weekly dosing. Both CJC-1134 and Naliglutide currently are being evaluated in phase 2 clinical studies.

Inhibition of DPP-4 Activity to Enhance Incretin Action for the Treatment of Type 2 Diabetes

The observation that native GLP-1 and GIP are cleaved rapidly by DPP-4 at the position 2 alanine leading to their inactivation has fostered considerable interest in the role of DPP-4 as a critical determinant of incretin action. Insight into the role of DPP-4 in the control of incretin biology has been derived from studies of rodents with inactivating mutations in the DPP-4 gene, and from the results of experiments using small-molecule chemical inhibitors of DPP-4 activity. Fischer (F344/DuCrj) rats harboring a point mutation in the DPP-4 gene produce normal levels of DPP-4 mRNA but a Gly633→Arg amino acid substitution produces a mutant DPP-4 protein that is retained within and rapidly degraded by the endoplasmic reticulum. F344/DuCrj rats exhibit improved glucose tolerance and increased levels of plasma GLP-1 and insulin after oral glucose challenge²³⁷; the DPP-4 inhibitor valine pyrrolidide had no effect on blood glucose levels in F344/DuCrj rats but lowered the level of blood glucose in control wild-type F344/Jcl rats.

Improved glucose tolerance also was observed in DPP-4 knockout mice, in association with increased levels of GIP and GLP-1, and enhanced insulin secretion after oral glucose challenge.⁷¹ Furthermore, DPP-4^{-/-} mice show resistance to diet-induced obesity, and preservation of insulin sensitivity after high-fat feeding.²³⁸ Complementary studies using DPP-4 inhibitors to treat rodents with experimental diabetes showed significant improvements in glucose tolerance, preservation of levels of intact GLP-1, and increased levels of plasma insulin after oral glucose challenge. The proliferative and anti-apoptotic actions of GLP-1 on islet β -cells have been mirrored by studies using DPP-4 inhibitors in diabetic rodents. High-fat–fed mice treated with low-dose streptozotocin followed by 2–3 months of treatment with des-fluoro-sitagliptin showed improvement in fasting and postprandial hyperglycemia, increased pancreatic insulin content, and

increased numbers of insulin-positive β -cells in association with normalization of β -cell mass and restoration toward normal of the β -cell: α -cell ratio.²³⁹

The large number of peptide substrates cleaved by DPP-4 has fostered investigations into the identity of the key glucoregulatory peptides essential for the antidiabetic actions of DPP-4 inhibitors. The inhibitor valine-pyrrolidide potentiated the acute insulinotropic response to co-administered exogenous GLP-1, GIP, pituitary adenylate cyclase-activating polypeptide 38, or GRP in mice.²⁴⁰ Similarly, DPP-4 inhibitors lower blood glucose levels after acute glucose challenge in mice with inactivating mutations in either the GLP-1 or GIP receptors.²⁴¹ In contrast, DIRKO mice with combined genetic mutations in both the GIP and GLP-1 receptors failed to exhibit a decrease in glucose after administration of 4 distinct DPP-4 inhibitors, implying that GLP-1 and GIP receptor signaling pathways are essential for transducing the glucoregulatory actions of DPP-4 inhibitors.²⁴¹ Taken together, these findings imply that GLP-1 and GIP are the dominant substrates essential for the glucose-lowering actions of DPP-4 inhibitors *in vivo*.

Proof of concept for the antidiabetic actions of DPP-4 inhibitors in human subjects with T2DM was obtained in experiments analyzing the actions of 1-[[[2-[(5-cyanopyridin-2-yl)amino]ethyl]amino]acetyl]-2-cyano-(S)-pyrrolidine (NVP DPP728). Treatment with NVP DPP728, 100 mg 3 times daily or 150 mg twice daily, was associated with good tolerability and significant improvements in fasting and meal-related glycemic excursion, and a significant reduction in HbA1c.²⁴² A subsequent 4-week study with the DPP-4 inhibitor Vildagliptin [(1-[[[3-hydroxy-1-adamantyl]amino]acetyl]-2-cyano-(S)-pyrrolidine), 100 mg/day, revealed improvements in both fasting glucose and glucose tolerance in association with reductions in plasma glucagon and improvements in β -cell function.²⁴³

Two DPP-4 inhibitors, Vildagliptin and Sitagliptin, have completed phase 3 clinical trial programs in subjects with T2DM. These agents were evaluated as monotherapy, or in combination with other antidiabetic agents. Vildagliptin and Sitagliptin were well tolerated, weight neutral, and not associated with hypoglycemia when used alone. Both agents increase plasma levels of GLP-1 and GIP after meal ingestion, and enhance glucose-stimulated insulin secretion and reduce ratios of proinsulin:insulin, consistent with an improvement in β -cell function. Minor gastrointestinal complaints and nasopharyngitis appear to be the most common adverse events associated with prolonged clinical use of DPP-4 inhibitors. The available clinical data suggest that both Sitagliptin and Vildagliptin significantly lower blood glucose and HbA1c when used as monotherapy, but were not as potent as

metformin alone in head-to-head studies.^{244–246} In contrast, addition of either Vildagliptin or Sitagliptin to metformin or thiazolidinediones (Rosiglitazone or Pioglitazone) produced significant improvements in HbA1c and resulted in a greater proportion of subjects achieving a HbA1c of less than 7%.²⁴⁷ Sitagliptin also was compared in a head-to-head study with glipizide over 52 weeks. Although no significant differences in HbA1c were noted over the course of the study, subjects treated with glipizide experienced a greater number of hypoglycemic events and more weight gain (2.4 kg) compared with patients treated with sitagliptin. Sitagliptin (Januvia) was approved for the treatment of T2DM in the United States in October 2006.

Summary and Future Directions

The unexpected success of Exenatide as a twice-daily injectable therapy is related in part to the ability of GLP-1R agonists to reduce HbA1c without associated weight gain in the majority of treated subjects. Indeed, most Exenatide-treated subjects experience weight loss, which in about 20% of patients can be substantial, and stands in marked contrast to the weight gain commonly seen with standard antidiabetic agents, including insulin, sulphonylureas, or thiazolidinediones. Whether chronic treatment with GLP-1R agonists will be associated with sustained improvement in and/or preservation of β -cell function remains uncertain. However, it seems reasonable to postulate that newer investigational agents such as Exenatide LAR or Liraglutide, which provide sustained GLP-1R activation over 24 hours, are more likely to achieve significant control of hepatic glucose production, and greater improvements in β -cell function, compared with results achieved with twice-daily Exenatide. Whether GLP-1R agonists will be associated with unexpected problems related to immunogenicity or gradual loss of therapeutic efficacy cannot be predicted with the currently available data. Much less is known about the consequences of long-term therapy with DPP-4 inhibitors. These agents have not demonstrated major adverse events in preclinical studies or in phase 3 clinical programs, however, the consequences of inhibiting DPP-4 activity for years in susceptible patients with chronic illness and T2DM are not yet known. Furthermore, each DPP-4 inhibitor exhibits a distinct selectivity profile that may be associated with a different potential for interaction with DPP-4-related enzymes, and hence each agent is likely to be associated with a unique adverse-event profile in both preclinical and clinical studies. The long-term consequences of DPP-4 inhibitor therapy on β -cell function remain unknown, and there is great interest in determining whether therapy with DPP-4 inhibitors or long-acting GLP-1R agonists may modify the natural history of T2DM, or prevent the transition from impaired glu-

cose tolerance to frank T2DM in at-risk individuals. Both GLP-1R agonists and DPP-4 inhibitors provide new mechanisms of action for physicians interested in the treatment of T2DM, and appear to provide additional therapeutic options that use novel mechanisms of action for achieving glycemic targets in subjects with T2DM.

References

1. Bayliss WM, Starling EH. On the causation of the so-called 'peripheral reflex secretion' of the pancreas. *Proc R Soc Lond Biol* 1902;69:352–353.
2. Moore B, Edie ES, Abram JH. On the treatment of diabetes mellitus by acid extract of duodenal mucous membrane. *Biochem J* 1906;1:28–38.
3. La Barre J. Sur les possibilites d'un traitement du diabete par l'incréline. *Bull Acad R Med Belg* 1932;12:620–634.
4. McIntyre N, Holsworth DC, Turner DS. New interpretation of oral glucose tolerance. *Lancet* 1964;2:20–21.
5. Elrick H, Stimmeler L, Hlad CJ Jr, Arai Y. Plasma insulin response to oral and intravenous glucose administration. *J Clin Invest* 1964;24:1076–1082.
6. Brown JC, Dryburgh JR, Ross SA, Dupre J. Identification and actions of gastric inhibitory polypeptide. *Recent Prog Horm Res* 1975;31:487–532.
7. Lauritsen KB, Moody AJ, Christensen KC, Lindkaer Jensen S. Gastric inhibitory polypeptide (GIP) and insulin release after small-bowel resection in man. *Scand J Gastroenterol* 1980;15:833–840.
8. Mojsov S, Weir GC, Habener JF. Insulintropin: 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* 1987;79:616–619.
9. Kreyman B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 1987;2:1300–1304.
10. White JW, Saunders GF. Structure of the human glucagon gene. *Nucl Acids Res* 1986;14:4719–4730.
11. 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* 1986;261:11880–11889.
12. Drucker DJ, Asa S. Glucagon gene expression in vertebrate brain. *J Biol Chem* 1988;263:13475–13478.
13. Efrat S, Teitelman G, Anwar M, Ruggiero D, Hanahan D. Glucagon gene regulatory region directs oncoprotein expression to neurons and pancreatic alpha cells. *Neuron* 1988;1:605–613.
14. St-Onge L, Sosa-Pineda B, Chowdhury K, Mansouri A, Gruss P. Pax6 is required for differentiation of glucagon-producing α -cells in mouse pancreas. *Nature* 1997;387:406–409.
15. Sander M, Neubuser A, Kalamaras J, Ee HC, Martin GR, German MS. Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. *Genes Dev* 1997;11:1662–1673.
16. Heller RS, Stoffers DA, Liu A, Schedl A, Crenshaw EB 3rd, Madsen OD, Serup P. The role of Brn4/Pou3f4 and Pax6 in forming the pancreatic glucagon cell identity. *Dev Biol* 2004;268:123–134.
17. McKinnon CM, Ravier MA, Rutter GA. FoxO1 is required for the regulation of preproglucagon gene expression by insulin in pancreatic alpha (TC1-9) cells. *J Biol Chem* 2006;281:39358–39369.
18. Drucker DJ, Brubaker PL. Proglucagon gene expression is regulated by a cyclic AMP-dependent pathway in rat intestine. *Proc Natl Acad Sci U S A* 1989;86:3953–3957.

19. Drucker DJ, Jin T, Asa SL, Young TA, Brubaker PL. Activation of proglucagon gene transcription by protein kinase-A in a novel mouse enteroendocrine cell line. *Mol Endocrinol* 1994;8:1646–1655.
20. Brubaker PL, Schloos J, Drucker DJ. Regulation of glucagon-like peptide-1 synthesis and secretion in the GLUTag enteroendocrine cell line. *Endocrinology* 1998;139:4108–4114.
21. Yi F, Brubaker PL, Jin T. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. *J Biol Chem* 2005;280:1457–1464.
22. Rountree DB, Ulshen MH, Selub S, Fuller CR, Bloom SR, Ghatei MA, Lund PK. Nutrient-independent increases in proglucagon and ornithine decarboxylase messenger RNAs after jejunoileal resection. *Gastroenterology* 1992;103:462–468.
23. Hoyt EC, Lund PK, Winesett DE, Fuller CR, Ghatei MA, Bloom SR, Ulshen MH. Effects of fasting, refeeding, and intraluminal triglyceride on proglucagon expression in jejunum and ileum. *Diabetes* 1996;45:434–439.
24. 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* 1996;137:3948–3956.
25. Tappenden KA, Thomson AB, Wild GE, McBurney MI. Short-chain fatty acids increase proglucagon and ornithine decarboxylase messenger RNAs after intestinal resection in rats. *JPN J Parenter Enteral Nutr* 1996;20:357–362.
26. Trinh DK, Zhang K, Hossain M, Brubaker PL, Drucker DJ. Pax-6 activates endogenous proglucagon gene expression in the rodent gastrointestinal epithelium. *Diabetes* 2003;52:425–433.
27. Hill ME, Asa SL, Drucker DJ. Essential requirement for Pax6 in control of enteroendocrine proglucagon gene transcription. *Mol Endocrinol* 1999;13:1474–1486.
28. 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* 1992;267:10705–10708.
29. Jin T, Drucker DJ. The proglucagon gene upstream enhancer contains positive and negative domains important for tissue-specific proglucagon gene transcription. *Mol Endocrinol* 1995;9:1306–1320.
30. Nian M, Drucker DJ, Irwin D. Divergent regulation of human and rat proglucagon gene promoters *in vivo*. *Am J Physiol* 1999;277:G829–G837.
31. Rouille Y, Martin S, Steiner DF. Differential processing of proglucagon by the subtilisin-like prohormone convertases PC2 and PC3 to generate either glucagon or glucagon-like peptide. *J Biol Chem* 1995;270:26488–26496.
32. Parker JC, Andrews KM, Allen MR, Stock JL, McNeish JD. Glycemic control in mice with targeted disruption of the glucagon receptor gene. *Biochem Biophys Res Commun* 2002;290:839–843.
33. Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C, Johansen PB, Rossetti L, Jelicks LA, Serup P, Nishimura E, Charron MJ. Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. *Proc Natl Acad Sci U S A* 2003;100:1438–1443.
34. Furuta M, Yano H, Zhou A, Rouille Y, Holst JJ, Carroll R, Ravazola M, Orci L, Furuta H, Steiner DF. Defective prohormone processing and altered pancreatic islet morphology in mice lacking active SPC2. *Proc Natl Acad Sci U S A* 1997;94:6646–6651.
35. Myojo S, Tsujikawa T, Sasaki M, Fujiyama Y, Bamba T. Trophic effects of glicentin on rat small-intestinal mucosa *in vivo* and *in vitro*. *J Gastroenterol* 1997;32:300–305.
36. Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Oxyntomodulin from distal gut. Role in regulation of gastric and pancreatic functions. *Dig Dis Sci* 1989;34:1411–1419.
37. Dakin CL, Gunn I, Small CJ, Edwards CM, Hay DL, Smith DM, Ghatei MA, Bloom SR. Oxyntomodulin inhibits food intake in the rat. *Endocrinology* 2001;142:4244–4250.
38. Baggio LL, Huang Q, Brown TJ, Drucker DJ. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology* 2004;127:546–558.
39. Sowden GL, Drucker DJ, Weinschenker D, Swoap SJ. Oxyntomodulin increases intrinsic heart rate in mice independent of the glucagon-like peptide-1 receptor. *Am J Physiol* 2006;292:R962–970.
40. Drucker DJ, Erlich P, Asa SL, Brubaker PL. Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proc Natl Acad Sci U S A* 1996;93:7911–7916.
41. Tang-Christensen M, Larsen PJ, Thulesen J, Romer J, Vrang N. The proglucagon-derived peptide, glucagon-like peptide-2, is a neurotransmitter involved in the regulation of food intake. *Nat Med* 2000;6:802–807.
42. Lovshin J, Estall J, Yusta B, Brown TJ, Drucker DJ. Glucagon-like peptide (GLP)-2 action in the murine central nervous system is enhanced by elimination of GLP-1 receptor signaling. *J Biol Chem* 2001;276:21489–21499.
43. Lovshin JA, Huang Q, Seaberg R, Brubaker PL, Drucker DJ. Extra-hypothalamic expression of the glucagon-like peptide-2 receptor is coupled to reduction of glutamate-induced cell death in cultured hippocampal cells. *Endocrinology* 2004;145:3495–3506.
44. 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* 1995;270:10136–10146.
45. Rouille Y, Kantengwa S, Irminger JC, Halban PA. Role of the prohormone convertase PC3 in the processing of proglucagon to glucagon-like peptide 1. *J Biol Chem* 1997;272:32810–32816.
46. Ugleholdt R, Poulsen ML, Holst PJ, Irminger JC, Orskov C, Pedersen J, Rosenkilde MM, Zhu X, Steiner DF, Holst JJ. Prohormone convertase 1/3 is essential for processing of the glucose-dependent insulinotropic polypeptide precursor. *J Biol Chem* 2006;281:11050–11057.
47. Mortensen K, Christensen LL, Holst JJ, Orskov C. GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. *Regul Pept* 2003;114:189–196.
48. Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K, Egan JM. Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. *Am J Physiol* 2006;290:E550–E559.
49. Brubaker PL. The glucagon-like peptides: pleiotropic regulators of nutrient homeostasis. *Ann N Y Acad Sci* 2006;1070:10–26.
50. Unger RH, Ohneda A, Valverde I, Eisentraut AM, Exton J. Characterization of the responses of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. *J Clin Invest* 1968;47:48–65.
51. Hermann C, Goke R, Richter G, Fehmann HC, Arnold R, Goke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 1995;56:117–126.
52. Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology* 1999;140:1687–1694.
53. 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* 1997;82:786–790.

54. Anini Y, Hansotia T, Brubaker PL. Muscarinic receptors control postprandial release of glucagon-like peptide-1: in vivo and in vitro studies in rats. *Endocrinology* 2002;143:2420–2426.
55. Anini Y, Brubaker PL. Muscarinic receptors control glucagon-like peptide 1 secretion by human endocrine L cells. *Endocrinology* 2003;144:3244–3250.
56. 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* 1993;91:301–307.
57. Roberge JN, Brubaker PL. Secretion of proglucagon-derived peptides in response to intestinal luminal nutrients. *Endocrinology* 1991;128:3169–3174.
58. Anini Y, Brubaker PL. Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes* 2003;52:252–259.
59. Reimann F, Gribble FM. Glucose-sensing in glucagon-like peptide-1-secreting cells. *Diabetes* 2002;51:2757–2763.
60. Gribble FM, Williams L, Simpson AK, Reimann F. A novel glucose-sensing mechanism contributing to glucagon-like peptide-1 secretion from the GLUTag cell line. *Diabetes* 2003;52:1147–1154.
61. Dhanvantari S, Seidah NG, Brubaker PL. Role of prohormone convertases in the tissue-specific processing of proglucagon. *Mol Endocrinol* 1996;10:342–355.
62. Orskov C, Wettergren A, Holst JJ. Biological effects and metabolic rates of glucagonlike peptide-1 7-36 amide and glucagon-like peptide-1 7-37 in healthy subjects are indistinguishable. *Diabetes* 1993;42:658–661.
63. Wettergren A, Pridal L, Wojdemann M, Holst JJ. Amidated and non-amidated glucagon-like peptide-1 (GLP-1): non-pancreatic effects (cephalic phase acid secretion) and stability in plasma in humans. *Regul Pept* 1998;77:83–87.
64. Orskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes* 1994;43:535–539.
65. Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide 1 are rapidly degraded from the NH₂-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 1995;44:1126–1131.
66. 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* 1993;214:829–835.
67. Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 1995;136:3585–3596.
68. 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* 1995;80:952–957.
69. Hansen L, Deacon CF, Orskov C, Holst JJ. Glucagon-like peptide-1(7-36)amide is transformed to glucagon-like peptide-1(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* 1999;140:5356–5363.
70. Mentlein R. Dipeptidyl-peptidase IV (CD26)—role in the inactivation of regulatory peptides. *Regul Pept* 1999;85:9–24.
71. Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, Ribel U, Watanabe T, Drucker DJ, Wagtmann N. Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci U S A* 2000;97:6874–6879.
72. Turner AJ, Isaac RE, Coates D. The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. *Bioessays* 2001;23:261–269.
73. Plamboeck A, Holst JJ, Carr RD, Deacon CF. Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both mediators of the degradation of glucagon-like peptide 1 in the anaesthetized pig. *Diabetologia* 2005;48:1882–1890.
74. Deacon CF. What do we know about the secretion and degradation of incretin hormones? *Regul Pept* 2005;128:117–124.
75. Ruiz-Grande C, Alarcon C, Alcantara A, Castilla C, Lopez Novoa JM, Villanueva-Penacarrillo ML, Valverde I. Renal catabolism of truncated glucagon-like peptide 1. *Horm Metab Res* 1993;25:612–616.
76. Orskov C, Andreasen J, Holst JJ. All products of proglucagon are elevated in plasma from uremic patients. *J Clin Endocrinol Metab* 1992;74:379–384.
77. Meier JJ, Nauck MA, Kranz D, Holst JJ, Deacon CF, Gaeckler D, Schmidt WE, Gallwitz B. Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes* 2004;53:654–662.
78. 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* 1993;138:159–166.
79. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001;50:609–613.
80. Xiao Q, Boushey RP, Drucker DJ, Brubaker PL. Secretion of the intestinotropic hormone glucagon-like peptide 2 is differentially regulated by nutrients in humans. *Gastroenterology* 1999;117:99–105.
81. Ranganath LR, Beety JM, Morgan LM, Wright JW, Howland R, Marks V. Attenuated GLP-1 secretion in obesity: cause or consequence? *Gut* 1996;38:916–919.
82. Ranganath L, Norris F, Morgan L, Wright J, Marks V. Inhibition of carbohydrate-mediated glucagon-like peptide-1 (7-36)amide secretion by circulating non-esterified fatty acids. *Clin Sci (Lond)* 1999;96:335–342.
83. Vaag AA, Holst JJ, Volund A, Beck-Nielsen HB. Gut incretin hormones in identical twins discordant for non-insulin-dependent diabetes mellitus (NIDDM)—evidence for decreased glucagon-like peptide 1 secretion during oral glucose ingestion in NIDDM twins. *Eur J Endocrinol* 1996;135:425–432.
84. Vilsboll T, Agerso H, Krarup T, Holst JJ. Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. *J Clin Endocrinol Metab* 2003;88:220–224.
85. Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B, Drucker DJ. International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol Rev* 2003;55:167–194.
86. Sandhu H, Wiesenthal SR, MacDonald PE, McCall RH, Tchipsavili V, Rashid S, Satkunarajah M, Irwin DM, Shi ZQ, Brubaker PL, Wheeler MB, Vranic M, Efendic S, Giacca A. Glucagon-like peptide 1 increases insulin sensitivity in depancreatized dogs. *Diabetes* 1999;48:1045–1053.
87. Montrose-Rafizadeh C, Avdonin P, Garant MJ, Rodgers BD, Kole S, Yang H, Levine MA, Schwindinger W, Bernier M. Pancreatic glucagon-like peptide-1 receptor couples to multiple G proteins and activates mitogen-activated protein kinase pathways in Chinese hamster ovary cells. *Endocrinology* 1999;140:1132–1140.
88. Hallbrink M, Holmqvist T, Olsson M, Ostenson CG, Efendic S, Langel U. Different domains in the third intracellular loop of the GLP-1 receptor are responsible for Galpha(s) and Galpha(i)/Galpha(o) activation. *Biochim Biophys Acta* 2001;1546:79–86.

89. Thorens B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci U S A* 1992;89:8641–8645.
90. 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 U S A* 1987;84:3434–3438.
91. 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 adenylyl cyclase and phospholipase-C. *Endocrinology* 1993;133:57–62.
92. Holz GG, Leech CA, Habener JF. Activation of a cAMP-regulated Ca²⁺-signaling pathway in pancreatic b-cells by the insulinotropic hormone glucagon-like peptide-1. *J Biol Chem* 1995;270:17749–17757.
93. Baggio LL, Kim JG, Drucker DJ. Chronic exposure to GLP-1R agonists promotes homologous GLP-1 receptor desensitization in vitro but does not attenuate GLP-1R-dependent glucose homeostasis in vivo. *Diabetes* 2004;53(Suppl 3):S205–S214.
94. Goke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, Goke 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 beta-cells. *J Biol Chem* 1993;268:19650–19655.
95. Scrocchi LA, Brown TJ, McCluskey 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 1 receptor gene. *Nat Med* 1996;2:1254–1258.
96. Holst JJ, Orskov C, Nielsen OV, Schwartz TW. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett* 1987;211:169–174.
97. 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* 1997;99:2883–2889.
98. Li Y, Cao X, Li LX, Brubaker PL, Edlund H, Drucker DJ. Beta-cell Pdx1 expression is essential for the glucoregulatory, proliferative, and cytoprotective actions of glucagon-like peptide-1. *Diabetes* 2005;54:482–491.
99. Alarcon C, Wicksteed B, Rhodes CJ. Exendin 4 controls insulin production in rat islet beta cells predominantly by potentiation of glucose-stimulated proinsulin biosynthesis at the translational level. *Diabetologia* 2006;49:2920–2929.
100. Fehmann H-C, Habener JF. Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma β TC-1 cells. *Endocrinology* 1992;130:159–166.
101. Wang X, Cahill CM, Pineyro MA, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 regulates the beta cell transcription factor, PDX-1, in insulinoma cells. *Endocrinology* 1999;140:4904–4907.
102. Wang H, Iezzi M, Theander S, Antinozzi PA, Gauthier BR, Halban PA, Wollheim CB. Suppression of Pdx-1 perturbs proinsulin processing, insulin secretion and GLP-1 signalling in INS-1 cells. *Diabetologia* 2005;48:720–731.
103. Holz GG, Kuhlreier WM, Habener JF. Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature* 1993;361:362–365.
104. Fehmann HC, Habener JF. Functional receptors for the insulinotropic hormone glucagon-like peptide-I(7-37) on a somatostatin secreting cell line. *FEBS Lett* 1991;279:335–340.
105. Heller RS, Kieffer TJ, Habener JF. Insulinotropic glucagon-like peptide I receptor expression in glucagon-producing alpha-cells of the rat endocrine pancreas. *Diabetes* 1997;46:785–791.
106. Creutzfeldt 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 I diabetic patients. *Diabetes Care* 1996;19:580–586.
107. Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R, Hufner M, Schmiegel WH. Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J Clin Endocrinol Metab* 2002;87:1239–1246.
108. Hui H, Nourparvar A, Zhao X, Perfetti R. Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5'-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. *Endocrinology* 2003;144:1444–1455.
109. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* 2003;278:471–478.
110. Buteau J, El-Assaad W, Rhodes CJ, Rosenberg L, Joly E, Prentki M. Glucagon-like peptide-1 prevents beta cell glucolipototoxicity. *Diabetologia* 2004;47:806–815.
111. Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Nourmehr H, Bertolotto C, Di Mario U, Harlan DM, Perfetti R. GLP-1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 2003;144:5149–5158.
112. Yusta B, Baggio LL, Estall JL, Koehler JA, Holland DP, Li H, Pipeleers D, Ling Z, Drucker DJ. GLP-1 receptor activation improves beta cell function and survival following induction of endoplasmic reticulum stress. *Cell Metab* 2006;4:391–406.
113. Holz GG, Chepurny OG. Diabetes outfoxed by GLP-1? *Sci STKE* 2005;2005:pe2.
114. Drucker DJ. Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis. *Mol Endocrinol* 2003;17:161–171.
115. Buteau J, Foisy S, Joly E, Prentki M. Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes* 2003;52:124–132.
116. Park S, Dong X, Fisher TL, Dunn S, Omer AK, Weir G, White MF. Exendin-4 uses Irs2 signaling to mediate pancreatic beta cell growth and function. *J Biol Chem* 2006;281:1159–1168.
117. Kodama S, Toyonaga T, Kondo T, Matsumoto K, Tsuruzoe K, Kawashima J, Goto H, Kume K, Kume S, Sakakida M, Araki E. Enhanced expression of PDX-1 and Ngn3 by exendin-4 during beta cell regeneration in STZ-treated mice. *Biochem Biophys Res Commun* 2005;327:1170–1178.
118. Buteau J, Spatz ML, Accili D. Transcription factor FoxO1 mediates glucagon-like peptide-1 effects on pancreatic beta-cell mass. *Diabetes* 2006;55:1190–1196.
119. Wang Q, Brubaker PL. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. *Diabetologia* 2002;45:1263–1273.
120. Jhala US, Canettieri G, Srean RA, Kulkarni RN, Krajewski S, Reed J, Walker J, Lin X, White M, Montminy M. cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. *Genes Dev* 2003;17:1575–1580.
121. Schirra J, Sturm K, Leicht P, Arnold R, Goke B, Katschinski M. Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans. *J Clin Invest* 1998;101:1421–1430.
122. Ling Z, Wu D, Zambre Y, Flamez D, Drucker DJ, Pipeleers DG, Schuit FC. Glucagon-like peptide 1 receptor signaling influences topography of islet cells in mice. *Virchows Arch* 2001;438:382–387.
123. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 1996;379:69–72.

124. 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* 1996;271:R848–R856.
125. Meeran K, O'Shea D, Edwards CM, Turton MD, Heath MM, Gunn I, Abusnana S, Rossi M, Small CJ, Goldstone AP, Taylor GM, Sunter D, Steere J, Choi SJ, Ghatei MA, Bloom SR. Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. *Endocrinology* 1999;140:244–250.
126. Szayna M, Doyle ME, Betkey JA, Holloway HW, Spencer RG, Greig NH, Egan JM. Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. *Endocrinology* 2000;141:1936–1941.
127. Abbott CR, Monteiro M, Small CJ, Sajedi A, Smith KL, Parkinson JR, Ghatei MA, Bloom SR. The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain Res* 2005;1044:127–131.
128. Talsania T, Anini Y, Siu S, Drucker DJ, Brubaker PL. Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. *Endocrinology* 2005;146:3748–3756.
129. Baggio LL, Huang Q, Brown TJ, Drucker DJ. A recombinant human glucagon-like peptide (GLP)-1-albumin protein (albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. *Diabetes* 2004;53:2492–2500.
130. Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ. Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am J Physiol* 1996;271:E458–E464.
131. Balkan B, Li X. Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. *Am J Physiol* 2000;279:R1449–R1454.
132. Ahren B. Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. *Am J Physiol* 2004;286:R269–R272.
133. Burcelin R, Da Costa A, Drucker D, Thorens B. Glucose competence of the hepatoportal vein sensor requires the presence of an activated glucagon-like peptide-1 receptor. *Diabetes* 2001;50:1720–1728.
134. Knauf C, Cani PD, Perrin C, Iglesias MA, Maury JF, Bernard E, Benhamed F, Gremeaux T, Drucker DJ, Kahn CR, Girard J, Tanti JF, Delzenne NM, Postic C, Burcelin R. Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. *J Clin Invest* 2005;115:3554–3563.
135. Perry T, Haughey NJ, Mattson MP, Egan JM, Greig NH. Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. *J Pharmacol Exp Ther* 2002;302:881–888.
136. During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, Bland RJ, Klugmann M, Banks WA, Drucker DJ, Haile CN. Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat Med* 2003;9:1173–1179.
137. 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 (noninsulin-dependent) diabetic patients. *J Clin Endocrinol Metab* 1996;81:327–332.
138. Meier JJ, Gallwitz B, Salmen S, Goetze O, Holst JJ, Schmidt WE, Nauck MA. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2003;88:2719–2725.
139. 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* 1995;44:626–630.
140. Wishart JM, Horowitz M, Morris HA, Jones KL, Nauck MA. Relation between gastric emptying of glucose and plasma concentrations of glucagon-like peptide-1. *Peptides* 1998;19:1049–1053.
141. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, Schmiegel WH. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol* 1997;273:E981–E988.
142. Meier JJ, Kemmeries G, Holst JJ, Nauck MA. Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. *Diabetes* 2005;54:2212–2218.
143. Barragan JM, Eng J, Rodriguez R, Blazquez E. Neural contribution to the effect of glucagon-like peptide-1-(7-36) amide on arterial blood pressure in rats. *Am J Physiol* 1999;277:E784–E791.
144. Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest* 2002;110:43–52.
145. Yamamoto H, Kishi T, Lee CE, Choi BJ, Fang H, Hollenberg AN, Drucker DJ, Elmquist JK. Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. *J Neurosci* 2003;23:2939–2946.
146. Toft-Nielsen MB, Madsbad S, Holst JJ. Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care* 1999;22:1137–1143.
147. Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, Ghatei MA, Bloom SR. Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. *Am J Physiol* 2001;281:E155–E161.
148. Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002;359:824–830.
149. Meier JJ, Nauck MA. Glucagon-like peptide 1 (GLP-1) in biology and pathology. *Diabetes Metab Res Rev* 2005;21:91–117.
150. Nystrom T, Gutniak MK, Zhang Q, Zhang F, Holst JJ, Ahren B, Sjolholm A. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol* 2004;287:E1209–E1215.
151. Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelis L, Stolarski C, Shen YT, Shannon RP. Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. *Circulation* 2004;110:955–961.
152. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes* 2005;54:146–151.
153. Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP. Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and post-ischemic isolated rat hearts. *J Pharmacol Exp Ther* 2006;317:1106–1113.
154. Gros R, You X, Baggio LL, Kabir MG, Sadi AM, Mungrue IN, Parker TG, Huang Q, Drucker DJ, Husain M. Cardiac function in mice lacking the glucagon-like peptide-1 receptor. *Endocrinology* 2003;144:2242–2252.
155. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D, Shannon RP. Effects of glucagon-like peptide-1 in patients with

- acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation* 2004;109:962–965.
156. Ruiz-Grande C, Alarcón C, Mzrida E, Valverde I. Lipolytic action of glucagon-like peptides in isolated rat adipocytes. *Peptides* 1992;13:13–16.
 157. Villanueva-Penacarrillo ML, Marquez L, Gonzalez N, Diaz-Miguel M, Valverde I. Effect of GLP-1 on lipid metabolism in human adipocytes. *Horm Metab Res* 2001;33:73–77.
 158. Young AA, Gedulin BR, Bhavsar S, Bodkin N, Jodka C, Hansen B, Denaro M. Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (ob/ob, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (*Macaca mulatta*). *Diabetes* 1999;48:1026–1034.
 159. Ding X, Saxena NK, Lin S, Gupta NA, Anania FA. Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. *Hepatology* 2006;43:173–181.
 160. Furnsinn C, Ebner K, Waldhauser W. Failure of GLP-1 (7-36) amide to affect glycogenesis in rat skeletal muscle. *Diabetologia* 1995;38:864–867.
 161. Nakagawa Y, Kawai K, Suzuki H, Ohashi S, Yamashita K. Glucagon-like peptide-1(7-36)amide and glycogen synthesis in the liver. *Diabetologia* 1996;39:1241–1242.
 162. D'Alessio DA, Kahn SE, Leusner CR, Ensinnck JW. Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. *J Clin Invest* 1994;93:2263–2266.
 163. Meneilly GS, McIntosh CH, Pederson RA, Habener JF, Gingerich R, Egan JM, Finegood DT, Elahi D. Effect of glucagon-like peptide 1 on non-insulin-mediated glucose uptake in the elderly patient with diabetes. *Diabetes Care* 2001;24:1951–1956.
 164. Egan JM, Meneilly GS, Habener JF, Elahi D. Glucagon-like peptide-1 augments insulin-mediated glucose uptake in the obese state. *J Clin Endocrinol Metab* 2002;87:3768–3773.
 165. Orskov L, Holst JJ, Møller J, Orskov C, Møller N, Alberti KG, Schmitz O. GLP-1 does not acutely affect insulin sensitivity in healthy man. *Diabetologia* 1996;39:1227–1232.
 166. Toft-Nielsen M, Madsbad S, Holst JJ. The effect of glucagon-like peptide I (GLP-I) on glucose elimination in healthy subjects depends on the pancreatic glucoregulatory hormones. *Diabetes* 1996;45:552–556.
 167. Larsson H, Holst JJ, Ahren B. Glucagon-like peptide-1 reduces hepatic glucose production indirectly through insulin and glucagon in humans. *Acta Physiol Scand* 1997;160:413–422.
 168. Ryan AS, Egan JM, Habener JF, Elahi D. Insulinotropic hormone glucagon-like peptide-1(7-37) appears not to augment insulin-mediated glucose uptake in young men during euglycemia. *J Clin Endocrinol Metab* 1998;83:2399–2404.
 169. Vella A, Shah P, Reed AS, Adkins AS, Basu R, Rizza RA. Lack of effect of exendin-4 and glucagon-like peptide-1(7,36)-amide on insulin action in non-diabetic humans. *Diabetologia* 2002;45:1410–1415.
 170. Prigeon RL, Quddusi S, Paty B, D'Alessio DA. Suppression of endogenous glucose production by glucagon-like peptide 1 independent of islet hormones: an extrapancreatic effect of an incretin hormone. *Am J Physiol* 2003;285:E701–E707.
 171. Larsen PJ, Tang-Christensen M, Jessop DS. Central administration of glucagon-like peptide-1 activates hypothalamic neuroendocrine neurons in the rat. *Endocrinology* 1997;138:4445–4455.
 172. MacLusky NJ, Cook S, Scrocchi L, Shin J, Kim J, Vaccarino F, Asa SL, Drucker DJ. Neuroendocrine function and response to stress in mice with complete disruption of glucagon-like peptide-1 receptor signaling. *Endocrinology* 2000;141:752–762.
 173. Yu M, Moreno C, Hoagland KM, Dahly A, Ditter K, Mistry M, Roman RJ. Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 2003;21:1125–1135.
 174. Gutzwiller JP, Tschopp S, Bock A, Zehnder CE, Huber AR, Kreyenbuehl M, Gutmann H, Drewe J, Henzen C, Goeke B, Beglinger C. Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. *J Clin Endocrinol Metab* 2004;89:3055–3061.
 175. Rolin B, Deacon CF, Carr RD, Ahren B. The major glucagon-like peptide-1 metabolite, GLP-1(9-36)-amide, does not affect glucose or insulin levels in mice. *Eur J Pharmacol* 2004;494:283–288.
 176. Deacon CF, Plamboeck A, Møller S, Holst JJ. GLP-1(9-36) amide reduces blood glucose in anesthetized pigs by a mechanism that does not involve insulin secretion. *Am J Physiol* 2002;282:E873–E879.
 177. Meier JJ, Gethmann A, Nauck MA, Gotze O, Schmitz F, Deacon CF, Gallwitz B, Schmidt WE, Holst JJ. The glucagon-like peptide-1 metabolite GLP-1(9-36) amide reduces postprandial glycemia independently of gastric emptying and insulin secretion in humans. *Am J Physiol* 2006;290:E1118–E1123.
 178. Vahl TP, Paty BW, Fuller BD, Prigeon RL, D'Alessio DA. Effects of GLP-1(7-36)NH₂, GLP-1(7-37), and GLP-1(9-36)NH₂ on intravenous glucose tolerance and glucose-induced insulin secretion in healthy humans. *J Clin Endocrinol Metab* 2003;88:1772–1779.
 179. Nikolaidis LA, Elahi D, Shen YT, Shannon RP. Active metabolite of GLP-1 mediates myocardial glucose uptake and improves left ventricular performance in conscious dogs with dilated cardiomyopathy. *Am J Physiol* 2005;289:H2401–H2408.
 180. Jepeal LI, Fujitani Y, Boylan MO, Wilson CN, Wright CV, Wolfe MM. Cell-specific expression of glucose-dependent-insulinotropic polypeptide is regulated by the transcription factor PDX-1. *Endocrinology* 2005;146:383–391.
 181. Buchan AM, Polak JM, Capella C, Solcia E, Pearse AGE. Electron immunohistochemical evidence for the K cell localization of gastric inhibitory polypeptide (GIP) in man. *Histochemistry* 1978;56:37–44.
 182. Besterman HS, Cook GC, Sarson DL, Christofides ND, Bryant MG, Gregor M, Bloom SR. Gut hormones in tropical malabsorption. *BMJ* 1979;2:1252–1255.
 183. Fushiki T, Kojima A, Imoto T, Inoue K, Sugimoto E. An extract of *Gymnema sylvestre* leaves and purified gymnemic acid inhibits glucose-stimulated gastric inhibitory peptide secretion in rats. *J Nutr* 1992;122:2367–2373.
 184. 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* 1996;31:665–670.
 185. Ross SA, Brown JC, Dupre J. Hypersecretion of gastric inhibitory polypeptide following oral glucose in diabetes mellitus. *Diabetes* 1977;26:525–529.
 186. Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ. Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J Clin Endocrinol Metab* 2000;85:3575–3581.
 187. Deacon CF, Danielsen P, Klarskov L, Olesen M, Holst JJ. Dipeptidyl peptidase IV inhibition reduces the degradation and clearance of GIP and potentiates its insulinotropic and antihyperglycemic effects in anesthetized pigs. *Diabetes* 2001;50:1588–1597.
 188. Vilsboll T, Agero H, Lauritsen T, Deacon CF, Aaboe K, Madsbad S, Krarup T, Holst JJ. The elimination rates of intact GIP as well as its primary metabolite, GIP 3-42, are similar in type 2 diabetic patients and healthy subjects. *Regul Pept* 2006;137:168–172.
 189. Yamada Y, Hayami T, Nakamura K, Kaisaki PJ, Someya Y, Wang CZ, Seino S, Seino Y. Human gastric inhibitory polypeptide

- receptor: cloning of the gene (GIPR) and cDNA. *Genomics* 1995;29:773–776.
190. Usdin TB, Mezey E, Button DC, Brownstein MJ, Bonner TI. Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. *Endocrinology* 1993;133:2861–2870.
 191. Lynn FC, Pamin N, Ng EH, McIntosh CH, Kieffer TJ, Pederson RA. Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* 2001;50:1004–1011.
 192. Wheeler MB, Gelling RW, Hinke SA, Tu B, Pederson RA, Lynn F, Ehses J, McIntosh CH. Characterization of the carboxyl-terminal domain of the rat glucose-dependent insulinotropic polypeptide (GIP) receptor. A role for serines 426 and 427 in regulating the rate of internalization. *J Biol Chem* 1999;274:24593–24601.
 193. Ding WG, Gromada J. Protein kinase A-dependent stimulation of exocytosis in mouse pancreatic beta-cells by glucose-dependent insulinotropic polypeptide. *Diabetes* 1997;46:615–621.
 194. Gromada J, Bokvist K, Ding WG, Holst JJ, Nielsen JH, Rorsman P. Glucagon-like peptide 1 (7-36) amide stimulates exocytosis in human pancreatic beta-cells by both proximal and distal regulatory steps in stimulus-secretion coupling. *Diabetes* 1998;47:57–65.
 195. Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H, Seino S. Critical role of cAMP-GEFII—Rim2 complex in incretin-potentiated insulin secretion. *J Biol Chem* 2001;276:46046–46053.
 196. Ehses JA, Lee SS, Pederson RA, McIntosh CH. A new pathway for glucose-dependent insulinotropic polypeptide (GIP) receptor signaling: evidence for the involvement of phospholipase A2 in GIP-stimulated insulin secretion. *J Biol Chem* 2001;276:23667–23673.
 197. Ehses JA, Pelech SL, Pederson RA, McIntosh CH. Glucose-dependent insulinotropic polypeptide activates the Raf-Mek1/2-ERK1/2 module via a cyclic AMP/cAMP-dependent protein kinase/Rap1-mediated pathway. *J Biol Chem* 2002;277:37088–37097.
 198. Kim SJ, Choi WS, Han JS, Warnock G, Fedida D, McIntosh CH. A novel mechanism for the suppression of a voltage-gated potassium channel by glucose-dependent insulinotropic polypeptide: protein kinase A-dependent endocytosis. *J Biol Chem* 2005;280:28692–28700.
 199. Wang Y, Montrose-Rafizadeh C, Adams L, Raygada M, Nadiv O, Egan JM. GIP regulates glucose transporters, hexokinases, and glucose-induced insulin secretion in RIN 1046-38 cells. *Mol Cell Endocrinol* 1996;116:81–87.
 200. Tseng CC, Kieffer TJ, Jarboe LA, Usdin TB, Wolfe MM. Postprandial stimulation of insulin release by glucose-dependent insulinotropic polypeptide (GIP). Effect of a specific glucose-dependent insulinotropic polypeptide receptor antagonist in the rat. *J Clin Invest* 1996;98:2440–2445.
 201. Gelling RW, Coy DH, Pederson RA, Wheeler MB, Hinke S, Kwan T, McIntosh CH. GIP(6-30amide) contains the high affinity binding region of GIP and is a potent inhibitor of GIP1-42 action in vitro. *Regul Peptides* 1997;69:151–154.
 202. Baggio L, Kieffer TJ, Drucker DJ. GLP-1 but not GIP regulates fasting and non-enteral glucose clearance in mice. *Endocrinology* 2000;141:3703–3709.
 203. Lewis JT, Dayanandan B, Habener JF, Kieffer TJ. Glucose-dependent insulinotropic polypeptide confers early phase insulin release to oral glucose in rats: demonstration by a receptor antagonist. *Endocrinology* 2000;141:3710–3716.
 204. Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, Kubota A, Fujimoto S, Kajikawa M, Kuroe A, Tsuda K, Hashimoto H, Yamashita T, Jomori T, Tashiro F, Miyazaki J, Seino Y. Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *Proc Natl Acad Sci U S A* 1999;96:14843–14847.
 205. Kim SJ, Winter K, Nian C, Tsuneoka M, Koda Y, McIntosh CH. GIP stimulation of pancreatic beta-cell survival is dependent upon phosphatidylinositol 3-kinase (PI3-K)/ protein kinase B (PKB) signaling, inactivation of the forkhead transcription factor Foxo1 and downregulation of bax expression. *J Biol Chem* 2005;280:22297–22307.
 206. Trumper A, Trumper K, Trusheim H, Arnold R, Goke B, Horsch D. Glucose-dependent insulinotropic polypeptide is a growth factor for beta (INS-1) cells by pleiotropic signaling. *Mol Endocrinol* 2001;15:1559–1570.
 207. Trumper A, Trumper K, Horsch D. Mechanisms of mitogenic and anti-apoptotic signaling by glucose-dependent insulinotropic polypeptide in beta(INS-1)-cells. *J Endocrinol* 2002;174:233–246.
 208. Ehses JA, Casilla VR, Doty T, Pospisilik JA, Demuth HU, Pederson RA, Winter KD, McIntosh CH. Glucose-dependent Insulinotropic Polypeptide (GIP) Promotes {beta}(INS-1) cell survival via cyclic AMP-mediated caspase-3 inhibition and regulation of p38 MAP kinase. *Endocrinology* 2003;144:4433–4445.
 209. Nyberg J, Anderson MF, Meister B, Alborn AM, Strom AK, Brederlau A, Illerskog AC, Nilsson O, Kieffer TJ, Hietala MA, Ricksten A, Eriksson PS. Glucose-dependent insulinotropic polypeptide is expressed in adult hippocampus and induces progenitor cell proliferation. *J Neurosci* 2005;25:1816–1825.
 210. Yip RGC, Boylan MO, Kieffer TJ, Wolfe MM. Functional GIP receptors are present on adipocytes. *Endocrinology* 1998;139:4004–4007.
 211. Creutzfeldt W, Ebert R, Willms B, Frerichs H, Brown JC. Gastric inhibitory polypeptide (GIP) and insulin in obesity: increased response to stimulation and defective feedback control of serum levels. *Diabetologia* 1978;14:15–24.
 212. Salera M, Giacomoni P, Pironi L, Cornia G, Capelli M, Marini A, Benfenati F, Miglioli M, Barbara L. Gastric inhibitory polypeptide release after oral glucose: relationship to glucose intolerance, diabetes mellitus, and obesity. *J Clin Endocrinol Metab* 1982;55:329–336.
 213. Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou H, Fujimoto S, Oku A, Tsuda K, Toyokuni S, Hiai H, Mizunoya W, Fushiki T, Holst JJ, Makino M, Tashita A, Kobara Y, Tsubamoto Y, Jinnouchi T, Jomori T, Seino Y. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med* 2002;8:738–742.
 214. Gault VA, Irwin N, Green BD, McCluskey JT, Greer B, Bailey CJ, Harriott P, O'Harte FP, Flatt PR. Chemical ablation of gastric inhibitory polypeptide receptor action by daily (Pro3)GIP administration improves glucose tolerance and ameliorates insulin resistance and abnormalities of islet structure in obesity-related diabetes. *Diabetes* 2005;54:2436–2446.
 215. Irwin N, Gault VA, Green BD, Greer B, McCluskey JT, Harriott P, O'Harte FP, Flatt PR. Effects of short-term chemical ablation of the GIP receptor on insulin secretion, islet morphology and glucose homeostasis in mice. *Biol Chem* 2004;385:845–852.
 216. Bollag RJ, Zhong Q, Phillips P, Min L, Zhong L, Cameron R, Mulloy AL, Rasmussen H, Qin F, Ding KH, Isales CM. Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors. *Endocrinology* 2000;141:1228–1235.
 217. Bollag RJ, Zhong Q, Ding KH, Phillips P, Zhong L, Qin F, Cranford J, Mulloy AL, Cameron R, Isales CM. Glucose-dependent insulinotropic peptide is an integrative hormone with osteotropic effects. *Mol Cell Endocrinol* 2001;177:35–41.
 218. Xie D, Cheng H, Hamrick M, Zhong Q, Ding KH, Correa D, Williams S, Mulloy A, Bollag W, Bollag RJ, Runner RR, McPherson JC, Insogna K, Isales CM. Glucose-dependent insulinotropic

- polypeptide receptor knockout mice have altered bone turnover. *Bone* 2005;37:759–769.
219. Zhong Q, Itokawa T, Sridhar S, Ding KH, Xie D, Kang B, Bollag WB, Bollag RJ, Hamrick M, Insogna K, Isaacs CM. Effects of glucose-dependent insulinotropic peptide on osteoclast function. *Am J Physiol* 2007;292:E543–E548.
 220. Henriksen DB, Alexandersen P, Bjarnason NH, Vilsboll T, Hartmann B, Henriksen EE, Byrjalsen I, Krarup T, Holst JJ, Christiansen C. Role of gastrointestinal hormones in postprandial reduction of bone resorption. *J Bone Miner Res* 2003;18:2180–2189.
 221. Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W. Lack of effect of synthetic human gastric inhibitory polypeptide and glucagon-like peptide 1 [7-36 amide] infused at near-physiological concentrations on pentagastrin-stimulated gastric acid secretion in normal human subjects. *Digestion* 1992;52:214–221.
 222. Mazzocchi G, Rebuffat P, Meneghelli V, Malendowicz LK, Tortorella C, Gottardo G, Nussdorfer G. Gastric inhibitory polypeptide stimulates glucocorticoid secretion in rats, acting through specific receptors coupled with the adenylate cyclase-dependent signaling pathway. *Peptides* 1999;20:589–594.
 223. Lacroix A, Bolte E, Tremblay J, Dupre J, Poitras P, Fournier H, Garon J, Garrel D, Bayard F, Taillefer R. Gastric inhibitory polypeptide-dependent cortisol hypersecretion—a new cause of Cushing's syndrome. *N Engl J Med* 1992;327:974–980.
 224. Zhong Q, Bollag RJ, Dransfield DT, Gasalla-Herraiz J, Ding KH, Min L, Isaacs CM. Glucose-dependent insulinotropic peptide signaling pathways in endothelial cells. *Peptides* 2000;21:1427–1432.
 225. Todd JF, Edwards CM, Ghatei MA, Mather HM, Bloom SR. Subcutaneous glucagon-like peptide-1 improves postprandial glycaemic control over a 3-week period in patients with early type 2 diabetes. *Clin Sci (Colch)* 1998;95:325–329.
 226. Meneilly GS, McIntosh CH, Pederson RA, Habener JF, Gingerich R, Egan JM, Elahi D. Glucagon-like peptide-1 (7-37) augments insulin release in elderly patients with diabetes. *Diabetes Care* 2001;24:964–965.
 227. 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* 1992;267:7402–7405.
 228. Fineman MS, Bicsak TA, Shen LZ, Taylor K, Gaines E, Varns A, Kim D, Baron AD. Effect on glycaemic control of exenatide (synthetic exendin-4) additive to existing metformin and/or sulfonylurea treatment in patients with type 2 diabetes. *Diabetes Care* 2003;26:2370–2377.
 229. DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycaemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 2005;28:1092–1100.
 230. Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycaemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care* 2004;27:2628–2635.
 231. Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycaemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. *Diabetes Care* 2005;28:1083–1091.
 232. Heine RJ, Van Gaal LF, Johns D, Mihm MJ, Widell MH, Brodows RG. Exenatide versus insulin glargine in patients with suboptimally controlled type 2 diabetes: a randomized trial. *Ann Intern Med* 2005;143:559–569.
 233. Nauck MA, Duran S, Kim D, Johns D, Northrup J, Festa A, Brodows R, Trautmann M. A comparison of twice-daily exenatide and biphasic insulin aspart in patients with type 2 diabetes who were suboptimally controlled with sulfonylurea and metformin: a non-inferiority study. *Diabetologia* 2007;50:259–267.
 234. Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, Rungby J, Landau BR, Schmitz O. One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycaemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. *Diabetes* 2004;53:1187–1194.
 235. Nauck MA, Hompesch M, Filipczak R, Le TD, Zdravkovic M, Gumprecht J. Five weeks of treatment with the GLP-1 analogue liraglutide improves glycaemic control and lowers body weight in subjects with type 2 diabetes. *Exp Clin Endocrinol Diabetes* 2006;114:417–423.
 236. Gedulin BR, Smith P, Prickett KS, Tryon M, Barnhill S, Reynolds J, Nielsen LL, Parkes DG, Young AA. Dose-response for glycaemic and metabolic changes 28 days after single injection of long-acting release exenatide in diabetic fatty Zucker rats. *Diabetologia* 2005;48:1380–1385.
 237. Nagakura T, Yasuda N, Yamazaki K, Ikuta H, Yoshikawa S, Asano O, Tanaka I. Improved glucose tolerance via enhanced glucose-dependent insulin secretion in dipeptidyl peptidase IV-deficient Fischer rats. *Biochem Biophys Res Commun* 2001;284:501–506.
 238. Conarello SL, Li Z, Ronan J, Roy RS, Zhu L, Jiang G, Liu F, Woods J, Zycband E, Moller DE, Thornberry NA, Zhang BB. Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc Natl Acad Sci U S A* 2003;100:6825–6830.
 239. Mu J, Woods J, Zhou YP, Roy RS, Li Z, Zycband E, Feng Y, Zhu L, Li C, Howard AD, Moller DE, Thornberry NA, Zhang BB. Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analogue preserves pancreatic β -cell mass and function in a rodent model of type 2 diabetes. *Diabetes* 2006;55:1695–1704.
 240. Ahren B, Hughes TE. Inhibition of dipeptidyl peptidase-4 augments insulin secretion in response to exogenously administered glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, pituitary adenylate cyclase-activating polypeptide, and gastrin-releasing peptide in mice. *Endocrinology* 2005;146:2055–2059.
 241. Hansotia T, Baggio LL, Delmeire D, Hinke SA, Yamada Y, Tsukiyama K, Seino Y, Holst JJ, Schuit F, Drucker DJ. Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. *Diabetes* 2004;53:1326–1335.
 242. Ahren B, Simonsson E, Larsson H, Landin-Olsson M, Torgeirsson H, Jansson PA, Sandqvist M, Bavenholm P, Efendic S, Eriksson JW, Dickinson S, Holmes D. Inhibition of dipeptidyl peptidase IV improves metabolic control over a 4-week study period in type 2 diabetes. *Diabetes Care* 2002;25:869–875.
 243. Ahren B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase-4 reduces glycaemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab* 2004;89:2078–2084.
 244. Charbonnel B, Karasik A, Liu J, Wu M, Meininger G. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled with metformin alone. *Diabetes Care* 2006;29:2638–2643.
 245. Aschner P, Kipnes MS, Lunceford JK, Sanchez M, Mickel C, Williams-Herman DE. Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycaemic control in patients with type 2 diabetes. *Diabetes Care* 2006;29:2632–2637.
 246. Raz I, Hanefeld M, Xu L, Caria C, Williams-Herman D, Khatami H. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy in patients with type 2 diabetes mellitus. *Diabetologia* 2006;49:2564–2571.

247. Rosenstock J, Brazg R, Andryuk PJ, Lu K, Stein P. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing pioglitazone therapy in patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther* 2006;28:1556–1568.

Received December 29, 2006. Accepted January 12, 2007.

Address requests for reprints to: Daniel J. Drucker, MD, Samuel Lunenfeld Research Institute, Room 975C, Mount Sinai Hospital, 600

University Avenue, Toronto, Ontario, Canada M5G 1X5. e-mail: d.drucker@utoronto.ca; fax: (416) 361-2669.

Dr Drucker has served as an advisor or consultant within the past 12 months to Amgen Inc., Amylin Pharmaceuticals, Arisaph Pharmaceuticals Inc., Bayer Inc., Chugai Inc., Conjuchem Inc., Eli Lilly Inc., Glaxo Smith Kline, Glenmark Pharmaceuticals, Johnson & Johnson, Merck Research Laboratories, Merck Fr., Novartis Pharmaceuticals, NPS Pharmaceuticals Inc., Phenomix Inc., Takeda, and Transition Pharmaceuticals Inc. Dr Baggio has served as a consultant to Merck.