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The Role of Incretins in Glucose Homeostasis and Diabetes Treatment

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Abstract

Incretins are gut hormones that are secreted from enteroendocrine cells into the blood within minutes after eating. One of their many physiological roles is to regulate the amount of insulin that is secreted after eating. In this manner, as well as others to be described in this review, their final common raison d'être is to aid in disposal of the products of digestion. There are two incretins, known as glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), that share many common actions in the pancreas but have distinct actions outside of the pancreas. Both incretins are rapidly deactivated by an enzyme called dipeptidyl peptidase 4 (DPP4). A lack of secretion of incretins or an increase in their clearance are not pathogenic factors in diabetes. However, in type 2 diabetes (T2DM), GIP no longer modulates glucose-dependent insulin secretion, even at supraphysiological (pharmacological) plasma levels, and therefore GIP incompetence is detrimental to β -cell function, especially after eating. GLP-1, on the other hand, is still insulinotropic in T2DM, and this has led to the development of compounds that activate the GLP-1 receptor with a view to improving insulin secretion. Since 2005, two new classes of drugs based on incretin action have been approved for lowering blood glucose levels in T2DM: an incretin mimetic (exenatide, which is a potent long-acting agonist of the GLP-1 receptor) and an incretin enhancer (sitagliptin, which is a DPP4 inhibitor). Exenatide is injected subcutaneously twice daily and its use leads to lower blood glucose and higher insulin levels, especially in the fed state. There is glucose-dependency to its insulin secretory capacity, making it unlikely to cause low blood sugars (hypoglycemia). DPP4 inhibitors are orally active and they increase endogenous blood levels of active incretins, thus leading to prolonged incretin action. The elevated levels of GLP-1 are thought to be the mechanism underlying their blood glucose-lowering effects.

I. Background and Introduction

Incretins are hormones that are released from the gut into the bloodstream in response to ingestion of food, and they then modulate the insulin secretory response to the products within the nutrients in the food. The insulin secretory response of incretins, called the incretin effect, accounts for at least 50% of the total insulin secreted after oral glucose. Therefore, by definition, incretin hormones are insulinotropic (i.e., they induce insulin secretion) at usual physiological concentrations seen in the plasma after ingestion. The concept of incretins is at least a century old (Table 1). In 1902, Bayliss and Starling published their landmark manuscript, "The Mechanism of Pancreatic Secretion." The authors found that acid infused into the digestive system caused pancreatic secretion of juices through the pancreatic duct from the pancreas, even after they cut the innervation to the intestine. Until that time, it was thought that nervous system signals controlled secretion of pancreatic juices. They carried out ground-breaking studies that led them to conclude that the nature of the signal to the pancreas was most likely

a chemical stimulus: they removed extracts from the intestinal wall after it had been stimulated by acid, injected the extracts into the bloodstream, and once again they could see juices coming from the pancreatic duct of the animal that had been injected. Therefore, they proved that the extracts must have contained a substance that must normally be secreted from the intestinal wall into the bloodstream to stimulate the flow of pancreatic juice. They called the substance “secretin.” In his “Cronian Lectures,” Starling introduced the word “hormone” (derived from the Greek word meaning “impetus”) for clinical factors that are released from one site and act on another (Starling, 1905). The example of this was that the intestinal extracts contained secretin, which induced obvious “exocrine” secretion of pancreatic juices. Moore wrote in 1906 that Bayliss and Starling considered the possibility that the duodenum also supplied a chemical excitant for the “internal” secretion of the pancreas. They wrote: “This line of argument seems to have occurred to the discoverers of secretin themselves, for Starling mentions a case of diabetes which was tested by Spriggs by injections of secretin solutions but with negative results.” Moore (1906) also described experiments carried out on individual young diabetic patients to whom he gave, by mouth, extracts of intestinal mucosa. This is therefore the first attempt at “incretin-based” therapies for treating diabetes, although, of course, the investigator did not call it that. He reported achieving some success, but his experiments were essentially doomed, because we now know that the chemical excitants are peptides that would have been degraded when given orally. After World War I ended and insulin was extracted from pancreatic islets by Banting and Best in 1921, there was further work on the possibility of food entering the gut leading to secretion of an excitant into the bloodstream that would ultimately lead to insulin secretion and lowering of blood glucose. Different groups published various and often contradictory results from experiments on the effects of extracts of duodenal mucosa on fasting blood glucose and/or on hyperglycemia induced by injection or ingestion of glucose. In 1932, La Barre used the word “incretin” to refer to an extract from upper gut mucosa that produces hypoglycemia but does not induce exocrine secretion, although he did not prove incontrovertibly that incretins existed. Progress on the incretin concept was rapidly made once radioimmunoassays for insulin became available. Between 1964 and 1967, at least three groups showed independently that glucose, given orally, induced a greater insulin response (by radioimmunoassay) than i.v. glucose injection even if the blood glucose levels attained were higher because of the i.v. glucose (Elrick et al., 1964; McIntyre et al., 1964; Perley and Kipnis, 1967). The three groups therefore knew that the oral glucose was indeed inducing release of “incretins” into the bloodstream that subsequently increased insulin secretion, more than did glucose itself. In 1971, John C. Brown isolated and deduced the amino acid structure of a peptide he had isolated from intestinal mucosa (Brown and Dryburgh, 1971). Exogenous administration of the peptide inhibited gastric acid secretion in dogs, so he called it gastric inhibitory polypeptide (GIP)¹ (Brown and Dryburgh, 1971). Brown and colleagues subsequently found that it had insulinotropic properties and suggested that it be called glucose-dependent insulinotropic peptide, retaining the acronym GIP (Dupre et al., 1973). They not only demonstrated GIP to be insulinotropic but also demonstrated the glucose dependence of

¹Abbreviations: 8-pT-2'-O-Me-cAMP, 8-(4-chlorophenylthio)-2'-O-methyladenosine-3',5'-cyclic monophosphate; AA, arachidonic acid; AP, area postrema; AR231453, 2-fluoro-4-methanesulfonyl-phenyl)-{6-[4-(3-isopropyl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-5-nitro-pyrimidin-4-yl}-amine; BBB, blood-brain barrier; bp, base pair(s); CNS, central nervous system; CRE, cAMP responsive element; CT, COOH-terminal tail; DPP4, dipeptidyl peptidase 4; DUSP14, dual-specificity phosphatase 14; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; Ex-4, exendin-4; FDA, Food and Drug Administration; FPG, fasting plasma glucose; GI, gastrointestinal; GIP, glucose-dependent insulinotropic peptide; GIPR, glucose-dependent insulinotropic peptide receptor; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; GLP-2, glucagon-like peptide-2; GLUT2, glucose transporter2; GPCR, G protein-coupled receptor; H89, N-[2-(4-bromocinnamylamino)ethyl]-5-iso-quinoline; HbA_{1c}, hemoglobin A_{1c}; IP₃R, inositol 1,4,5 triphosphate receptor; kb, kilobase pair(s); LY294002, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one; MAPK, mitogen-activated protein kinase; NEP, neutral endopeptidase; NFAT, nuclear factor of activated T cells; NTS, nucleus of the solitary tract; PC, proconvertase; PDX-1, pancreatic duodenal homeobox-1; PI-3K, phosphatidylinositol-3 kinase; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; PLA2, phospholipase A2; PPG, postprandial plasma glucose; RyR, ryanodine receptor; STZ, streptozotocin; T2DM, type 2 diabetes; TCF7L2, T-cell factor-like 2; TNF, tumor necrosis factor; TZD, thiazolidinedione; U0126, 1,4-diamino-2,3-dicyano-1,4-bis-(methylthio)butadiene; ZDF, diabetic-fatty Zucker.

the insulinotropic activity; i.e., plasma glucose must be elevated in order for GIP to induce insulin secretion. GIP was therefore the first incretin to be isolated and its properties characterized.

As late as 1985, another peptide produced in gut, glucagon-like peptide-1 (GLP-1) (7–36), was found to be a fragment of the recently sequenced proglucagon molecule and was shown to be a potent insulinotropic factor (Schmidt et al., 1985). It was therefore the second and final incretin to be characterized. As with GIP, glucose dependence of its insulin-secreting capacity also applies to GLP-1. GIP and GLP-1 together account for the full incretin effect (Fig. 1). Subsequently, clinical investigators demonstrated that GLP-1 could increase insulin secretion and normalize blood glucose in subjects with type 2 diabetes mellitus (T2DM) when given intravenously so as to raise plasma GLP-1 to supraphysiological levels: supraphysiological levels of GIP did not do so, and thus were sown the seeds of new therapies based on the actions of GLP-1 for treating T2DM.

II. General Description of Incretins

A. Glucose-Dependent Insulinotropic Peptide

The first incretin hormone described, GIP, is a single 42-amino acid peptide derived from the post-translational processing of a 153-amino acid precursor encoded by the *gip* gene (Takeda et al., 1987) (Fig. 2) and a member of a family of structurally related hormones that includes secretin, glucagon, and vasoactive intestinal peptide. GIP was originally observed to inhibit gastric acid secretion and gastrointestinal (GI) motility in dogs, predominantly at supraphysiological dosages (Brown et al., 1975). Other exciting studies uncovered that GIP exerts glucose-dependent stimulatory effects on insulin secretion, thereby ensuring prompt insulin-mediated uptake of glucose into tissues. Furthermore, this action occurred at physiological plasma levels of GIP (Dupre et al., 1973). It is synthesized in and released in response to nutrients from enteroendocrine cells (called K cells) primarily in the proximal small intestine (duodenum and jejunum). In the fasted state, circulating levels of GIP are low relative to levels attained after eating, and GIP release into the bloodstream is then stimulated by food ingestion containing glucose or fat (Dupre et al., 1973; Pederson et al., 1975; Elliott et al., 1993). Oral fat alone (e.g., oral corn oil), without any carbohydrate being present, induces GIP secretion, but this is not sufficient to stimulate insulin secretion at fasting glucose concentrations, indicating that the effects of GIP on insulin release do not occur if plasma levels of glucose are also not concurrently increasing; i.e., GIP-mediated insulin secretion is glucose-dependent (Ross and Dupre, 1978). GIP achieves its insulinotropic effects by binding to its specific receptor (GIPR), which is positively coupled to increases in intracellular cAMP and Ca²⁺ levels in β cells. In addition to being insulinotropic, GIP is involved in fat metabolism in adipocytes: it enhances insulin-stimulated incorporation of fatty acids into triglycerides, stimulates lipoprotein lipase activity, modulates fatty acid synthesis (Yip and Wolfe, 2000), and promotes β -cell proliferation and cell survival (Trümper et al., 2001, 2002). GIP is degraded very quickly by dipeptidyl peptidase 4 (DPP4). In T2DM, plasma concentrations are reported to be normal or increased (Ross et al., 1977; Vilsbøll et al., 2001), but the insulinotropic effect is deficient. Although the mechanisms underlying the reduced β -cell response to GIP remain unclear, more recent studies suggest that hyperglycemia alters the physiological response as a result of down-regulation of GIPR expression/activity (Lynn et al., 2001; Zhou et al., 2007). Therefore, GIP/GIPRs have not become targets for treating T2DM.

B. Glucagon-Like Peptide-1

GLP-1, deduced during cloning and characterization of the *proglucagon* gene (Bell et al., 1983), is a post-translational cleavage product of the *proglucagon* gene by the prohormone convertase PC1/3 (Zhu et al., 2002; Ugleholdt et al., 2004) (Fig. 2) and is a second peptide

with incretin activity that potently stimulates glucose-dependent insulin secretion (Schmidt et al., 1985). In addition to encoding glucagon, the *proglucagon* gene encodes two glucagon-like peptides that have approximately 50% amino acid homology to glucagon; these are designated GLP-1 and glucagon-like peptide-2 (GLP-2, which is not insulinotropic, has no glucose-lowering properties, and is therefore not an incretin). These two peptides are mainly produced in enteroendocrine L cells that are scattered among the enterocytes throughout the small bowel and ascending colon, where they are secreted into the bloodstream in response to nutrient ingestion (Doyle and Egan, 2007; Jang et al., 2007). In humans, GLP-1 exists in multiple forms. The majority (at least 80%) of circulating biologically active GLP-1 in humans is the COOH-terminally amidated form, GLP-1 (7–36) amide, with lesser amounts of the minor glycine extended form, GLP-1 (7–37), also detectable (Orskov et al., 1986). Like GIP, GLP-1 achieves its insulinotropic effects by binding to its specific receptor (GLP-1R) that is positively coupled to increases in intracellular cAMP and Ca²⁺ levels in β cells. In addition to its insulinotropic effects, it inhibits gastric emptying, decreases food intake (Willms et al., 1996), inhibits glucagon secretion (Komatsu et al., 1989), and slows the rate of endogenous glucose production (Prigeon et al., 2003), all of which should help to lower blood glucose in T2DM. It has also been shown to protect β cells from apoptosis (Farilla et al., 2002) and to stimulate β -cell proliferation by up-regulation of the β -cell transcription factor pancreatic duodenal homeobox-1 protein (PDX-1) (Perfetti et al., 2000; Stoffers et al., 2000), which is known to augment *insulin* gene transcription and up-regulate glucokinase and glucose transporter2 (GLUT2) (Wang et al., 1999). There is no evidence that defective secretion of GLP-1 is a cause of T2DM (further discussed in depth in section III.C). Continuous GLP-1 treatment in T2DM can normalize blood glucose, improve β -cell function, and restore first-phase insulin secretion and “glucose competence” to β cells (Holz et al., 1993; Zander et al., 2002); hence, GLP-1/GLP-1Rs are therapeutic targets for treating T2DM. Continuous GLP-1 administration is required for maintenance of glucose homeostasis because of its short half-life (1.5–2 min); similar to GIP, it is degraded by DPP4. The following sections provide our current understanding of the physiology, the therapeutic potential, and the targets of incretin-based therapies.

III. Synthesis, Secretion, and Degradation of Incretins

A. Synthesis, Secretion, and Degradation of Glucose-Dependent Insulinotropic Peptide

1. Pro-Glucose-Dependent Insulinotropic Peptide Gene Structure, Expression and Post-Translational Processing—As already stated, GIP is synthesized within and secreted from K cells in the small intestine, the highest density of K cells being in duodenum and jejunum; few, if any, K cells are present in distal ileum (Buffa et al., 1975; Buchan et al., 1978). Human GIP is a single 42-amino acid peptide derived from the processing by PC1/3 of proGIP, a 153-amino acid precursor (Fig. 2) that is encoded by a 459-bp open reading frame and whose gene is localized to chromosome 17q. It is composed of six exons, and the majority of GIP-encoding sequences are in exon 3 (reviewed extensively in Fehmann et al., 1995). This sequence includes a 51-amino acid N-terminal peptide containing a signal peptide with a cleavage site at glycine and a 60-amino acid C-terminal peptide flanking the 42-amino acid GIP hormone, which presently seems to be the only biologically active peptide derived from proGIP (Fig. 2). The rat GIP cDNA has a 432-bp open reading frame encoding a 144-amino acid polypeptide, and the figure is similar but not identical to the human gene (Higashimoto et al., 1992). There is a >90% amino acid homology of GIP between human, porcine, bovine, mouse, and rat. Expression of the *gip* gene in the gut is regulated by nutrients (Tseng et al., 1994; Kieffer et al., 1995). Glucose and lipid administrations into the rat GI tract increase GIP mRNA levels (Higashimoto et al., 1995). Likewise, Northern blot analysis and radioimmunoassays have shown that duodenal GIP mRNA, as well as serum and tissue peptide levels, increase in strepto-zotocin (STZ)-induced diabetic rats, suggesting that hyperglycemia

can up-regulate *gip* gene expression; STZ, which is a β -cell toxin often used to cause insulin-dependent diabetes for research purposes and to screen for compounds that might have protective effects on β -cell apoptosis, produces a progressive increase in blood glucose levels over time as β cells die. Studies of the rodent *gip* promoter show that the first 193 bp upstream of the transcription initiation site are sufficient to direct specific expression through the binding of transcription factors that include GATA-4, Isl-1, and PDX-1 (Boylan et al., 1997; Jepeal et al., 2003, 2005, 2008). GATA-4 binds to a consensus motif for the GATA family, located between bp -193 and -182, that is highly conserved across rat, mouse, and human species and Isl-1 binds to a second *cis*-regulatory region (between bp -156 and -151) downstream of the GATA-4 binding site. Isl-1 seems to function in conjunction with GATA-4 to promote *gip* gene expression (Jepeal et al., 2003). The essential β -cell transcription factor, PDX-1, also is essential to direct cell-specific expression of GIP within K cells. In the embryo, PDX-1 is required for pancreatic development and ultimately for development of the β cells within it. The *pdx1* gene is first expressed in a well delineated domain in the foregut that gives rise to the pancreas; this is the same area that contains the GIP-expressing cells (Offield et al., 1996). PDX-1 protein is expressed in the nucleus of GIP-expressing K cells in both embryos and adult mice, and PDX-1(-/-) mice have a greater than 90% reduction in GIP-expressing cells compared with wild-type mice in the foregut mucosa at embryonal day 18.5 (Jepeal et al., 2005). The number of some types of enteroendocrine cells, such as gastrin-expressing cells, was also reported reduced in PDX-1(-/-) newborn mice; however, GIP-expressing cells were not specifically quantified, but presumably because they were so severely reduced in the embryo, they would also be reduced in the newborn (Larsson et al., 1996; Offield et al., 1996). Other experiments performed on transgenic mice also point to the necessity of PDX-1 for the full complement of GIP-expressing cells (Boyer et al., 2006), illustrating the importance of PDX-1 not only for its well known effects on pancreatic and β -cell development, but also for K-cell development. PDX-1 is capable of binding to the second *cis*-regulatory element located between bp -156 and -151 of the *gip* promoter, and overexpression of PDX-1 in transient transfection assays leads to a specific increase in the activity of *gip*/Luc reporter constructs (Jepeal et al., 2005). In addition, the human *gip* gene promoter contains potential binding sites for a number of transcription factors: these include Sp1, activator proteins-1 and -2, and DNase I footprinting with the recombinant cAMP responsive element (CRE) binding proteins. Mutation analyses actually show that this promoter contains two CREs that are required for basal promoter activity (Someya et al., 1993). Pax6 is another transcription factor that is essential for GIP expression as shown by the fact that its deletion prevented K cell development as well as many other classic gut hormones (Larsson et al., 1998). There are reports in the literature that some enteroendocrine cells contain both GIP and *proglucagon* gene products (so called K/L cells) in rodents and humans (Mortensen et al., 2003; Theodorakis et al., 2006) and in those cells PDX-1 is also expressed. In a report by Fujita et al. (2008), the investigators found that Pax6 and PDX-1 are both expressed in K/L cells and they bind to the proximal sequence (-184 to -145 bp) of the human *gip* promoter. *gip* promoter activity was enhanced by exogenous Pax6 or PDX-1 and diminished by inhibition of endogenous Pax6 or PDX-1 by dominant-negative forms.

It is noteworthy that the rat salivary gland also expresses the *gip* gene (Tseng et al., 1993); other species have not been reported on. However, based on feeding experiments in humans, there is indirect evidence that GIP is produced in salivary glands and its secretion into saliva is reduced after feeding, as opposed to small bowel-derived GIP, whose secretion increases after feeding (Messenger et al., 2003) (there is no evidence that proglucagon or any of its usual products are produced in salivary glands). Lens epithelial cells, again in rats, are reported to contain the *gip* gene (Nakajima et al., 2002); finally, a subpopulation of adult rat hippocampal cells express both the *gip* gene and protein, where it may have a role in proliferation of progenitor cell turnover (Nyberg et al., 2005).

Proconvertases (PCs) are endoproteinases that cleave prohormones to biologically active ones and studies using specific PC-null mice and cell lines using adenovirus-mediated overexpression of PC enzymes indicate that PC1/3 is both essential and sufficient for the cleavage of proGIP to the biologically active 42-amino acid peptide; it obviously colocalizes with GIP in K cells (Ugleholdt et al., 2006).

2. Glucose-Dependent Insulinotropic Peptide Secretion and Degradation—GIP secretion from the gut occurs in a regulated manner; circulating levels of GIP are low in the fasted state and rise within minutes of food ingestion. Amino acids are weak stimuli. Oral or intraduodenal infusion of glucose and/or fat increases GIP secretion in a dose-dependent manner (Schirra et al., 1996). The degree to which nutrients regulate GIP secretion is species-dependent because fat is a more potent stimulator of GIP secretion than carbohydrates in humans, whereas, in the rodent and pig, carbohydrates are more potent than fat (Baggio and Drucker, 2007). Sucrose, galactose, and fructose also stimulate GIP secretion, although mannose does not (Flatt et al., 1989). The postprandial level of circulating GIP is dependent on meal size (Hampton et al., 1986; Vilsbøll et al., 2003c). GIP secretion is reduced in patients with intestinal malabsorption (Besterman et al., 1978, 1979), presumably as a result of insufficient K cells, as well as in chronic pancreatitis (Ebert et al., 1976). GIP secretion coupling pathways are poorly understood and have not been studied to the same extent as has secretion from L cells, because GIP has not become a therapeutic target for T2DM, but presumably elevation of intracellular Ca^{2+} levels is required. In addition, some K cells contain sweet receptors, which are G protein-coupled (GPCRs), and their activation by sugars and sweeteners may lead to GIP secretion (Jang et al., 2007; Egan and Margolskee, 2008). Other GPCRs that are activated by fats and may be responsible for initiating GIP secretion are being described as mechanisms by which GLP-1 is secreted are being studied, and these will be discussed below in section III.B.2.

GIP secretion in response to oral glucose ingestion or different test meals has been quantified in numerous studies. In humans, fasting plasma GIP levels, assayed from peripheral veins, are approximately 9 to 11 pM (total GIP) and peak plasma concentrations of 50 to 120 pM are achieved after eating, depending on health status of the subject and the amount and quality of the food consumed (Vilsbøll et al., 2001).

Once released, GIP is subject to degradation by DPP4, which is bound to lymphocytes (where it is called CD-26) and endothelial cells of blood vessels of gut and liver as well as being present in soluble form in the circulation. The first two amino acids (Tyr Ala) at the N terminus of full-length GIP (1–42) are cleaved in 1 to 2 min in rodents and 5 to 7 min in humans by DPP4 and converted to GIP (3–42), which has insignificant, if any, insulinotropic activity (Kieffer et al., 1995; Deacon et al., 2000). It is then excreted by the kidney. The elimination rates of GIP (1–42) and GIP (3–42) are similar in subjects with T2DM and those without (Vilsbøll et al., 2006); hence, more rapid degradation/elimination of GIP is unlikely to be a factor in defective insulinotropic effects seen in T2DM.

B. Synthesis, Secretion and Degradation of Glucagon-Like Peptide-1

1. Proglucagon Gene Structure and Expression—The human *proglucagon* gene located on the long arm of chromosome 2 has six exons, of which exons 2 to 5 encode distinct functional domains (Bell, 1986), and five introns. It spans approximately 9.4 kb (White and Saunders, 1986). Just a single gene encodes the proglucagon sequence in mammals and proglucagon, 180 amino acids long, is very similar in all mammalian species (greater than 90% amino acid sequence homology). Glucagon is encoded in exon 3, and GLP-1 and -2 are encoded in exons 4 and 5, respectively (White and Saunders, 1986). A single size structurally identical mRNA transcript is produced in all tissues that contain proglucagon (Novak et al., 1987;

Drucker and Asa, 1988). These tissues are the L cells of the intestine, the α cells of islets of Langerhans, some taste cells in the tongue, and some neurons in the brainstem and hypothalamus (Drucker and Asa, 1988; Eissele et al., 1992; Fehmann et al., 1995; Shin et al., 2008).

It seems that the 5'-flanking regions of human and rat *proglucagon* genes are essential for their tissue-specific expression in the pancreas, brain, and intestine (White and Saunders, 1986; Lee et al., 1992; Jin and Drucker, 1995; Nian et al., 1999). Indeed, the DNA sequence located between -1.3 and -2.3 kb of rat *proglucagon* promoter, the *proglucagon* gene upstream enhancer element (GUE), contains multiple *cis*-acting positive and negative transcriptional elements contributing to the transcriptional control of *proglucagon* gene expression in intestinal L cells (Jin and Drucker, 1995), and initial studies of *proglucagon* gene expression using transgenic mice have shown that -1.3 kb of 5'-flanking sequences are responsible for the pancreatic α cell- and brain-specific expression of rat *proglucagon* gene (Efrat et al., 1988). Further studies identified the first 300 bp of the 5'-flanking region of the *proglucagon* gene as containing a minimum promoter region (G1) and four enhancer elements (G2-G5) contributing to the pancreatic α cell specificity of *glucagon* gene expression (Philippe et al., 1988; Herzog et al., 2000), via the binding to these elements to a cocktail of transcription factors, including Isl-1, Pax6, Cdx2/3, Brn4, Pbx, Foxa1, and c-Maf. Study of the human *proglucagon* gene transcription using transfected reporter genes and cell lines in vitro and transgenic mice in vivo has indicated that -1.6 kb of the 5'-flanking region of human *proglucagon* promoter is required for *proglucagon* gene transcription in the brain and intestine, but not in pancreatic islets, whereas -6 kb of human *proglucagon* promoter are required for expression in islet cell lines (Nian et al., 1999).

A typical CRE also exists in the 5'-flanking region of the rat *proglucagon* gene (Philippe et al., 1988), and increased levels of cAMP stimulate *proglucagon* gene expression in both pancreatic α cells and intestinal L cells via protein kinase A (PKA)- or Epac/MAPK-dependent pathway (Knepel et al., 1990; Drucker et al., 1994; Brubaker et al., 1998; Lotfi et al., 2006; Jin, 2008). In humans, however, whether cAMP signaling is capable of stimulating human *proglucagon* gene expression is unknown. Pax6, a critical determinant of islet cell development (Habener et al., 2005), as well as K cell development, as described in section III.A.1, also activates *proglucagon* gene transcription in the intestine and pancreas, via binding to the G1, G3, and G5 elements in the *proglucagon* promoter (Jin, 2008). This is supported by the following two observations: 1) the dominant-negative (*SEY^{Neu}*) form of Pax6 in mice leads to marked reduction of *proglucagon* mRNA transcripts in the intestine and pancreatic α cells (Sander et al., 1997), and neither GLP-1 nor GLP-2-immunopositive enteroendocrine cells are detected in the intestinal mucosa (Hill et al., 1999); 2) adenoviral-expressed Pax6 activated both *proglucagon* promoter-luciferase activity and expression of the endogenous *proglucagon* gene in enteroendocrine cell lines, and furthermore, increased levels of endogenous *proglucagon* gene expression were observed in primary rat intestinal cell cultures in vitro, and in rat colonic epithelium in vivo, after adenoviral-mediated overexpression of Pax-6 (Trinh et al., 2003). Similar to α cells in islets, L cells do not express PDX-1: only the K/L cells require it, as well as Pax6 (Fujita et al., 2008). Recent studies have indicated that the Wnt signaling pathway via β -catenin/T-cell factor-4, the major effector of the Wnt signaling pathway, is a potent mediator of *proglucagon* gene expression and GLP-1 production in L cells but not in islets (Ni et al., 2003; Yi et al., 2005). In these studies, *proglucagon* gene expression and GLP-1 production were activated by inhibition of glycogen synthase kinase-3 β , a major negative modulator of the Wnt pathway, and by β -catenin overexpression in L cells but not in islets, and this effect was mediated by binding of the transcription factor TCF-4 to the *proglucagon* gene promoter G2 enhancer that is abundantly expressed in the gut but not in pancreatic islets. More recently, Yi et al. (2008) suggested the existence of cross-talk between the insulin and Wnt signaling pathways and the regulation of *proglucagon* gene expression and GLP-1 production by this

cross-talk in the L cells. Insulin has been shown to repress *proglucagon* gene transcription in the pancreatic islets by regulation of binding of FoxO1 to the G3 element of the *proglucagon* gene promoter (Philippe, 1989, 1991; McKinnon et al., 2006). It is noteworthy that the opposite may be true in enteroendocrine cells of the gut. Insulin was shown to actually activate *proglucagon* gene expression and GLP-1 production in a murine L cell line and in primary rat L cells as well as in the mouse intestine *in vivo*, and this effect of insulin was thought to be mediated by nuclear β -catenin accumulation and binding of β -catenin/T-cell factor-4 to the *proglucagon* gene promoter, suggesting a potential novel function for insulin—namely up-regulation of GLP-1 (and GLP-2) production, via cross-talk with Wnt signaling pathway (Yi et al., 2008). This dichotomy of insulin effect has not been looked for in humans. It also seems that different downstream signaling pathways are in action in the L cells and pancreatic α cells, because protein kinase B (PKB) activity is involved in *proglucagon* gene expression in the α cells (Schinner et al., 2005), but not in the L cell lines (Yi et al., 2008). In an α cell line, orexin-A, a neuropeptide hormone that regulates food intake and energy homeostasis, inhibits *proglucagon* gene expression via activation of the PKB/PI-3K/FoxO1-dependent pathway, and also inhibits glucagon secretion via decreases of intracellular α cell cAMP and Ca^{2+} concentration (Göncz et al., 2008). However, the effect of orexin-A, if any, in the L cells has not been determined. (The players in the regulation of *proglucagon* expression in brain and taste cells are also virtually unknown.)

2. Tissue-Specific Post-Translational Processing of Proglucagon, Product Secretion, and Degradation—Peptide products with different physiological properties in tissues arise as a consequence of tissue-specific, differential, post-translational processing of *proglucagon*, and this allows one protein to serve a variety of functions (Mojsov et al., 1986; Orskov et al., 1986). Seven PCs have been identified (von Eggelkraut-Gottanka and Beck-Sickinger, 2004); two, PC1/3 and PC2, seem localized to only neuroendocrine cells, whereas a third, furin, is found in both neuroendocrine and nonendocrine cells. Recent findings have shown that the expression level of PC2 is high in the pancreatic α cells and absent in the gut, whereas PC1/3 is definitely expressed in gut endocrine cells (Rouillé et al., 1994; Scopsi et al., 1995). The activity of the specific PC ultimately determines entirely different peptide products. It seems that of all the PCs, only PC1/3 and PC2 are essential for *proglucagon* processing (Rothenberg et al., 1995; Rouillé et al., 1995). PC2, in conjunction with 7B2, a chaperone protein that is responsible for maturation of PC2 as well as its enzymatic activity, is responsible for the pancreatic α cell-specific processing of *proglucagon* to glucagon (Rouillé et al., 1995). Consistent with this, mice lacking active PC2 exhibit multiple endocrine disorders, have severely impaired processing of *proglucagon* to glucagon, and consequently become severely hypoglycemic (Furuta et al., 1997). The *proglucagon*-derived peptides from the pancreatic α cells include glucagon, glicentin-related pancreatic peptide, intervening peptide-1, and the major *proglucagon* fragment (Fig. 3). In addition to being present in K cells, where, as stated above, it is required for the cleavage of proGIP, PC1/3 is also present in intestinal L cells and is responsible for the processing of *proglucagon* to GLP-1 (and GLP-2) (Rothenberg et al., 1995; Rouillé et al., 1995) (Fig. 2). In agreement with this, mice with a targeted deletion of the *PC1/3* gene cannot process *proglucagon* to GLP-1 and GLP-2 (Zhu et al., 2002; Ugleholdt et al., 2004). In a human case of PC1/3 deficiency, however (of which there are just three cases described in the literature), the patient suffered from a plethora of endocrinopathies and had some mature GLP-1 in her plasma, indicating a lack of absolute dependence for PC1/3 in humans (Jackson et al., 2003). It is noteworthy that Wideman et al. (2006) recently demonstrated that up-regulation of PC1/3 expression using adenovirus system in pancreatic α cells leads to increase of islet GLP-1 secretion, resulting in improved glucose-stimulated insulin secretion and enhanced survival of islets in response to cytokine treatment. Similar effects have been observed also in PC1/3-expressing α cells derived from mice lacking active PC2 (Wideman et al., 2007). Moreover, this ability of PC1/3-expressing α cells was attenuated

in *GLP-1R(-/-)* mice and transplantation of PC1/3-expressing α cells prevented STZ-induced hyperglycemia by preserving β -cell area and islet morphology via increased β -cell proliferation (Wideman et al., 2007). Besides GLP-1 and GLP-2, the other proglucagon-derived peptides from the L cells include glicentin, oxyntomodulin, and intervening peptide-2 (Fig. 3). Although proglucagon exists in the brain and processing of proglucagon also occurs (Drucker and Asa, 1988), the PC enzymes have not been specifically studied in brain. In taste cells of the tongue, however, both PC1/3 and PC2 (as well as 7B2) are present; consequently, GLP-1, GLP-2, and glucagon are all present (Shin et al., 2008).

The primary physiological stimuli for the secretion of GLP-1 are fat- and carbohydrate-rich meals, but mixed meals or individual nutrients, including glucose and other sugars, sweeteners, fatty acids, amino acids, and dietary fiber, also can stimulate GLP-1 secretion (Baggio and Drucker, 2007). Although functional GLP-1 has been found in taste buds and brain, the majority if not all of the GLP-1 measured in peripheral blood is synthesized in L cells, stored in granules, and released after eating from the L cells that are distributed throughout the small and large intestine (Eissele et al., 1992). More L cells are located in the distal ileum and colon than in the duodenum and jejunum, in contrast to GIP-secreting K cells. K/L cells seem most abundant in terminal ileum (Fujita et al., 2008). Plasma levels of GLP-1 increase rapidly within just a few minutes after oral glucose in rodents and humans. Meal ingestion results in a biphasic pattern of GLP-1 secretion, with an early phase beginning within 5 to 15 min and a prolonged second phase following within 30 to 60 min (Herrmann et al., 1995). Thus, the early phase and the prolonged second phase of GLP-1 secretion may be due to both direct nutrient contact with the L cells and K/L cells in upper small intestine and by nutrient (most likely fat, as nutrient-derived carbohydrate should have been absorbed before arriving in the ileum), neural, and other gut-derived (and even non-gut-derived) endocrine factors activating L cells in the distal bowel (Roberge and Brubaker, 1991; Rocca and Brubaker, 1999; De León et al., 2006). It now seems probable that the mechanism by which the early phase of GLP-1 secretion occurs is that sugars activate sweet taste receptors on L and or K/L cells (Theodorakis et al., 2006; Jang et al., 2007). The L cell (as well as the K cell) is an open-type intestinal epithelial endocrine cell that is in direct contact with nutrients (Eissele et al., 1992) through its microvilli on the luminal surface, in contact with the enteric nervous system as well as the central nervous system (CNS) via the vagus, and in contact with the microvasculature through its basolateral membrane (Hansen et al., 1999; Anini et al., 2002). This allows GLP-1 secretion from L cells, as well as GIP from K cells, to be regulated by a variety of nutrient, neural, and endocrine signals. Indeed, several studies have postulated that GLP-1 secretion from the L cells is regulated by a complex proximal-distal loop that involves both endocrine and neural factors with the vagus nerves having an essential role in this loop (Balks et al., 1997; Rocca and Brubaker, 1999; Anini and Brubaker, 2003a). In this loop also, GIP, acetylcholine, and gastrin-releasing peptide act as mediators: the afferent vagus nerve is activated by GIP, which subsequently stimulates GLP-1 secretion through the efferent vagus nerve and enteric neurons that release acetylcholine and gastrin-releasing peptide (Roberge et al., 1996; Rocca and Brubaker, 1999; Persson et al., 2000; Anini et al., 2002; Anini and Brubaker, 2003a). It seems that GLP-1 secretion is also affected by other neurotransmitters and peptides, including GABA and *calcitonin* gene-related peptide (Brubaker, 1991; Gameiro et al., 2005). Furthermore, non-nutrient factors, including leptin (Anini and Brubaker, 2003b) and insulin (Lim and Brubaker, 2006), have also been identified as stimulators of GLP-1 secretion. Conversely, somatostatin, which is produced from the intestinal enteroendocrine D cells (as well as from δ cells in islets of Langerhans) and whose secretion is increased by GLP-1, has been identified as the inhibitor of GLP-1 secretion, implicating the existence of a negative local feedback loop in the gut (Brubaker, 1991; Hansen et al., 2000; Chisholm and Greenberg, 2002).

Recent studies have uncovered some of the intracellular signaling pathways that mediate nutrient-induced GLP-1 secretion from L cells, which is then most likely modified by the neural

and endocrine factors mentioned above. Several studies show that specific GPCRs present on L cells are necessary for GLP-1 secretion. In particular, long-chain free fatty acids and lipids stimulate GLP-1 secretion through interaction with GPCRs, including GPR120 (Hirasawa et al., 2005), GPR119 (Chu et al., 2008), and GPR40 (Edfalk et al., 2008). GPR120 is highly expressed in the intestine and the stimulation of GPR120 by free fatty acids promotes the secretion of GLP-1 via increase of intracellular Ca^{2+} levels and activation of p42/44 MAPK (Hirasawa et al., 2005). GPR119 mRNA is found in intestinal subregions of humans and mouse and in cultured L cell lines, and in situ hybridization studies also show that most GLP-1-producing cells express GPR119 mRNA. The engagement of GPR119 by phospholipids and fatty acid amides stimulates GLP-1 secretion (Chu et al., 2008). AR231453, a potent GPR119 agonist, stimulated intracellular cAMP accumulation and GLP-1 release from the GLUTag L cell line and, when given by oral gavage before oral glucose, plasma levels of GLP-1 were higher than in those animals not given AR231453. It also increased plasma levels of GIP even though GPR119 was undetectable by in situ hybridization in K cells. Furthermore, the effects of AR231453 on incretin secretion were absent in GPR119-deficient mice (Chu et al., 2008). More recently, Edfalk et al. (2008) suggested that GPR40 modulated FFA-stimulated insulin secretion from β cells not only directly but also indirectly via regulation of incretin secretion. In that study, GPR40 expression was uncovered in endocrine cells of the GI tract as well as in β cells, and PDX-1 was required for its expression in β cells, stomach, and duodenum. However, in distal GI, such as ileum, where PDX-1 is not expressed and where the greatest number of L cells reside, GPR40 expression was also present, indicating that PDX-1 expression is not absolutely necessary for its expression. Secretion of GLP-1 and GIP were both diminished, though not absent, in GPR40-null mutant mice in response to a fat diet (Edfalk et al., 2008). Therefore, it seems likely that many different GPRs present on both K and L cells complement one another to bring about fatty acid-mediated incretin secretion. It is also likely that different GPRs reside in the various parts of the bowel so that fat, in various stages of digestion as it moves through the bowel, can continue to bring about enteroendocrine hormone secretion.

With regard to glucose stimulation of GLP-1 secretion, and analogous to β cells, expression of glucokinase, the glucose sensor in β cells, has been found in the mouse intestinal L cells (Jetton et al., 1994), and GLUT2, the most abundant glucose transporter in β cells, seems to be required for GLP-1 secretion (Cani et al., 2007). In addition, it is likely that both GIPR and GLP-1R are required for the full regulation of oral glucose-induced GLP-1 and GIP secretion (Cani et al., 2007). The regulation of glucose-induced GLP-1 secretion by taste transduction elements was identified by our recent studies. Jang et al. (2007) demonstrated that glucose (and the sucrose analog, sucralose, commonly used as a noncaloric sweetener) led to secretion from L cells via a signaling pathway quite similar to that used by taste cells in the tongue. The human L cell line NCI-H716 and duodenal L cells expressed taste transduction elements, including sweet taste receptors, the taste G protein gustducin, and several other signaling elements. Ingestion of glucose by α -gustducin-null mice revealed deficiencies in secretion of GLP-1 and isolated small bowel and intestinal villi from these mice showed markedly defective GLP-1 secretion in response to glucose. Furthermore, GLP-1 release from NCI-H716 cells was promoted by the sugar sucralose and blocked by the sweet receptor antagonist lactisole. Down-regulation of α -gustducin expression by small interfering RNA also lessened glucose-induced GLP-1 secretion. In conclusion, the L cells of the gut “taste” glucose through the same mechanisms used by taste cells of the tongue, and this has an essential role in glucose-induced GLP-1 secretion (Jang et al., 2007).

Typical basal (fasting) levels of bioactive GLP-1, measured from peripheral veins, are in the range of 5 to 10 pM and increase by 2- to 3-fold after meal ingestion, depending on the size and composition of meal (Elliott et al., 1993). The first two N-terminal amino acids (His Ala) of native GLP-1 are rapidly cleaved by DPP4, and the resulting GLP-1 (9–36) fragment is not insulinotropic (Hansen et al., 1999). GLP-1 is also degraded by neutral endopeptidase 24.11

(NEP-24.11) (Plamboeck et al., 2005), which is a membrane-bound zinc metallopeptidase (Turner et al., 2001), and six potential cleavage sites in GLP-1 have been identified (Hupe-Sodmann et al., 1995). High levels of this enzyme are found in the kidney and GLP-1, and its metabolites are rapidly cleared through the kidneys (Ruiz-Grande et al., 1993), implying the involvement of NEP-24.11 in renal clearance of GLP-1. Therefore, many modifications have been made to synthetic GLP-1 so as to increase its biological half-life and consequently its efficacy *in vivo*, and therapeutic strategies based on modulating GLP-1 levels and GLP-1 activity through administration of GLP-1 and its analogs or by inhibiting its degradation have been tested and/or are under development for treating T2DM. The elimination rates of GLP-1 are similar in T2DM and nondiabetic subjects, and, as with GIP, more rapid degradation of GLP-1 is unlikely to be a contributory cause for glucose-responsiveness in T2DM (Vilsbøll et al., 2003a).

Exenatide, a synthetic form of exendin-4 (Ex-4, a GLP-1R agonist that is naturally synthesized in the salivary glands of the Gila monster lizard and does not possess a DPP4 recognition site), and sitagliptin, a small, orally active DPP4 inhibitor, are now FDA-approved and are being used for lowering blood glucose in T2DM. For example, in the ACCORD study, 12.1% of the intensively treated subjects with diabetes received exenatide (Gerstein et al., 2008).

C. Incretin Secretion in Type 2 Diabetes

T2DM is characterized by a severely impaired or absent GIP insulinotropic effect (Nauck et al., 1986) (to be further discussed in section V) that most likely results in worsening insulin secretion. However, T2DM seems unlikely to result from deficient incretin secretion. One of the reasons frequently given for using exenatide or DPP4 inhibitors is that they lead to “normalization” of incretin levels that are supposedly reduced compared with nondiabetic subjects (Drucker and Nauck, 2006). However, on closer analysis of all the data in print, it is far from certain that incretin secretion is reduced in this condition. For GIP, the data seem to actually favor the opposite conclusion. Based on results obtained in the course of oral glucose tolerance testing and during meal testing, GIP secretion and fasting levels seem to be actually increased, in both the impaired and diabetic state (Theodorakis et al., 2004; Vollmer et al., 2008) (Fig. 4), whereas the insulinotropic effect is almost totally lost in T2DM. Although different radioimmunoassays have been used over the years, most studies seem to agree that the secretion of GIP is normal or even higher in patients with T2DM compared with healthy control subjects (Crockett et al., 1976; Ross et al., 1977; Ebert and Creutzfeldt, 1980; Jones et al., 1989; Vilsbøll et al., 2001).

For GLP-1 secretion in T2DM, the data has been confusing, especially those from earlier studies, although a consensus is finally emerging thanks to studies performed in newly diagnosed subjects and subjects with impaired glucose tolerance before receiving any treatment for glucose control (Vollmer et al., 2008) (Fig. 4). In addition, better assays are now available and better methodologies, such as proper storage of samples and not repeated assaying of plasma samples that have been thawed and refrozen (this leads to degradation of incretins), have lead to more reliable quantification. In general, GLP-1 levels reach maximum secretion 17 to 20 min after oral glucose administration, followed by a slow decline toward fasting levels; unfortunately, many older studies began sampling 30 min after oral glucose, thereby missing peak secretion. In contrast, peak secretion occurs 60 to 90 min after a mixed meal. Data from the Baltimore Longitudinal Study of Aging shows that GLP-1 secretion is not deficient in either the fasting state or after oral glucose in glucose-impaired or diabetic subjects not taking any drugs affecting glucose homeostasis (Fig. 4). Vollmer et al. (2008) actually found a trend toward higher plasma GLP-1 levels in 17 well controlled subjects with T2DM [mean hemoglobin A_{1c} (HbA_{1c}) level of 6.8%] after a mixed meal (Vollmer et al., 2008). HbA_{1c} is a measure used in clinical practice to monitor long-term blood glucose control in diabetes

management. Levels of <7% indicate good control. Therefore, it is evident that T2DM develops in the setting of normal incretin secretion and reduced secretion cannot be evoked as causing the disease. Older studies, using patients with worse metabolic control, on multiple drugs and suffering from diabetes for longer times, have found impairments in GLP-1 secretion (Toft-Nielsen et al., 2001; Vilsbøll et al., 2001) and so chronic hyperglycemia and its metabolic consequences may be the cause of the slightly impaired GLP-1 secretion seen in those earlier studies. In addition, in T2DM, insulin secretion is typically reduced just after ingestion of glucose or a meal (this is called defective early-phase insulin secretion), but studies showing deficient GLP-1 secretion found reductions in secretion at 60 to 150 min, which is well after early-phase insulin secretion has occurred and at a time where there is actually exaggerated insulin secretion; therefore, defective insulin secretion did not coincide in time with defective GLP-1 secretion, so one cannot postulate cause and effect (Vilsbøll et al., 2001). It seems fair therefore to conclude that abnormalities of incretin secretion are unlikely to be a primary pathogenic factor in the development of T2DM and are instead a consequence of the diabetic state (Vaag et al., 1996; Meier et al., 2005; Knop et al., 2007). In addition, it is clear that the incretin effect of GLP-1 in T2DM is better preserved, in contrast to that of GIP (Nauck et al., 1993a), because an infusion of GLP-1 in T2DM so as to reach pharmacologic concentrations in plasma can normalize fasting (Nauck et al., 1993b; Gutniak et al., 1997; Rachman et al., 1997) and postprandial (Gutniak et al., 1997; Rachman et al., 1997; Meier et al., 2003a) glucose concentrations, resulting from increase of glucose-stimulated insulin secretion, decrease of glucagon secretion and slowing of gastric emptying (Gutniak et al., 1992; Nauck et al., 1993b; Holst and Gromada, 2004). Continuous i.v. infusion of GLP-1 also lowers postprandial plasma glucose (PPG) levels in subjects with type 1 diabetes by delaying gastric emptying (Gutniak et al., 1992). These effects of GLP-1 have been consistently shown in a number of human studies (Nathan et al., 1992; Nauck et al., 1993a,b; Elahi et al., 1994). In particular, continuous subcutaneous infusion (4.8 pmol/kg/min) of GLP-1 for 6 weeks in T2DM subjects was associated with significant reductions in both fasting plasma glucose (FPG) and PPG as well as HbA_{1c} with a slight decrease of body weight (Zander et al., 2002). Prolonging the GLP-1 infusion (3.2 pmol/kg/min) for 3 months in patients with T2DM resulted in a restoration of first-phase insulin secretion as well as an improvement of late-phase secretion during a glucose clamp, but no significant changes in body weight and plasma glucagon levels were noted (Meneilly et al., 2003). In another study, repeated i.v. infusion (1 ~ 1.2 pmol/kg/min) of GLP-1 also normalized FPG in patients with T2DM (Nauck et al., 1997). Thus, there has been considerable interest in an incretin-based therapeutic approach for treating T2DM. However, continuous GLP-1 infusion or repeated GLP-1 injections are impractical and expensive ways to lower blood glucose and so the strategies mentioned above have been developed.

IV. Incretin Receptors

A. Glucose-Dependent Insulinotropic Peptide Receptor

The presence of GIPRs was first demonstrated in a transplantable hamster insulinoma (Maletti et al., 1984) and an insulin-secreting hamster β -cell line In111 (Amiranoff et al., 1984, 1985), followed by cloning from rat cerebral cortex cDNA library (Usdin et al., 1993), and subsequently hamster (Yasuda et al., 1994) and human GIPRs (Yamada et al., 1995) were cloned. The gene encoding rat and mouse GIPRs contains 15 exons (Boylan et al., 1999). The human *gipr* gene comprises 14 exons that span approximately 14 kb (Yamada et al., 1995) and is localized to chromosome 19, band q13.3. Its protein exists as two isoforms of 466 and 493 amino acids. Northern blot, reverse transcription/polymerase chain reaction, and in situ hybridization studies have shown that the *gipr* gene is expressed in both α and β cells in pancreatic islets (Moens et al., 1996), GI tract, adipose tissue, adrenal cortex, pituitary, heart, testis, endothelium of major blood vessels, bone, trachea, spleen, thymus, lung, kidney, thyroid, and several brain areas (Usdin et al., 1993; Yasuda et al., 1994; McIntosh et al., 1996; Yip et

al., 1998). Both GIP (1–42) and several GIP fragments, truncated at the N and C termini, bind to the GIPR with high affinity, but none of the related peptides from the glucagon family do so (Wheeler et al., 1995). The GIPR is a glycoprotein belonging to the class II G protein-coupled receptor superfamily that includes receptors for glucagon, GLP-1, secretin, vasoactive intestinal polypeptide, and pituitary adenylyl cyclase-activating protein. As with other GPCRs of this class, GIPR comprises an N-terminal extracellular domain that is essential for high-affinity GIP binding and receptor activation; a central transmembrane domain (the first transmembrane domain of which is important for receptor activation and cAMP coupling); and a C-terminal cytoplasmic domain that mediates intracellular signaling by physical association with G_s protein (Usdin et al., 1993; Gelling et al., 1997; Wheeler et al., 1999). Although the majority of the cytoplasmic domain of the GIPR mediates intracellular signal transduction, a minimum length of approximately 405 amino acids is required for efficient transport and plasma membrane insertion (Baggio and Drucker, 2007). Ligand binding to the GIPR activates a heterotrimeric G_s protein that in turn activates adenylate cyclase, elevates intracellular cAMP and Ca²⁺ levels, and activates PKA, as well as a host of other signaling pathways, including PI-3K, PKB, MAPK, and phospholipase A2, which result in a cascade of intracellular events that mediate the potentiation of glucose- and depolarization-stimulated exocytosis of insulin-containing granules.

The regulators of GIPR expression are not all known. Rat *gipr* gene contains a TATA-less promoter, and the first 100 bp of this promoter directed high levels of gene expression (Boylan et al., 1999). The 5'-flanking region of the *gipr* gene is sufficient to direct transcription in a rat insulinoma cell line 2 (RIN38), and this region contains negative regulatory sequences distal to the transcription start site controlling the cell-specific expression of the *gipr* gene (Boylan et al., 1999). In addition, the 5'-flanking region of *gipr* gene contains the binding sites for Oct-1, Sp1, and Sp3 transcription factors; binding of these transcription factors is critical not only for high levels of *gipr* transcription but also for cell-specific expression of the GIPR (Boylan et al., 2006).

B. Glucagon-Like Peptide-1 Receptor

GLP-1R is in the same class of receptors as GIPR. Although, as stated above, GLP-1, GLP-2, and glucagon result from post-translational modifications of the proglucagon molecule encoded by one gene, ligand binding of the three hormones to their unique receptors is highly specific with no relevant cross-reactivity to receptors for the other two peptides. Glucagon, for example, binds GLP-1R with 100- to 1000-fold less affinity than does GLP-1 (Thorens, 1992; Fehmann et al., 1994; Doyle and Egan, 2007). Ex-4 and its N-terminally truncated peptide exendin (9–39) (Ex-9-39) bind GLP-1R and act as potent and specific GLP-1R agonists and antagonists, respectively (Göke et al., 1993). Thus Ex-9-39 is also used as an investigational tool to uncover the physiological effects of GLP-1 signaling. The *glp-1r* gene was first cloned from rat pancreatic islets cDNA library in 1992 (Thorens, 1992), and the highly homologous human receptor was next cloned from a human pancreatic insulinoma (Dillon et al., 1993; Thorens et al., 1993) and a gut tumor cell line (Graziano et al., 1993). The human *glp-1r* gene is located on chromosome 6p21.1 (Stoffel et al., 1993). The rat and human GLP-1Rs are 463 amino acids in length and are 90% identical (Thorens, 1992; Thorens et al., 1993), differing at 42 amino acid positions (Tibaduiza et al., 2001). Although alternate splicing results in two different transcripts for GLP-1R in rat and human (Thorens, 1992; Dillon et al., 1993), and numerous attempts have been made to identify alternative GLP-1R, only one structurally and functionally identical GLP-1R has been described. Ex-4 is also a ligand for the known GLP-1R and yet the lizard also synthesizes GLP-1 in its gut, but it does not have a unique Ex-4 receptor. GLP-1R has, over the years, been reported to be expressed in a variety of tissues: pancreatic ducts, many cell types within pancreatic islets, thyroid C cells, kidney, lung, heart, gastrointestinal track, skin, pituitary, and multiple regions of the peripheral and

central nervous system, including hypothalamus, hippocampus and cortex. In islets, it is clear that GLP-1Rs are expressed in all β cells, whereas much contradictory information has been published with regard to their presence in the other islet cell types (Fehmann and Habener, 1991; Heller and Aponte, 1995; Heller et al., 1997). A recent study using in situ hybridization and double and triple fluorescence microscopy in mouse, rat, and human pancreas seems to have cleared the waters regarding which islet cell types express GLP-1Rs (Tornehave et al., 2008). Judging from those data, GLP-1R may be almost exclusively restricted to the β cells, because it was not observed in α cells and was rarely observed in δ (somatostatin-secreting) cells, and is present in cells lining the pancreatic ducts. Therefore, additional studies are required to determine whether GLP-1R actually exists in δ cells [The presence of GLP-1Rs (or GIPRs for that matter) on pancreatic polypeptide- or ghrelin-expressing cells in islets was not investigated.] It is noteworthy that functional GLP-1Rs are also detected on intragemmal nerve fibers of taste buds of rodent and monkey (Shin et al., 2008). There is considerable controversy with respect to the presence of functional GLP-1Rs in human and rodent muscle, adipose tissue, and liver, although GLP-1R expression was detected in muscle and adipose tissue of dog (Sandhu et al., 1999) and in muscle, liver, and fat of rodents (Campos et al., 1994; Egan et al., 1994). GLP-1R mRNA transcripts have also been detected in spleen, thymus, and lymph nodes from nondiabetic and diabetic mice (Hadjiyanni et al., 2008). In brain, activated GLP-1Rs are involved in regulation of satiety and food intake, memory and learning, and, similar to GIP, hippocampal cell turnover.

Like other GIPRs in its superfamily, GLP-1R comprises an N-terminal extracellular region that is important for GLP-1 recognition and binding, seven α -helical transmembrane domains (the second and fourth trans-membrane domains is also important for GLP-1 binding), and a C-terminal, cytoplasmic region that contains the major determinants required for specific G protein coupling. GLP-1R is capable of signaling through $G\alpha_s$ subunit as well as additional G protein subunits such as $G\alpha_q$, $G\alpha_o$, and $G\alpha_i$ (Montrose-Rafizadeh et al., 1999; Hällbrink et al., 2001). Under physiological conditions studied to date, however, it seems that GLP-1R activation leads to increased intracellular cAMP and Ca^{2+} concentrations and activation of downstream pathways, including PKA, PKC, PI-3K, Epac2, and MAPK signaling pathway (Drucker et al., 1987; Thorens, 1992; Wheeler et al., 1993; Holz et al., 1995; Montrose-Rafizadeh et al., 1999). Distinct domains within the third intracellular loop of GLP-1R are responsible for the activation of the different G proteins (Hällbrink et al., 2001). However, the coupling of additional G proteins to GLP-1R in vivo remains to be clarified, although the coupling of the receptor to specific G proteins could be explained by the existence of subtypes of GLP-1R (to be discussed in section VII.B.3) (which have not been shown to be present in humans), or could result from a consequence of tissue-specific distribution of G proteins (Hällbrink et al., 2001). It is likely that glycosylation of GLP-1R also regulates and optimizes its function by facilitating its correct insertion into the cell membrane. Treatment with tunicamycin, which prevents glycosylation, resulted in a decrease in the number of GLP-1 binding sites in the membrane of rat insulinoma RINm5F cells without an inhibition of transcription of mRNA but a reduction in cAMP production occurred in response to GLP-1 stimulation (Göke et al., 1994). The signal transduction activity of GLP-1R is also regulated by palmitoylation at the cysteine 438 residue (Vázquez et al., 2005b). However, the significance of these effects in vivo is unknown.

V. The Incretin Effect

A. The Incretin Effect of Glucose-Dependent Insulinotropic Peptide and Its Impact in Type 2 Diabetes

The incretin function of GIP has been identified in studies using GIP antagonists (Tseng et al., 1996b; Lewis et al., 2000) and GIPR antisera (“immunoneutralization studies”) (Ebert and Creutzfeldt, 1982). Treatment of animals with these compounds caused a reduction in the

insulin response to oral glucose that resulted in impaired glucose tolerance. In vitro studies of treatment of isolated islets or perfused pancreas with GIP indicate that it increases insulin secretion (Dupre et al., 1973; Schauder et al., 1975). Other studies demonstrate that the elevated GIP concentrations elicited by oral glucose can almost completely account for the added insulin secretory response seen in oral versus intravenous glucose administration (Nauck et al., 1989). Studies using the specific GIPR antagonist (Pro3)GIP in *Lep^{ob}/Lep^{ob}* mice (mice with defective leptin that become obese) show that daily administration of (Pro3)GIP reduced pancreatic insulin content (Gault et al., 2005) and that GIP might be responsible for as much as 80% of the incretin effect seen after oral ingestion (Gault et al., 2002, 2003). This seems to confirm the pre-eminence of GIP as an incretin. *GIPR(-/-)* mice have fasting blood glucose levels comparable with those of wild-type mice, and after intraperitoneal glucose challenge, glucose-stimulated insulin secretion also seems comparable with that of wild-type mice. In addition, glucose-stimulated insulin secretion from isolated islets is preserved in *GIPR(-/-)* mice (Miyawaki et al., 1999). However, in response to oral glucose administration, *GIPR(-/-)* mice have impaired glucose tolerance as a result of a 50% reduction in insulin secretion compared with wild-type animals. These results demonstrate that GIP's primary role is that of an incretin and that insulin secretion from β cells is regulated not only by glucose but also by GIP, especially in the postprandial state (Miyawaki et al., 1999). It is noteworthy that isolated islets from *GIPR(-/-)* mice show increased responsiveness to GLP-1, although serum GLP-1 levels in *GIPR(-/-)* mice are unaltered (Pamir et al., 2003). Furthermore, these mice exhibit a decrease in intrapancreatic insulin content and *insulin* gene expression (Pamir et al., 2003). Although it has been reported that intracerebroventricular administration of GIP does not affect food intake (Woods et al., 1981) and there was no significant difference in food intake between WT and *GIPR(-/-)* mice, *GIPR(-/-)* mice fed a high-fat diet do not become obese or insulin-resistant. Moreover, *GIPR(-/-)* mice with defective leptin [*GIPR(-/-), Lep^{ob}/Lep^{ob}*] are protected from diet-induced obesity and its associated complications that occur with defective leptin signaling, including T2DM and fatty liver (Miyawaki et al., 2002).

The incretin effect of GIP in stimulating insulin secretion is almost lost in T2DM (Nauck et al., 1993a; Vilsbøll et al., 2002), and many studies indicate the existence of a specific defect in GIP action in these patients. It has been speculated that this loss of insulinotropic action of GIP may occur as a result of either chronic desensitization of GIPRs (Tseng et al., 1996a) or a reduction in the expression of GIPRs on pancreatic β cells (Holst et al., 1997; Lynn et al., 2001; Xu et al., 2007; Zhou et al., 2007). Several studies have examined the expression of GIPR in experimental models of diabetes. Indeed, it has been shown that the expressions of both *GIPR* mRNA and protein are decreased in β cells of diabetic-fatty Zucker (ZDF) rats containing a leptin receptor missense mutation (Lynn et al., 2001; Piteau et al., 2007). Another study suggested a role for circulating free fatty acids in down-regulation of GIPR expression (Lynn et al., 2003). Zhou et al. (2007) suggested that ubiquitination may also be critical for the hyperglycemia-associated down-regulation of GIPR expression. Hyperglycemia triggered the association of GIPR and ubiquitin ligase complexes, resulting in reduction of GIPR protein levels on β -cell membranes (but not GLP-1R) and down-regulation of GIP action.

Receptor desensitization has been described for several receptors, including glucagon, somatostatin, β -adrenergic receptor, and GLP-1. The GIPR is susceptible to very rapid and reversible homologous desensitization in vitro (Jones et al., 1989; Tseng et al., 1996a; Wheeler et al., 1999). This desensitization process has been shown to be mediated by a number of separate mechanisms, including receptor internalization, down-regulation, and uncoupling from G proteins. Upon agonist stimulation, GIPRs are phosphorylated by protein kinase, resulting in their uncoupling from interaction with G protein (Premont et al., 1995), and regulators of G protein signaling and G protein receptor kinase 2 proteins have been demonstrated to mediate this desensitization mechanism (Druey et al., 1996; Koelle and Horvitz, 1996; Neill et al., 1997; Tseng and Zhang, 1998). Functional studies on the role of

the COOH-terminal tail (CT) of GPCRs have been implicated in receptor desensitization and endocytosis (Reneke et al., 1988; Hausdorff et al., 1990), and the CTs of glucagon receptor (Buggy et al., 1997) and GLP-1R (Widmann et al., 1997) have been shown to be required for receptor desensitization. In the case of GIPRs, CT deletion analyses demonstrate that the majority of the CT of GIPR is important for regulating the rate of receptor internalization but not for GIPR expression and signaling (Wheeler et al., 1999). Specific serine residues within the CT, particularly serines 426 and 427, play an important role in regulating the rate of receptor internalization, whereas serines 406 and 411 are important for receptor desensitization (Wheeler et al., 1999; Baggio and Drucker, 2007). In summary, the incretin effect of GIP is deficient in T2DM; a unifying consensus has not yet emerged, but increasing evidence suggests that persistent hyperglycemia leads to reduced GIPR protein levels. A recent study in ZDF animals supports the role of hyperglycemia in GIPR down-regulation. Piteau et al. (2007) treated ZDF rats with phlorizin, bringing blood glucose levels down from 28 to 10 mM. Nontreated ZDF rats had GIPR mRNA levels that were 94% less than those of lean rats, and insulin secretion did not increase in response to exogenous GIP in the ZDF animals. But after phlorizin treatment, mRNA levels returned to those of lean rats. In addition, insulinotropic responsiveness to GIP was restored in the treated rats. GIP also promotes insulin biosynthesis as well as β -cell proliferation and survival, and whether these pleiotropic effects (to be discussed in section VI.A and Table 2) as well as the insulinotropic effects of GIP are also deficient in humans with T2DM is not known.

B. The Incretin Effect of Glucagon-Like Peptide-1 and Its Impact in Type 2 Diabetes

Blockade of endogenous GIP action by injection of its antiserum resulted in partial preservation of incretin activity. This observation led to suspicion of the existence of additional incretin hormones, and insulinotropic effects of GLP-1 were first described in rodent in 1985 (Schmidt et al., 1985). In humans, insulinotropic effects of GLP-1 were first described in 1987 (Kreymann et al., 1987). In that study, the ability of GLP-1 to enhance glucose-dependent insulin secretion was of such magnitude that the authors believed that its effects, in conjunction with those of GIP, were sufficient to account for the total incretin effect. GLP-1 (7–37) and GLP-1 (7–36) amide seem to be equipotent insulinotropic factors (Orskov et al., 1986). Although GLP-1 is circulating in much lower concentrations than GIP, GLP-1 is one of the most potent insulinotropic factors that stimulate insulin secretion in a glucose-dependent manner, far exceeding GIP's effects on a molar basis. In animals and humans, it is essential for normal postprandial glucose homeostasis and the complete early/first-phase insulin response (Otonkoski and Hayek, 1995). Secretion of GLP-1 highly correlates with glucose level and insulin secretion, and its insulinotropic effects are observed at increased circulating glucose concentrations, but disappear once plasma glucose returns to normal (Nauck et al., 2002).

Evidence for the functional importance of GLP-1 as a physiologically relevant incretin derives from studies that have inactivated GLP-1 signaling using immune-neutralizing antisera, or GLP-1R antagonists or knockout mice. A specific antibody to immunoneutralize circulating GLP-1 and the GLP-1R antagonist Ex-9-39 have been used to demonstrate the essential physiological role of endogenous GLP-1 in the control of glucose-dependent insulin secretion and glucose homeostasis in rodent and human subjects. Removal of GLP-1 action, whether by Ex-9-39 or by its immunoneutralization, causes increases in both fasting and postprandial glycemia and reduces glucose-stimulated insulin release in both animals and humans (Kolligs et al., 1995; Wang et al., 1995; D'Alessio et al., 1996; Edwards et al., 1999; Baggio et al., 2000). Administration of Ex-9-39 to rodents or humans results in a suppression of the incretin effect by 50 to 70% (Kolligs et al., 1995; Flamez et al., 1999; Schirra et al., 2006). In agreement with these results, *GLP-1R(-/-)* mice with a targeted deletion of all GLP-1Rs have mild fasting hyperglycemia and abnormally high blood glucose levels after oral and intraperitoneal glucose

challenges that are associated with reduced circulating levels of glucose-stimulated insulin secretion, whereas *GLP-1R(-/-)* mice have normal body weight, food intake, and fasting and postprandial plasma levels of glucagon (Scrocchi et al., 1996; Scrocchi et al., 1998). However, GLP-1R signaling is not required for actual glucose responsiveness of pancreatic β cells, as demonstrated by the fact that isolated islets from *GLP-1R(-/-)* mice still have a well preserved glucose response in terms of insulin secretion, and both islet and total pancreatic insulin content are not significantly lower than in wild-type mice (Flamez et al., 1998). Pancreatic insulin mRNA transcripts are also similar in wild-type and *GLP-1R(-/-)* mice (Scrocchi et al., 1998). Moreover, *GLP-1R(-/-)* islets exhibit reduced basal but enhanced GIP-stimulated cAMP production and abnormalities in basal and glucose-stimulated intracellular Ca^{2+} concentration (Flamez et al., 1998). In these mice, the lack of GLP-1R signaling is compensated for, at least in part, via increased GIP secretion and up-regulation of the GIP-insulin axis on pancreatic islets (Pederson et al., 1998). In addition, *GIPR(-/-)* mice exhibit an enhanced GLP-1-mediated insulin secretory response in pancreatic islets (Pamir et al., 2003). Thus, disruption of single incretin receptor genes in mice causes only modest glucose intolerance, with a well preserved insulin-secretory response to glucose that is partially modified as a result of complementary up-regulation of the remaining intact incretin hormone. However, double incretin receptor knockout mice, which have disruption of both *glp-1r* and *gipr*, surprisingly show only a modest and obviously incomplete perturbation in glucose homeostasis, suggesting that other mechanisms are capable of compensating for the absence of the actions of GIP and GLP-1 (Hansotia et al., 2004; Preitner et al., 2004).

The GLP-1R is no exception to the susceptibility of members of its subfamily to undergo desensitization, at least in vitro. It undergoes rapid internalization, primarily via mechanisms dependent on clathrin-coated pits (Widmann et al., 1995, 1997; Vázquez et al., 2005a) and caveolin (Syme et al., 2006). This internalization is mediated by phosphorylation of specific residues in the GLP-1R C-terminal tail (Widmann et al., 1997) that regulates receptor subcellular localization, trafficking, and signaling. In addition, GLP-1R also undergoes homologous and heterologous desensitization in vitro, which correlates with phosphorylation of GLP-1R C-terminal tail (Widmann et al., 1996a,b, 1997; Baggio et al., 2004c). Xu et al. (2007) suggested that protein levels of GLP-1R are significantly decreased in β cells exposed to high blood glucose concentrations in vivo and in vitro, and insulin response to GLP-1 is reduced in this system. But research in our laboratory (Zhou et al., 2007) did not find this to be the case in human islets. Even after long-term GLP-1R agonist administration, such as with exenatide treatment, there does not seem to be any attenuation of GLP-1R-dependent effects on glucose homeostasis in vivo (Klonoff et al., 2008), and an elevated steady state of plasma GLP-1 for 6 to 12 weeks in clinical trials has resulted in effective reduction in blood glucose levels without any loss of potency (Zander et al., 2002; Meneilly et al., 2003). Therefore, no physiological significance for either the homologous or heterologous in vitro desensitization has been established in vivo. In T2DM, the insulinotropic properties of GLP-1 seem to be largely intact (Nauck et al., 1993a). In addition, in support of fully functional GLP-1Rs in T2DM, an acute infusion of exenatide perfectly restores first-phase insulin secretion in response to i.v. glucose in subjects with T2DM (Fehse et al., 2005).

In addition to stimulating insulin secretion, GLP-1 also promotes insulin biosynthesis as well as β -cell proliferation and survival, thereby augmenting supplies of insulin for secretion. It should be noted that GLP-1, and not GIP, also suppresses glucagon secretion in a glucose-dependent manner, contributing to the glucose-lowering effect of GLP-1. Deceleration of gastric emptying by GLP-1, but not GIP (Meier et al., 2004), also adds to the glucose-lowering effect of incretins after meal ingestion in humans. In addition, GLP-1 may enhance peripheral glucose disposal and insulin sensitivity (Todd and Bloom, 2007).

GLP-1 and GIP in β -cell lines have additive effects on insulin secretion but only at concentration that have submaximal insulinotropic effects (Montrose-Rafizadeh et al., 1994). Therefore, the final pathway to insulin secretion is most likely a common one. But under usual postprandial physiological conditions, they are most likely each acting at submaximal concentrations. GLP-1 is at least 2 orders of magnitude more potent than GIP as an insulinotropic agent. There is some evidence that GLP-1 can recruit cells into a secretory mode in response to glucose (i.e., make the cells glucose-competent), whereas there is no such evidence for GIP (Holz et al., 1993; Montrose-Rafizadeh et al., 1994). It is conceivable, however, GLP-1 might prime the cells for GIP actions, although this was found not to be the case in elderly patients with T2DM (Meneilly and Elahi, 2006).

VI. The Pleiotropic Effect of Incretin in Pancreas

A. The Pleiotropic Effects of Glucose-Dependent Insulinotropic Peptide in Pancreas

1. Effects on β Cells—GIP binding to its specific receptor on pancreatic β cells facilitates the secretion of insulin in the presence of PPG concentration via activation of proximal signal transduction pathways, and the ability of GIP to promote glucose-stimulated insulin secretion in pancreatic β cells is attributed to GIPR activation leading to membrane depolarization and increases in the intracellular Ca^{2+} concentration as well as direct effects on insulin exocytosis (Fig. 5). Ligand binding to GIPR activates several mechanisms, including stimulation of the adenylyl cyclase/cAMP/PKA, inhibition of K_{ATP} channels, increases in intracellular Ca^{2+} , activation of phospholipase A2 (PLA2) (Ehse et al., 2001), specific protein kinase signaling pathways, including PKB (Trümper et al., 2001), mitogen-activated protein kinases (Ehse et al., 2002), and stimulation of exocytosis (Ding and Gromada, 1997). The rise in cAMP significantly up-regulates activity of PKA, which leads to a cascade in which phosphorylation of regulatory proteins, including GLUT2 (a low K_m glucose transporter), SUR1 (subunits of the pancreatic β -cell K_{ATP} channel), α -SNAP (a vesicle-associated protein), and other ion channels (Ca^{2+} channels, for example), induces membrane depolarization and subsequent exocytosis of insulin, as well as phosphorylation of critical transcription factors, which results in significant up-regulation of *insulin* gene promoter activity (Doyle and Egan, 2003). A PKA-independent mechanism is also involved in GIP-mediated insulin secretion. The cAMP-binding protein cAMP-GEFII (Epac2) mediates cAMP-dependent, PKA-independent insulin secretion, and this effect of Epac2 on insulin secretion is mediated by Rim2 and depends on intracellular Ca^{2+} as well as on cAMP (Kashima et al., 2001). GIP stimulation of insulin secretion from pancreatic β cells is also mediated by PLA2 (Ehse et al., 2001). Metabolism of glucose has been shown to induce hydrolysis of membrane phospholipids, leading to the accumulation of arachidonic acid (AA), which amplifies insulin secretion (Turk et al., 1987; Turk et al., 1993). GIP stimulates AA release from clonal β cells ($\beta\text{TC-3}$), and coupling of GIPR to AA production in β cells is mediated by cAMP and a Ca^{2+} -independent PLA2 (Ehse et al., 2001). Incretin- and glucose-stimulated insulin secretion is also mediated via opening of voltage-dependent Ca^{2+} channels as well as activation of several species of K^+ channels, including K_{ATP} channels, Ca^{2+} -activated K^+ (K_{CA}) channels, and voltage-gated K^+ (K_{V}) channels (for review, see Doyle and Egan, 2003). Although all K^+ channels are present in pancreatic β cells, most of the studies have concentrated on studying K_{ATP} and K_{CA} channels, and less is known about the mechanisms involved in insulin modulation by K_{V} channels. However, one study identified a potential role for GIP in the regulation of $\text{K}_{\text{V}1.4}$ endocytosis and GIP-induced down-regulation of $\text{K}_{\text{V}1.4}$ via PKA-dependent phosphorylation and endocytosis of the channel protein, resulting in the modulation of insulin secretion (Kim et al., 2005b).

In addition to potentiating the release of insulin from β cells, GIP replenishes insulin in β cells by increasing *insulin* gene transcription and biosynthesis, and enhances the glucose-sensing system by increasing the expression of components of β -cell glucose sensors. Acute treatment

of GIP synergizes with glucose, causing more insulin to be released from β cells both in vivo and in vitro, and the amount of insulin released from β cells is proportional to the rate of glucose metabolism (Newgard, 1994), which is determined by the plasma membrane glucose transporters, the enzymes responsible for glucose metabolism and their activity (Matschinsky et al., 1993). In the RIN 1046–38 rat insulinoma cell line, extended exposure to GIP up-regulates the expression of both GLUT1-mediated glucose uptake and hexokinases catalyzing the initial phosphorylation of glucose, allowing glycolysis to proceed (Wang et al., 1996). In addition, *GIPR*($-/-$) mice exhibit decreased *insulin* gene transcription and protein biosynthesis (Pamir et al., 2003), confirming the role of GIP in insulin biosynthesis. Together, these results suggest that GIP confers long-term beneficial effects on insulin secretion and cellular health by replenishing insulin stores.

Some evidence indicates that GIP acts synergistically with glucose as a growth- and antiapoptotic factor for β cells, providing another mechanism by which GIP promotes long-term accommodation of β -cell function to changing nutritional status. GIP potentiates glucose-induced β -cell proliferation and protects β cells from cell death induced by various stimuli, including exposure to wortmannin, STZ, glucolipotoxicity, or serum or glucose deprivation (Ehse et al., 2003; Kim et al., 2005c). Moreover, overexpression of a dominant-negative human GIPR in murine β cells results in diminished islet size (Herbach et al., 2005), and GIP reduces glucolipotoxicity-induced cell death in islets from wild-type but not *GIPR*($-/-$) mice (Kim et al., 2005c). Recent studies have begun to unravel the molecular signaling pathways that mediate the proliferative and antiapoptotic actions of GIP in the β -cell, and it is clear that growth and antiapoptotic pathways rely on similar pathways, including activation of cAMP/PKA, PKA/CREB, MAPK, and PI-3K-dependent activation of PKB (Trümper et al., 2001, 2002; Ehse et al., 2002, 2003; Kim et al., 2005c) (Fig. 6). These signaling modules are dependent on glucose metabolism and Ca^{2+} influx, and cAMP-PKA-Rab1-extra-cellular signal-regulated kinase 1/2 pathway may also be one way in which GIP regulates β -cell proliferation (Ehse et al., 2002). In particular, GIP phosphorylates several transcription factors such as FoxO1 and p70^{S6K}, crucial regulators of translation known to be involved in glucose-induced β -cell mitogenesis, suggesting possible mediators of GIP-mediated β -cell growth (Ehse et al., 2002). FoxO1 has been shown to play a critical role in GIP-mediated β -cell survival and apoptosis. GIP improved cell survival in INS-1 (clone 832/13) cells and murine islets via GIP-induced phosphorylation of FoxO1, resulting in inactivation of the pro-apoptotic *bax* gene (Kim et al., 2005c). In addition, a 2-week infusion of GIP into diabetic (ZDF) rats was shown to significantly reduce β -cell apoptosis because of down-regulation of the *bax* gene and up-regulation of the antiapoptotic *bcl-2* gene: these effects were mediated by activation of PI-3K/PKB, and subsequent phosphorylation and nuclear exclusion of FoxO1 (Kim et al., 2005c). The protective effects of GIP on β -cell apoptosis in the diabetic state are very intriguing to us. It is clear from many studies that GIP is not insulinotropic in T2DM and that in animal models, such as the ZDF rat discussed above (section V.A), GIP does not stimulate insulin secretion, and its receptor is severely down-regulated (94% reduction compared with lean nondiabetic rats) (Piteau et al., 2007). So, if the GIP receptor is so severely down-regulated in the ZDF rats, how did 2 weeks of GIP infusion have its antiapoptotic effects? Unlike the more recent work, in which the authors found that reduction in hyperglycemia by phlorizin treatment reversed GIPR down-regulation (Piteau et al., 2007), Kim et al. (2005c) did not first lower hyperglycemia before giving exogenous GIP. Is there a case to be made that the antiapoptotic effect, in vivo, is not through the known GIPR on β cells? Could this mean that by not developing GIPR agonists to treat T2DM, we are missing the other potential effect of GIPR stimulation? Is there a case to be made that GLP-1R and GIPR stimulation, together, might be beneficial in T2DM? In β -cell lines at least, a direct antiapoptotic effect of GIP seems to hold up, because another study from the same research group corroborated that GIP has antiapoptotic properties and that the antiapoptotic effect of GIP is, at the very least, partially due to GIP-stimulated increases in *bcl-2* gene expression (Kim et al., 2008b). In that study, stimulation of

INS-1 (clone 832/13) cells with GIP resulted in enhanced expression of *bcl-2*, and this was mediated by a pathway involving AMP-activated protein kinase, cAMP-responsive CREB activator 2, and CREB. GIP treatment also reduces caspase 3 activation via cAMP and MAPK regulation (Ehses et al., 2003; Kim et al., 2008b), and there is a possibility that GIP improves β -cell survival by reducing ER stress (Yusta et al., 2006).

2. Effects on Glucagon Secretion—Several studies have suggested that GIPR seems to be also present in pancreatic α cells (Moens et al., 1996) and compatible with this observation, exposure of α cells to GIP increases cAMP content to levels that are required for nutrient-induced glucagon secretion (Pipeleers et al., 1985; Moens et al., 1996), and GIP stimulates Ca^{2+} -induced exocytosis in isolated rat α cells by a PKA-mediated mechanism (Ding and Gromada, 1997). However, several lines of evidence indicate that GIP stimulates glucagon secretion in human and animal models but only under certain circumstances. For example, a glucagonostatic GIP effect has been observed in patients with liver cirrhosis and basal hyperglucagonemia (Dupre et al., 1991) and in human and animal models during euglycemic conditions (Pederson and Brown, 1978; Meier et al., 2003b), but in healthy persons under hyperglycemic conditions or in T2DM, no GIP effects on glucagon secretion were reported (Nauck et al., 1993a; Meier et al., 2001; Vilsbøll et al., 2003b). Most experiments in the literature were carried out under hyperglycemic conditions to increase GIP-mediated insulin secretion, suggesting that an absence of GIP effect on glucagon secretion in those studies may be due to increased glucose concentrations and insulin-mediated counteraction of effects to stimulate glucagon secretion.

B. Pleiotropic Effects of Glucagon-Like Peptide-1 in Pancreas

1. Effects on β Cells—GLP-1 is one of the most potent substances known to stimulate glucose-dependent insulin secretion from islet β cells and, like GIP, its stimulatory activity is exerted via binding to its receptor on β cells. This binding results in activation of adenylyl cyclase with consequent production of cAMP and subsequent activation of PKA and the Epac family, which led to inhibition of K_{ATP} channels, elevation of intracellular Ca^{2+} levels, increases in mitochondrial ATP synthesis, and enhanced exocytosis of insulin from insulin-secretory vesicles (Fig. 5).

PKA, a key component in the regulation of insulin secretion by cAMP, mediates many of the phosphorylation reactions required for insulin secretion including K_{ATP} channels, the L-type Ca^{2+} channels on the ER and GLUT2 glucose transporters. GLP-1-mediated activation of PKA results in phosphorylation of S1448 on the SUR1 K_{ATP} channel subunit via an ADP-dependent mechanism, facilitating its closure (Light et al., 2002). This is followed by membrane depolarization and triggering of the insulin secretory pathway. Treatment with the PKA inhibitor 8-bromoadenosine-3',5'-cyclic mono-phosphorothioate, *Rp*-isomer (Gromada et al., 1998) or H89 (Light et al., 2002) abolishes GLP-1-induced inhibition of the K_{ATP} channels and SUR1(−/−) islets lack an insulin secretory response but exhibit a normal rise in cAMP to GLP-1 and GIP, implicating cAMP-dependent PKA-independent signal transduction pathway by which incretins exert their effects (Nakazaki et al., 2002; Shiota et al., 2002). It is now evident that the action of cAMP produced by GLP-1 signaling is mediated not just by PKA, but also by Epac2 (de Rooij et al., 1998; Kawasaki et al., 1998), and Epac2 also inhibits the function of K_{ATP} channels in rodent and human β cells via interaction with SUR1 (Shibasaki et al., 2004; Kang et al., 2006a). A recent study has also demonstrated that the scaffold protein β -arrestin-1 facilitates GLP-1-stimulated cAMP production via interaction with GLP-1Rs (Sonoda et al., 2008).

GLP-1 signaling also antagonizes voltage-dependent K^+ (K_v) channels via cAMP/PKA-dependent pathway in β cells, which prevents β -cell repolarization by reducing K_v currents

(MacDonald et al., 2002, 2003). However, K_v currents are not antagonized by treatment with cAMP analog or the constitutively active PKA catalytic sub-unit, and the Epac-selective activator 8-pT-2'-O-Me-cAMP, implying a role for additional signaling pathways required for antagonism of the K_v current (MacDonald et al., 2003). This study has identified a role of PI-3K with subsequent activation of PKC ζ in the antagonism of the K_v current by GLP-1 and that this occurs via epidermal growth factor receptor (EGFR) transactivation, not via the G protein-regulated isoform p110 γ (MacDonald et al., 2003).

L-type Ca^{2+} channels are also phosphorylated by PKA, leading to increase of their open probability and enhancement of Ca^{2+} influx (Lester et al., 1997; Fraser et al., 1998; Hulme et al., 2003). As well as effects on Ca^{2+} influx, activation of GLP-1R increases intracellular Ca^{2+} through two intracellular Ca^{2+} -releasing receptors in the endoplasmic reticulum (ER): the inositol 1,4,5 triphosphate receptors (IP $_3$ R) activated by PKA and the ryanodine receptors (RyR) activated by Epac2 (Kang et al., 2003; Tsuboi et al., 2003; Doyle and Egan, 2007). Phosphorylation of IP $_3$ R by PKA alters Ca^{2+} release properties of IP $_3$ Rs (Bugrim, 1999), contributing to increasing intracellular Ca^{2+} concentrations. Pretreatment of β cells with ryanodine, and not with *Xestospongina C*, an IP $_3$ R inhibitor (Kang et al., 2001), blocked Ca^{2+} release from ER in response to 8-pT-2'-O-Me-cAMP (Kang et al., 2003), suggesting that Epac2 increases the release of Ca^{2+} from ER into cytosol through RyR. A recent study has indicated that GLP-1 elevates intracellular Ca^{2+} concentration via stimulation of the nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose production (Kim et al., 2008a), catalyzed by cyclic ADP-ribose cyclases, that stimulates glucose-induced Ca^{2+} mobilization followed by insulin secretion (Kato et al., 1995, 1999). GLP-1-induced increase in intracellular Ca^{2+} concentrations is a key trigger for fusion of insulin-containing vesicles to the plasma membrane, and exocytosis of insulin from β cells rapidly follows.

Direct effects of GLP-1 signaling on insulin-laden vesicles have also been described. PKA and Epac2 directly regulate exocytosis of insulin-containing vesicles via interaction with the regulators of exocytosis. Lester et al. (2001) proposed a mechanism by which changes in insulin secretion are associated with phosphorylation of the vesicle-associated protein synapsin-1, a protein that is also a key regulator of neuronal exocytosis, by PKA followed by dephosphorylation by calcineurin (Lester et al., 2001). PKA may also regulate the vesicle priming through the phosphorylation of RIM proteins (Lonart et al., 2003). It is known that RIM proteins bind Rab3a, which serves to tether the vesicle to the plasma membrane (Wang et al., 1997b) and also bind Munc13-1 to create a link between synaptic vesicle tethering and priming (Betz et al., 2001). RIM proteins also bind Epac2, and this binding participates in the regulation of docking and fusion of insulin-containing vesicles to the plasma membrane (Ozaki et al., 2000). Islet β cells isolated from haplodeficient Munc13-1(+/-) mice exhibit impaired first- and second-phase insulin secretion mimicking T2DM, with defects in priming and refilling of a releasable pool of insulin-containing vesicles (Kang et al., 2006b; Kwan et al., 2006). GLP-1 stimulation normalizes the defects caused by Munc13-1 deficiency via cAMP-dependent activation of PKA and Epac, and the rescue requires RIM2-Munc13-1 interaction (Kwan et al., 2007). In addition, Epac2 interacts with Piccolo, a RIM2-interacting protein on insulin-containing vesicles, and it forms both homodimers and heterodimers with RIM2 in a Ca^{2+} -dependent manner (Fujimoto et al., 2002). In that study, treatment of pancreatic islets with antisense oligodeoxynucleotides against Piccolo inhibited 8-pT-2'-O-Me-cAMP- and glucose-induced insulin secretion. GLP-1 has been shown to counteract abnormalities, including decreased insulin secretion and increased glucagon secretion, caused by excessive islet NO generation in a rat model of T2DM through a cAMP/PKA-dependent mechanism (Salehi et al., 2008) and to augment islet adaptation to high fat diet-induced insulin resistance (Winzell and Ahrén, 2008).

Glucose, entering via GLUT2 transporters, is rapidly phosphorylated to glucose 6-phosphate by glucokinase and is subsequently metabolized in mitochondria, leading to ATP formation, changes in the ATP/ADP ratio, and closure of the K_{ATP} channels, as described above. ATP has other roles in β cells; it seems to be a major factor in movement of insulin-containing vesicles toward the plasma membrane and in priming of exocytosis (Eliasson et al., 1997). Because GLP-1 also stimulates the expression of GLUT2 transporters and glucokinase, which determine the rate of glycolysis, it helps to confer glucose sensitivity to β cells and thereby increase the efficacy (maximal effect) and potency (threshold concentration) of glucose as a stimulus for insulin secretion (Holz et al., 1993; Holz and Chepurny, 2005).

GLP-1 replenishes β -cell insulin stores and prevents exhaustion of β -cell reserves via increased insulin mRNA stability, gene transcription, and biosynthesis. It stabilizes mRNA encoding insulin via stimulation of cytoplasmic translocation of polypyrimidine tract binding protein by cAMP/PKA-dependent phosphorylation, in which polypyrimidine tract binding protein binds to the 3'-untranslated region of insulin mRNA, thereby stabilizing and up-regulating its expression (Tillmar et al., 2002; Knoch et al., 2004, 2006). GLP-1 also increases *insulin* gene transcription and biosynthesis via activation of PKA-dependent and -independent signaling pathways (Fig. 6). Ca^{2+} /calcineurin/nuclear factor of activated T cells (NFAT) is a mediator of these pathways on *insulin* gene transcription as well as β -cell growth and function (Lawrence et al., 2002; Heit et al., 2006). Intracellular Ca^{2+} elevations activate calcineurin, leading to dephosphorylation and subsequent nuclear localization of NFATc proteins. Through direct binding to *cis*-regulatory sequences in target genes in cooperation with partner transcription factors (NFATn), NFATc proteins activate transcription of genes involved in insulin production and β -cell growth. PDX-1, the most extensively studied insulin transcription factor and, as described above, necessary for development of the pancreas and K cells, is a key effector for the GLP-1R signaling pathway on *insulin* gene transcription and biosynthesis, as well as differentiation, proliferation, and survival of the β -cell (Fig. 6). GLP-1 has been shown, both in vitro and in vivo, to be involved in regulation of PDX-1 by increasing its total protein levels, and its translocation to the nucleus, followed by its binding to and resultant increase in activity of the *insulin* gene promoter in β cells (Buteau et al., 1999; Wang et al., 1999, 2001; Stoffers et al., 2000). PDX-1 directly binds to the A-box element and the GG2 element of the rat and human insulin promoters, which is apparently critical for the activation of the *insulin* gene (Ohlsson et al., 1993; Le Lay and Stein, 2006). The regulation of PDX-1 by GLP-1 mainly occurs via cAMP/PKA-dependent signaling pathway (Wang et al., 2001). In addition to PKA-dependent pathway influencing PDX-1, GLP-1-induced expression and nuclear localization of PDX-1 involves the phosphorylation of FoxO1 via transactivation of the EGFR and PI-3K/PKB pathway, resulting in deactivation and nuclear exclusion of FoxO1 and consequent disinhibition of Foxa2-dependent *pdx-1* gene promoter activity (Kitamura et al., 2002; Buteau et al., 2006). In addition, FoxO1 and PDX-1 mutually exclude each other from the nucleus of the β -cell (Kitamura et al., 2002). The GLP-1R signaling pathway also mediates *insulin* gene transcription via basic region-leucine zipper transcription factors that are related structurally but are not identical to the transcription factor CREB, and these directly bind to CRE sites on the *insulin* gene promoter; this effect is independent of $G_s\alpha$, cAMP/PKA, and PKC and may be mediated by the 90-kDa ribosomal S6 kinase and mitogen- and stress-activated protein kinase family of CREB kinases (Skoglund et al., 2000; Chepurny et al., 2002). In addition, treatment with p38 MAPK inhibitor leads to an increase in *insulin* transcription in the presence of either GLP-1 or Ex-4 and this effect is mediated via the CRE site (Kemp and Habener, 2001).

Studies in rodents and humans have continued to illustrate that GLP-1R signaling plays a central role in the homeostasis of pancreatic β -cell mass as well as function through stimulation of β -cell proliferation and neogenesis, and inhibition of β -cell apoptosis. Long-term treatment of both normal and diabetic rodents with GLP-1R agonists stimulates β -cell proliferation and

neogenesis, and slows the rate of β -cell apoptosis, leading to an expansion of β -cell mass. Furthermore, a transient treatment of GLP-1 or Ex-4 in STZ-treated newborn Wistar rats results in improved β -cell mass (Tourrel et al., 2001) and a transient treatment of Ex-4 in the intrauterine growth retarded rat also leads to an increase in β -cell number and prevents the development of diabetes (Stoffers et al., 2003). GLP-1 induced proliferation in β cells of old glucose-intolerant rats, and it also improved their glucose tolerance (Perfetti et al., 2000). It has become clear that GLP-1 acts by means of $G_s\alpha$ and PI-3K/PKB to stimulate β -cell proliferation and survival (Fig. 6). A β -cell-specific $G_s\alpha$ deficiency in mice results in insulin-deficient diabetes characterized by reduced insulin secretion and β -cell mass with the primary defect being in decreased β -cell proliferative capacity (Xie et al., 2007b). GLP-1 activation of PI-3K/PKB facilitates acute nuclear translocation of existing PDX-1 and the expression of further PDX-1 via stimulation of FoxO1 phosphorylation and GLP-1 exerts at least some of its stimulatory effects on β -cell proliferation and differentiation through PDX-1. Indeed, mice with a β -cell-specific inactivation of PDX-1 do not display a proliferative response to Ex-4 treatment (Li et al., 2005). GLP-1 activation of PI-3K is mediated by transactivation of EGFRs via GLP-1R-mediated activation of c-Src that in turn activates a membrane-bound metalloproteinase, with concomitant release of the soluble ligand betacellulin, which is a ligand of EGFRs (Buteau et al., 2003). This is also followed by activation and translocation to the nucleus of PKC ζ , resulting in enhancement of the stimulatory effect of GLP-1 on β -cell proliferation (Buteau et al., 2001). GLP-1 also exerts its stimulatory effects on β -cell proliferation through CREB-mediated *insulin receptor substrate-2* (*Irs2*) gene expression, leading to activation of PI-3K/PKB (Jhala et al., 2003). Indeed, Ex-4 did not rescue *Irs2*($-/-$) mice from a failure of β -cell proliferation (Park et al., 2006). As discussed above, the action of GLP-1 to promote β -cell proliferation and function is also likely to be mediated through Ca^{2+} /calcineurin/NFAT signaling pathways. Mice with a β -cell-specific deletion of the calcineurin phosphatase regulatory sub-unit, calcineurin b1 (*Cnb1*), exhibit age-dependent diabetes with reduced β -cell proliferation and mass, decreased pancreatic insulin content and hypoinsulinemia, and expression of active NFAT c_1 in these mice rescues these defects through promoting the expression of essential β -cell factors that regulate proliferation and insulin production such as PDX-1, cyclin D1 and D2, c-Myc and cyclin-dependent kinase 4 (Heit et al., 2006). In agreement with this result, GLP-1R activation has been shown to up-regulate the expression of cyclin D1 (Friedrichsen et al., 2006; Kim et al., 2006), and this effect is likely to be mediated by PKA-dependent activation of CREB (Kim et al., 2006). GLP-1-mediated expression of cyclin D1 was also observed in human islets from patients with type 2 diabetes (Lupi et al., 2008). Another study shows that GLP-1R signaling via cAMP/PKA activates β -catenin/T-cell factor-like 2 (TCF7L2)-dependent Wnt signaling in isolated mouse islets and INS-1 β cells and that Wnt signaling is involved in β -cell proliferation through up-regulation of cyclin D1 (Liu and Habener, 2008). In addition, TCF7L2 seems to regulate *insulin* gene expression and glucose- and incretin-mediated insulin secretion in mature β cells (Loder et al., 2008). This is very interesting from a clinical standpoint because genetic studies have linked the risk for T2DM to TCF7L2 polymorphisms (Lyssenko et al., 2007).

GLP-1R activation reduces β -cell apoptosis in purified rodent and human islets, and β -cell lines after exposure to several proapoptotic agents, and this activation also promotes preservation and expansion of β -cell mass in type 1 and 2 diabetic rodent models through protecting β cells against the deleterious effects of the diabetic milieu (i.e., increased cytokine toxicity, glucose toxicity, and lipotoxicity). Ex-4 treatment of *Lep^{db}/Lep^{db}* mice (these mice have defective leptin receptors and, as a result, become obese and develop diabetes accompanied by β -cell failure and apoptosis) decreases activation of caspase-3 and prevents β -cell apoptosis through PKB and MAPK (Wang and Brubaker, 2002) and infusion with GLP-1 drastically reduced the number of apoptotic β cells in islets of Zucker diabetic rats (Farilla et al., 2002). After STZ administration, *GLP-1R*($-/-$) mice exhibit increased β -cell apoptosis relative to the wild-type STZ-treated and control mice; conversely, STZ-induced apoptosis was

significantly reduced by coadministration of Ex-4 (Li et al., 2003). Ex-4 also reduces biochemical markers of islet ER stress in vivo and ER stress-associated β -cell death in a PKA-dependent manner (Yusta et al., 2006; Tsunekawa et al., 2007). In addition to these studies, GLP-1 was shown to inhibit apoptosis in β cells exposed to many cytotoxic agents, including reactive oxygen species, glucose, free fatty acid, palmitate, cytokines, tumor necrosis factor α (TNF- α), immunosuppressive reagents, and dexamethasone (for review, see Doyle and Egan, 2007). Similar to GLP-1-induced β -cell proliferation, antiapoptotic effects of GLP-1 in β cells are mediated by promotion of FoxO1 nuclear exclusion and consequent up-regulation of PDX-1 and Foxa2 expression via EGFR- and PI-3K-dependent activation of PKB and cAMP/PKA-dependent activation of CREB, leading to up-regulation of Irs2 protein expression and activation of PKB (Fig. 6). Moreover, cAMP/PKA-dependent activation of CREB can mediate antiapoptotic effects of GLP-1 in β cells through up-regulation of Bcl-2 and Bcl-xL expression (Farilla et al., 2003; Hui et al., 2003; Ranta et al., 2006). This protective effect of GLP-1 on β -cell glucolipototoxicity is also mediated by PKB activation and possibly its downstream target nuclear factor- κ B (Buteau et al., 2004). In that study, GLP-1 enhanced nuclear factor- κ B DNA binding activity via activation of PKB and stimulated the expression of inhibitor of apoptosis protein-2 and Bcl-2. In addition, recent studies suggest that GLP-1R agonists protect β cells from pro-inflammatory cytokine-induced apoptosis by inhibiting the c-Jun NH₂-terminal kinase pathway via up-regulation of islet-brain 1, a potent blocker of the c-Jun NH₂-terminal kinase pathway (Ferdaoussi et al., 2008), and activating the extracellular signal-regulated kinase 1/2-dependent pathway (Blandino-Rosano et al., 2008). In addition, combined treatment with GLP-1R agonists and insulin analogs results in additive activity to enhanced to protect against cytokine- and fatty acid-induced apoptosis in INS-1 cells (Tews et al., 2008).

Klinger et al. (2008) shed light on cellular mechanisms that limit the proliferative effect of GLP-1 in β cells. If GLP-1R agonists were to be used continuously to treat T2DM, then uncontrolled β -cell proliferation would become an issue unless there were brakes on the system. As it happens, GLP-1 provides its own brakes because it leads to the rapid and strong expression of four negative regulators of intracellular signaling, which included regulator of G protein signaling 2, dual-specificity phosphatase 14 (DUSP14), also called mitogen-activated protein kinase phosphatase, a negative feedback regulator of the mitogen-activated protein kinase signaling cascade, and inducible cAMP early repressor, cAMP responsive element modulator α , and knockdown of cAMP responsive element modulator α or DUSP14 or expression of a dominant-negative form of DUSP14 increased the proliferation of β -cell lines in an additive manner and of GLP-1-treated primary β cells, suggesting that β cells have evolved very tight, supremely elegant mechanisms for limiting their own growth (after all, insulin is a death molecule; too much circulating insulin leads to death from neuroglycopenia), and that preventing the expression of these negative regulators may lead to a more exuberant effect of GLP-1 on β -cell mass (Klinger et al., 2008).

2. Effects on Glucagon Secretion—GLP-1 infusions and exenatide treatment cause reductions in plasma levels of glucagon in both healthy subjects as well as subjects with type 1 and type 2 diabetes (Gutniak et al., 1992). This effect of GLP-1 contributes to lowering plasma glucose levels as a result of reduction in hepatic glucose output. Indeed, exogenous GLP-1 in patients with type 1 diabetes led to reductions even in fasting hyperglycemia via its glucagonostatic action (Creutzfeldt et al., 1996). Similar to the GLP-1 effects on insulin secretion, this glucagonostatic action of GLP-1 depends on glucose levels (Nauck et al., 2002). Its inhibitory effect on glucagon secretion is lost below normal fasting glucose levels, thereby reducing the risk for developing hypoglycemia.

Unlike GIPRs, which have been shown to be present on α cells (Moens et al., 1996), there is controversy concerning the presence of GLP-1Rs on α cells, as discussed in section IV.B. Several older studies demonstrated the presence of GLP-1Rs on at least some α cells (Orskov

and Poulsen, 1991; Thorens, 1992; Heller and Aponte, 1995; Ding and Gromada, 1997; Heller et al., 1997), whereas others were unable to detect them (Fehmann and Habener, 1991; Bullock et al., 1996; Moens et al., 1996; Franklin et al., 2005). Tornehave et al. (2008) were unable to identify GLP-1Rs on α cells of mice and humans using a sensitive in situ hybridization protocol for GLP-1R mRNA as well as double and triple fluorescence microscopy techniques and reached the conclusion that GLP-1Rs were present only on β cells. It is note-worthy that in glucagonoma cells (INR1-G9) transfected with human GLP-1R, GLP-1 treatment led to glucagon secretion via increases in intracellular cAMP concentration (Dillon et al., 2005). Therefore, if GLP-1Rs are endogenously expressed on α cells, one would expect glucagon secretion to result from GLP-1 stimulation, just as it does with GIP stimulation.

The stimulatory effect of GLP-1 on somatostatin secretion has been shown in both perfused rat pancreas (Schmid et al., 1990) and isolated islets (Fehmann et al., 1995), and this effect was thought to be mediated by direct interaction with GLP-1Rs on pancreatic δ cells (Fehmann et al., 1995). Both somatostatin antibodies and somatostatin receptor 2 antagonists abolished the GLP-1-induced inhibition of glucagon secretion in the perfused rat pancreas (Holst, 2007), leading to the conclusion that GLP-1 treatment, by inducing somatostatin secretion, suppresses glucagon secretion. Once again, however, the recent data from Tornehave et al. (2008) cast fresh doubt on the direct effect of GLP-1 on somatostatin secretion, because the presence of GLP-1Rs on δ cells could not be demonstrated (Tornehave et al., 2008). Therefore, the in vivo suppression of glucagon secretion by GLP-1 leads us to conclude that it results from paracrine effects via insulin and/or another β -cell-derived mediator(s). Against this, the intact suppression of glucagon secretion by GLP-1 seemed to be preserved in patients with type 1 diabetes (Creutzfeldt et al., 1996), as noted above, seeming to show the presence of a β -cell-independent mechanism. But those subjects did have some residual β -cell function, because C-peptide levels increased after GLP-1 treatment, so a β -cell mediator still cannot be totally ruled out as a cause of glucagon suppression. In addition, β -cell-specific inactivation of the *pdx-1* gene led to loss of Ex-4-induced inhibition of glucagon secretion (PDX-1 is not expressed in α cells, as discussed above, and is not necessary for their development in the embryo), which also favors the conclusion that glucagon suppression requires a β -cell product (Li et al., 2005).

3. Effects on Pancreatic Exocrine and Ductal Cells—Our study using the AR42J acinar cell line derived from a rat pancreatic tumor has shown that GLP-1Rs exist on these cells and that treatment with GLP-1 and Ex-4 causes increases in both intracellular cAMP and Ca^{2+} levels (Zhou et al., 1999a). GLP-1Rs were recently confirmed to be present in pancreatic ducts in mouse, rat, and human by the same group that found GLP-1Rs to be present in only β cells within islets (Tornehave et al., 2008).

Activation of GLP-1R signaling either in ductal or acinar cell lines or in vivo in rodents has resulted in differentiation of a fraction of these cells toward an islet-like phenotype, in association with activation of PKC and MAPK, and transcription factors necessary for an endocrine phenotype such as PDX-1, as well as the glucose-sensing factors glucokinase and GLUT2 (Zhou et al., 1999b, 2002; Hui et al., 2001). GLP-1R activation of those cells also affects transforming growth factor- β signaling pathways, resulting in reduced Smad activity that is conducive to endocrine phenotypes (Yew et al., 2004, 2005; Tei et al., 2005). AR42J cells, even without GLP-1R activation, have the potential to be converted into endocrine (Mashima et al., 1996) or exocrine cells (Christophe, 1994) but are negative for islet hormones and their transcripts under usual culture conditions (Zhou et al., 1999b). When these cells were exposed to GLP-1 or Ex-4, approximately 20% of the cells contained insulin protein and were capable of releasing insulin in a glucose-mediated fashion (Zhou et al., 1999b). This GLP-1 effect was also observed in Capan-1 cell line (Zhou et al., 2002) and rat ARIP and human PANC-1 cell lines; similar to the AR42J cells, GLUT2 and glucokinase transcripts were

induced in these cell lines (Hui et al., 2001). In particular, the differentiation-promoting activity of GLP-1 requires the expression of PDX-1, because PANC-1 cells, which lack endogenous PDX-1, differentiate only when transfected with PDX-1, whereas rat ARIP cells that express PDX-1 are susceptible to undergoing differentiation into insulin-secreting cells (Hui et al., 2001). In the Capan-1 cell line, differentiation to insulin-producing cells was also seen when they were transfected with PDX-1, and PDX-1 antisense totally inhibited such conversion (Zhou et al., 2002). Moreover, both nondiabetic and diabetic *Lep^{db}/Lep^{db}* mice treated with Ex-4 for 2 weeks show enhanced expression of PDX-1 in the ducts (favoring the presence of GLP-1Rs in ductal cells, as referenced above) and the exocrine tissue (Stoffers et al., 2000), which means that GLP-1R agonists aid in islet neogenesis, because ductal cells have been thought to be the main source for endocrine neogenesis (Egan et al., 2003).

VII. Extrapancreatic Effects of Incretins

A. Extrapancreatic Effects of Glucose-Dependent Insulinotropic Peptide

Several studies have demonstrated that GIPRs are present in the central nervous system, adipose tissues, and osteoblasts, but GIP actions, unlike those of GLP-1 on extrapancreatic tissues, have not received much attention.

1. Central Nervous System—GIPRs have been detected in many areas of the brain, including the hippocampus and hippocampal progenitor cells (Usdin et al., 1993; Kaplan and Vigna, 1994; Nyberg et al., 2005). Although for years it was assumed that GIP was expressed only in K cells and salivary glands, GIP mRNA and protein are also expressed in rat retina and the olfactory system (Cho et al., 2002; Nyberg et al., 2005). Hippocampus, Purkinje cells, amygdala, substantia nigra, striatum, and several areas of the thalamus, hypothalamus and brainstem (Nyberg et al., 2005, 2007; Sondhi et al., 2006) are all positive for GIP mRNA and/or protein. Exogenous administration of GIP increased hippocampal progenitor cell proliferation in vivo and in vitro (Nyberg et al., 2005). Conversely, *GIPR(-/-)* mice exhibit significantly reduced numbers of new proliferating cells in the hippocampal dentate gyrus compared with wild-type mice, implying a role for GIP in neurogenesis (Nyberg et al., 2005). Moreover, transgenic mice that overexpress the GIPR display enhanced exploratory behavior in the open-field locomotor activity test and also increased performance in some of the motor function tests compared with wild-type mice, suggesting that GIPR plays a role in the regulation of locomotor activity and exploration (Ding et al., 2006). Thus, GIP and GIPR are indeed expressed in the CNS and seem to play a role in neural progenitor cell proliferation and behavior modification (Nyberg et al., 2005, 2007; Ding et al., 2006). Whether this is true for humans is unknown.

2. Gastrointestinal Tract—"Gastric inhibitory polypeptide," as already stated, was originally characterized for its inhibition of gastric acid secretion (Brown and Dryburgh, 1971; Pederson and Brown, 1972). However, although its inhibitory effect on gastric acid secretion is seen under physiologic conditions in dogs (Wolfe et al., 1983), humans require supraphysiologic plasma concentrations to bring about gastric acid inhibition (Nauck et al., 1992). Upper and lower GI motility is inhibited by GIP at supraphysiologic plasma concentrations in healthy human subjects (Thor et al., 1987), but the rate of gastric emptying is unaffected by GIP, even at highly supraphysiologic plasma levels (Meier et al., 2004).

3. Adipose Tissue—GIP seems to have effects on insulin sensitivity of adipocytes as well as being involved in fat metabolism and the development of obesity. Fat intake stimulates GIP secretion in humans, and plasma GIP concentrations have been shown to be elevated in obese persons (Falko et al., 1975; Creutzfeldt et al., 1978; Ross and Dupre, 1978; Flatt et al., 1983). Functional GIPRs are present on adipocytes, and their numbers increase when preadipocyte

3T3-L1 cells undergo differentiation to adipocytes (Yip et al., 1998). Thus, GIP possibly plays a role as an anabolic regulator of fat metabolism (Yip and Wolfe, 2000). GIP also inhibits glucagon-stimulated lipolysis and enhances lipoprotein lipase activity. In isolated adipocytes or adipocyte cell lines, GIP potentiates the effects of insulin by promoting glucose and fatty acid uptake and fatty acid storage in triglycerides. More recent work has confirmed earlier findings and identified some GIP-stimulated signaling pathways involved in increasing fat storage in adipocytes (Kim et al., 2007b, c; Song et al., 2007). Kim et al. (2007b,c) show that GIP increases lipoprotein lipase activity and triglyceride accumulation through a pathway involving increased PKB phosphorylation and decreases in LKB1 and AMP-activated protein kinase phosphorylation in differentiated 3T3-L1, cultured subcutaneous human adipocytes, and epidermal fat tissue of the VDF Zucker rat (Kim et al., 2007b) and these effects of GIP are mediated through the direct regulation of adipokine resistin release (Kim et al., 2007c). Moreover, GIPR expression is enhanced during the differentiation process of adipocytes, similar to that described in 3T3-L1 cells (Yip et al., 1998), and activation of GIPR up-regulates the expression of aP2, a fat cell marker, and enhances adipocyte glucose uptake through PKB activation and subsequent membrane translocation of GLUT4 (Song et al., 2007).

Of great interest to diabetes and obesity researchers are data generated using *GIPR(-/-)* mice suggesting that GIP is an obesity-promoting factor linking overnutrition to obesity (Miyawaki et al., 2002). In normal mice, high-fat diet leads to increased adipocyte mass and insulin resistance, whereas *GIPR(-/-)* mice fed high-fat diets do not become obese or develop insulin resistance. The mice seem to have a higher energy expenditure rather than lower energy intake compared with wild-type mice. Moreover, double homozygous [*GIPR(-/-)*, *Lep^{ob}/Lep^{ob}*] mice, as stated earlier, have a lower adipocyte mass and do not become obese (Miyawaki et al., 2002). Consistent with these results, daily administration of the GIPR antagonist (Pro³) GIP to *Lep^{ob}/Lep^{ob}* mice improves glucose tolerance, prevents onset of insulin resistance, and prevents abnormalities of islet structure and function, suggesting that antagonizing GIP's actions might serve as an effective means of controlling body weight (Gault et al., 2005). In addition, normal mice given a high-fat diet for 160 days and then given (Pro³) GIP for an additional 50 days while being maintained on the high-fat diet demonstrate amelioration of their insulin resistance accompanied by a drop in liver and muscle levels of triglycerides, a drop in circulating levels of triglycerides, and a significant drop in their body weight, without a decrease in food intake (McClellan et al., 2007).

Some of these effects resulting from absence of GIPR in adipose tissue are similar to what occurs with peroxisome proliferator-activated receptor γ activation by thiazolidinediones (TZDs). TZDs cause a decrease in visceral fat, increased insulin sensitivity, increased triglyceride storage in the subcutaneous space, and decreased plasma triglyceride levels (Rizos et al., 2008). TZDs, however, lead to weight gain, most likely because of increased subcutaneous fat and fluid retention. But it is interesting to speculate that GIPR signaling influences the actions of adipose peroxisome proliferator-activated receptors, and this area of interaction might be interesting to study.

On assimilation all of the in vitro and in vivo work, and to understand how GIPR blockage might lead to increased energy expenditure, one can hypothesize that when GIPRs are blocked in adipocytes, uptake and feed-forward oxidation of fatty acids by liver and muscle is favored, because these tissues, lacking GIPRs, are not directly affected by the antagonist. However, one must be aware of how whole-body glucose homeostasis may be affected, because antagonism of GIP action also leads to elevated blood glucose levels, as reported from several animal studies (Tseng et al., 1996b; Miyawaki et al., 1999) as a result of the inhibition of the insulinotropic action of GIP in β cells. In addition, no direct link between obesity and GIP in humans has been reported, and no studies of GIPR antagonism have been performed in humans.

Thus, additional studies are required to fully delineate and characterize the biological significance and the complex nature of GIP or its analogs.

4. Bone—Functional GIPRs are present on bone cells, including osteoblasts and osteocytes in bone proper as well as in established osteoblast-like cell lines, and GIP seems to have a function in these cells (Bollag et al., 2000). GIPRs have also been detected in osteoclasts, and administration of GIP inhibited bone resorption and the resorptive activity of mature osteoclasts in an organ culture system (Zhong et al., 2007). Addition of GIP to osteoblast-like cells also leads to an increase in cAMP content, intracellular Ca^{2+} , collagen type I expression, and alkaline phosphatase activity, all of which are associated with bone formation (Bollag et al., 2000). Consistent with an anabolic effect of GIP on bone, administration of GIP prevents bone loss in an ovariectomized rat model (Bollag et al., 2001), and GIP-overexpressing transgenic mice have a significant increase in bone mass, decreased biochemical markers of bone resorption and increased markers of bone formation, all once again suggesting that GIP inhibits bone resorption and stimulates bone formation (Xie et al., 2007a). Conversely, absence of GIPR results in decreased bone size and mass, increased bone breakdown, and reduced bone turnover (Xie et al., 2005; Tsukiyama et al., 2006). In addition, GIPR(−/−) mice show increased plasma Ca^{2+} concentration after meal ingestion, suggesting that when GIP is not present, efficient storage in bone of the meal-derived Ca^{2+} does not occur (Tsukiyama et al., 2006). However, these effects of GIP have not been described in human studies.

B. Extraprancreatic Effects of Glucagon-Like Peptide-1

1. Central and Peripheral Nervous System Effects on Food Intake and Glucose Homeostasis—A number of studies have proposed the reciprocal systems, “the gut-brain axis” (as opposed to brain-gut signaling), between the GI tract and the CNS to regulate short- and long-term energy homeostasis, such as the appetite, food intake, and body weight, in which the GI tract senses the presence of food and signals to the hypothalamus and brainstem, key brain regions involved in feeding control, via neural and endocrine mechanisms. It is clear that GLP-1 is a key regulator of the appetite, food intake, and body weight in this axis. Rodent studies indicate that GLP-1R agonists reduce short-term food intake when injected either peripherally or into the CNS, and their repeated injection significantly inhibits not only food intake but also weight gain (Tang-Christensen et al., 1996; Turton et al., 1996; Meeran et al., 1999; Baggio et al., 2004a; Abbott et al., 2005), implying that both peripheral GLP-1 from the intestinal L cells and central GLP-1 from neurons of the nucleus of the solitary tract (NTS) in the brainstem (Jin et al., 1988) regulate food intake and body weight. These effects of GLP-1 are observed in lean and obese as well as diabetic humans (Flint et al., 1998; Näslund et al., 1999; Verdich et al., 2001; Zander et al., 2002). Conversely, antagonist of GLP-1R, such as with Ex-9-39, inhibits the effect of GLP-1 (Turton et al., 1996; Meeran et al., 1999) and exenatide has no effect on feeding in *GLP-1R*(−/−) mice (Baggio et al., 2004a). In addition, GLP-1R is present in the nodose ganglion neurons of the vagus nerve and several regions of CNS, including the NTS of brainstem and hypothalamus (Göke et al., 1995a; Larsen et al., 1997; Nakagawa et al., 2004). Thus, these suggest that GLP-1 has direct effects through GLP-1Rs in the brain.

Peripherally administered GLP-1 regulation of appetite and food intake seems to involve both the brainstem-vagus complex and the hypothalamus. As stated above, GLP-1R is expressed in the nodose ganglion neurons of the vagus nerve, brainstem, and hypothalamus. Like central GLP-1, peripherally injected GLP-1 activates neural c-Fos expression, a marker of neural activation (Sagar et al., 1988), in the paraventricular nucleus (PVN) of the hypothalamus (Baggio et al., 2004a; Dakin et al., 2004; Abbott et al., 2005). Feeding inhibition of GLP-1 is abolished by ablation of vagal-brainstem-hypothalamic pathway through either subdiaphragmatic total truncal vagotomy or surgical transection of brainstem-hypothalamic

pathway in rodents (Abbott et al., 2005), and also by capsaicin, which causes selective degeneration of small diameter unmyelinated sensory neurons, including the nodose ganglion and the vagus nerve (Talsania et al., 2005). In addition, recombinant GLP-1-albumin (albugon) (Baggio et al., 2004b) and Ex-4-albumin (Baggio et al., 2008) fusion proteins that are unable to cross the blood-brain barrier (BBB) activate c-Fos expression in the multiple regions of CNS, including the area postrema (AP), NTS, and PVN, and inhibit food intake, gastric emptying, and weight gain when peripherally administered. It has been also demonstrated that circulating GLP-1 in the portal vein is converted to neural information by the hepatic vagus, implying that this information may be conveyed to various parts of the CNS so as to further distribute a variety of information to target organs, including the pancreas (Nishizawa et al., 1996). This is substantiated by the observation that direct intraportal GLP-1 injections facilitate the activation of the pancreatic vagal efferents in normal rats (Nakabayashi et al., 1996). Consistent with these studies, and further substantiating indirect effects of GLP-1 even on β cells, GLP-1-dependent insulin secretion was blocked by the ganglionic blocker chlorisondamine (Balkan and Li, 2000) and by capsaicin (Ahrén, 2004). Central GLP-1 and Ex-4 infusion, but not Ex-9-39, also promote glucose-stimulated insulin secretion (Knauf et al., 2005; Sandoval et al., 2008). Moreover, GLP-1 injections into the portal vein stimulate peripheral glucose disposal through activation of the gut-glucose sensors, and this pathway is mediated by central GLP-1R signaling because increased glucose clearance after glucose infusion into the portal vein is abrogated by coinfusion with Ex-9-39 and absent in *GLP-1R* ($-/-$) mice (Burcelin et al., 2001). It is also possible that peripheral GLP-1 directly interacts with central GLP-1Rs, because GLP-1 is a small molecule that can cross the BBB. Indeed, several regulatory peptides from the periphery access the brain via diffusion (Larsen and Holst, 2005), and circulating GLP-1 is accessible to GLP-1Rs in the subformal organ and the AP (Orskov et al., 1996). In addition, ghrelin (Chelikani et al., 2006) and leptin (Goldstone et al., 1997; Goldstone et al., 2000) may exert their effects on food intake at least in part through attenuating or enhancing the effects of GLP-1, respectively.

It is noteworthy that Knauf et al. (2005) have suggested a novel function of central GLP-1 in the control of whole-body and tissue-specific glucose homeostasis that is distinct and independent from that of peripheral GLP-1 and GLP-1Rs. They demonstrate that during hyperglycemia, blockade of the central GLP-1R by administration of Ex-9-39 into the CNS increased muscle glycogen deposition independent of muscle insulin action, whereas insulin secretion and hepatic glycogen levels were reduced. Therefore, centrally acting GLP-1 favors glucose uptake into liver at the expense of muscle uptake. In addition, central exenatide infusion induced muscle/adipose insulin resistance and increased insulin secretion and liver glycogen storage. More recently, they suggest that control of whole-body glucose utilization and muscle glycogen synthesis are regulated by enteric glucose sensors and that the glucose sensors require GLP-1R signaling in the brain. Low-rate intragastric infusion of glucose leads to c-Fos activation in both brainstem and hypothalamus in wild-type mice but not in *GLP-1R* ($-/-$) mice (Knauf et al., 2008).

2. Gastrointestinal Tract and Gastric Emptying—Unlike GIP, which does not affect to gastric emptying in humans (Meier et al., 2004), GLP-1 has been identified as a potent inhibitor of several gastrointestinal functions, such as gastric acid secretion, gastric emptying, and motility, thereby slowing the entry of nutrients into the circulation and contributing to the normalization of blood glucose levels. Given its inhibitory function of gastric acid secretion and motility, GLP-1 is thought to be an important mediator of “ileal brake effect”—the endocrine inhibition of upper gastrointestinal functions activated by the presence of nutrients in the ileum (Layer et al., 1992; Holst, 1997; Wettergren et al., 1997a,b). Deceleration of gastric emptying by GLP-1 is observed in both rodents and humans (Wettergren et al., 1993; Wishart et al., 1998; Chelikani et al., 2005), and this effect is blocked by administration of the Ex-9-39 (Imeryüz et al., 1997). Moreover, inhibition of gastric emptying also lowers blood glucose

levels in patients with type 1 or type 2 diabetes (Dupre et al., 1995; Willms et al., 1996; Meier et al., 2003a).

The mechanism(s) whereby GLP-1R activation causes slowing of gastric emptying is not fully worked out but are most likely neurally mediated. Peripherally administered Albugon and Ex-4-albumin activate c-Fos expression in the multiple regions of CNS and inhibit gastric emptying (Baggio et al., 2004b, 2008). In addition, Wettergren et al. (1994) have demonstrated that GLP-1 in physiological concentrations inhibits vagally stimulated acid secretion in humans during modified sham feeding, and the inhibitory effect is impaired in subjects that were vagotomized for duodenal ulcer disease (Wettergren et al., 1997b). Further studies also show that vagal afferent denervation abolished the central and peripheral action of GLP-1 on gastric emptying, whereas intracerebroventricular administration of Ex-9-39 was ineffective (Imeryüz et al., 1997). Thus, the mechanism by which GLP-1 inhibits gastric emptying is thought to involve the gut-brain axis mediated by neural mechanisms via vagal nerves dependent on GLP-1Rs. However, after vagal deafferentation, GLP-1 still inhibits antral motility in pigs (Nagell et al., 2006), and GLP-1 causes contraction of smooth muscle cells from the human colon, which is blocked by Ex-9-39 (Ayachi et al., 2005). Moreover, GLP-1Rs are present in the GI tract, including stomach and intestine, and recent studies show that genetic variation in stomach GLP-1R expression is associated with altered rates of gastric emptying in mice (Kumar et al., 2008). Thus, it remains possible that GLP-1 also regulates gastric emptying at least in part via a direct mechanism in the gut.

3. Muscle, Adipose Tissue, and Liver—GLP-1 seems to have insulin-like effects in major extrapancreatic tissues, participating in glucose homeostasis and lipid metabolism in tissues such as muscle, liver, and adipose tissues. However, GLP-1 treatment did not seem to be associated with intracellular cAMP generation in those tissues but with inositol phosphoglycan generation. Therefore, GLP-1 actions seem different from that in the pancreas (Galera et al., 1996; Trapote et al., 1996; Márquez et al., 1998; Yang et al., 1998; Luque et al., 2002). In L6 myotubes transfected with pancreatic GLP-1R, GLP-1 inhibits glycogen synthesis, whereas, in parental L6 myotubes, GLP-1 enhances insulin-stimulated glycogen synthesis (Yang et al., 1998). However, there is considerable controversy with respect to the presence of functional GLP-1Rs in these tissues, and it is unclear whether or not GLP-1 acts independently of the islet hormones, such as insulin, glucagon, and somatostatin.

In human skeletal muscle strips and primary culture myotubes, GLP-1 and Ex-4, but not Ex-9-39, stimulated glycogen synthase α activity and glycogen synthesis in association with activation of PI-3K/PKB and p42/44 MAPK pathways (Acitores et al., 2004; González et al., 2005), and they also stimulated glucose oxidation and utilization, as did insulin (Alcántara et al., 1997; Luque et al., 2002). These effects are preserved in STZ-treated diabetic rats (Morales et al., 1997). GLP-1R agonists also stimulate glucose uptake along with increases in glucose transporter levels via pathways that involve PI-3K activation in normal and diabetic rats (Villanueva-Peñacarrillo et al., 2001; Idris et al., 2002; González et al., 2005). It is noteworthy that Ex-9-39 also has effects similar to GLP-1 in normal human myocytes (Luque et al., 2002; González et al., 2005) and L6 myotubes (Yang et al., 1998).

GLP-1 also stimulates glucose uptake, lipogenesis, and lipolysis in adipocytes and has the ability to stimulate fatty acid synthesis in omental adipose tissue culture. Several studies show that injection of a recombinant adenovirus expressing GLP-1 into obese diabetic mice increases insulin-stimulated glucose uptake in adipocytes (Lee et al., 2007), and GLP-1 also augments insulin-stimulated glucose uptake along with an increase in glucose transporter levels in 3T3-L1 adipocytes (Wang et al., 1997a; Gao et al., 2007). In normal human adipocytes, GLP-1 and Ex-4, but not Ex-9-39 increase glucose uptake in association with activation of PI-3K and MAPK, and this effect is impaired in obese patients (Sancho et al., 2007). PI-3K and MAPK

pathways are also involved in GLP-1-mediated lipogenesis and lipolysis in rat adipocytes (Sancho et al., 2005). GLP-1 infusion in rat also reduces intestinal lymph flow, triglyceride absorption, and apolipoprotein production, although it does not affect cholesterol absorption, suggesting the effect of GLP-1 to limit the excursion of lipids, like glucose, after meals (Qin et al., 2005).

Stimulating effects of GLP-1 on glycogen synthase α activity and glycogen synthesis have been shown in hepatocytes isolated from normal and diabetic rats (Morales et al., 1997; Redondo et al., 2003), and these effects seem to occur through a cellular signaling pathway that involves activation of PI-3K, PKB and PKC (Redondo et al., 2003). Recent studies have shown that transduction of GLP-1 using adenovirus system into obese diabetic mice reduces hepatic gluconeogenesis and hepatic expression of phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and fatty acid synthase and improves insulin signaling and insulin sensitivity (Lee et al., 2007).

4. Bone—In contrast to GIPRs, GLP-1Rs has not been found in bone. However, recent studies have shown that *GLP-1R(-/-)* mice have cortical osteopenia and enhanced bone fragility that is likely to be attributable to increased bone resorption by osteoclasts (Yamada et al., 2008). Unlike GIP, GLP-1 has no direct effects on osteoclasts and osteoblasts in in vitro experiments, so the *GLP-1R(-/-)* mice may have osteopenia because of reduced levels of calcitonin, which is a known inhibitor of bone resorption that is produced by thyroid C cells (Zaidi et al., 2002); before common usage of bisphosphonates to treat Paget's disease, it was the drug of choice in inhibiting osteoclast activity in that disease. It has been reported that GLP-1Rs are present in rat and murine thyroid C cells and that GLP-1 stimulates the secretion of calcitonin via a cAMP-dependent mechanism in cultured murine C cells (Crespel et al., 1996; Lamari et al., 1996). Consistent with these results, Ex-4 administration into normal mice increases *calcitonin* gene expression in thyroid glands. Thus, the authors suggest that GLP-1R signaling may regulate bone resorption and turnover through a calcitonin-dependent pathway (Yamada et al., 2008).

5. Cardiovascular System—GLP-1Rs are present on rodent and human cardiac myocytes and endothelial cells and vascular smooth muscle cells (Richter et al., 1993; Wei and Mojsov, 1995; Bullock et al., 1996; Nyström et al., 2004; Ban et al., 2008) and the deduced amino acid sequences of the GLP-1Rs from heart are the same as those from pancreas (Wei and Mojsov, 1995). *GLP-1R(-/-)* mice have structural and functional cardiac abnormalities, including cardiac contractility and diastolic dysfunction and changes in heart weight, wall thickness, and resting heart rate (Gros et al., 2003). In addition, activation of GLP-1Rs in brain may modulate cardiovascular functions. Centrally and/or peripherally administered GLP-1R agonists increase blood pressure and heart rate (Barragán et al., 1994, 1999; Yamamoto et al., 2002), and these effects are blocked by Ex-9-39 (Barragán et al., 1999). In rats, bilateral vagotomy blocks the stimulating effect of central GLP-1 administration on blood pressure and heart rate and also prevents the blocking effect of central but not peripheral Ex-9-39 (Barragán et al., 1999). Administration of GLP-1 activates GLP-1R-expressing neurons in autonomic regulatory sites in the rat brain that modulate cardiovascular function (Yamamoto et al., 2002), and experiments show that GLP-1R-expressing catecholamine neurons in the AP link peripheral GLP-1 and central autonomic control sites that mediate diverse neuroendocrine and autonomic actions of peripheral GLP-1 (Yamamoto et al., 2003). Based on these studies, the effects of GLP-1 on heart rate and blood pressure are mediated by neuroendocrine and autonomic control via vagal nerves dependent on GLP-1Rs. Besides acute effects on cardiac hemodynamics, GLP-1 seems to have beneficial effects on cardiac function. A 72-h infusion of GLP-1 improves regional and global left ventricular function in patients with acute myocardial infarction and severe systolic dysfunction after successful primary angioplasty (Nikolaidis et al., 2004b). The same authors also demonstrate that a 48-h infusion of GLP-1

in conscious dogs with advanced dilated cardiomyopathy improves left ventricular function and systemic hemodynamics in association with increase in myocardial glucose uptake and decreases in plasma norepinephrine and glucagon levels, despite similar insulin levels in the saline control group, suggesting that GLP-1 exerts myocardial metabolic effects that are independent of insulinotropic effects of GLP-1 (Nikolaidis et al., 2004a).

Besides cardiac contractility effects, GLP-1 has been demonstrated to improve endothelial dysfunction. In particular, GLP-1 infusion significantly ameliorates endothelial dysfunction in patients with T2DM with an established coronary artery disease (Nyström et al., 2004). Recent studies have identified potential molecular mechanisms responsible for this effect on endothelial function (Liu et al., 2008). GLP-1 attenuates TNF- α -induced expression of plasminogen activator inhibitor-1, a regulator of plasminogen activation implicated in endothelial cell dysfunction (Norata et al., 2006), in a spontaneously transformed vascular endothelial cell line (C11-STH) and in primary human vascular endothelial cells. In addition, GLP-1 inhibits TNF- α -mediated expression of Nur77, an orphan nuclear receptor, and PKB phosphorylation. The authors suggest that GLP-1 may improve endothelial dysfunction through inhibition of TNF- α -mediated plasminogen activator inhibitor-1 induction in vascular endothelial cells, and this effect may involve PKB-mediated signaling pathways and the modulation of Nur77 expression (Liu et al., 2008). However, recent studies also show that GLP-1 agonists have cardiovascular effects independent of the autonomic nervous system (Gardiner et al., 2008) and even independent of known GLP-1R-linked pathways (Ban et al., 2008).

Besides affecting myocardial performance, GLP-1R signaling has been also shown to exert cardioprotective effects against ischemic damage or heart failure. In studies using both isolated perfused rat heart and whole-animal models of ischemia/reperfusion, GLP-1 significantly reduces infarction size compared with the saline control group, and this protection is abolished by Ex-9-39, the cAMP inhibitor Rp-cAMP, the PI3K inhibitor LY294002, and the p42/44 MAPK inhibitor U0126 in the in vitro hearts (Bose et al., 2005). GLP-1 also increases myocardial glucose uptake by increasing nitric oxide production and GLUT1 translocation and enhances recovery of cardiac function after low-flow ischemia-reperfusion injury in rat heart (Zhao et al., 2006) and recovery from ischemic myocardial stunning after ischemia/reperfusion in conscious canines (Nikolaidis et al., 2005). Although the effect is small, GLP-1 infusion in patients with T2DM and heart failure improves metabolic control and left ventricular function (Thrainsdottir et al., 2004). Consistent with this study, long-term infusion of GLP-1 in patients with severe heart failure significantly improves left ventricular function, and the effect is also seen in patients with and without diabetes (Sokos et al., 2006). More recently, studies using isolated, perfused rat heart have also shown that GLP-1R agonists protect the heart against myocardial ischemic-reperfusion injury (Bose et al., 2007; Huisamen et al., 2008; Sonne et al., 2008), and this protection is blocked by Ex-9-39 (Sonne et al., 2008). Moreover, other studies also suggest the involvement of PI3K/PKB pathways in this protection (Bose et al., 2007; Huisamen et al., 2008). However, some cardioprotective and vasodilatory actions of GLP-1 are preserved in *GLP-1R(-/-)* mice after ischemia/reperfusion (Ban et al., 2008) and are thought to be mediated, at least in part, by its insulinotropically inactive metabolite, GLP-1 (9–36) (Ban et al., 2008; Sonne et al., 2008), consistent with previous findings in dogs (Nikolaidis et al., 2005). Thus, these findings suggest that GLP-1 (9–36) is a biologically active substance, at least in the cardiovascular system, and there exists an alternative pathway of action for GLP-1 (9–36) that must be independent of the known GLP-1R.

6. Hypothalamic-Pituitary Axis—Besides affecting food intake, several studies have shown that GLP-1 is involved in the regulation of the hypothalamic pituitary axis. In addition to being present in multiple regions of hypothalamus, GLP-1R is also present in the rat pituitary (Göke et al., 1995b). It is likely that GLP-1-expressing nerve terminals establish direct synaptic

contacts with corticotropin-releasing hormone neurons in the PVN, suggesting that GLP-1 may modulate the hypothalamic pituitary axis through direct effect (Shughrue et al., 1996; Larsen et al., 1997; Rinaman, 1999; Sarkar et al., 2003) and in line with this thought, an infusion of GLP-1 to humans was shown to increase plasma adrenocorticotropin secretion after 30 min, with a resultant increase in serum cortisol levels (Ryan et al., 1998). In rodents, central injection of GLP-1 increases levels of adrenocorticotropin and corticosterone (Larsen et al., 1997; Kinzig et al., 2003), and GLP-1 antagonists block increases in these hormones induced by the LiCl (Kinzig et al., 2003). GLP-1 stimulates thyroid-stimulating hormone release from a rodent thyrotrope cell line as well as from rat primary anterior pituitary cells (Beak et al., 1996) and also stimulates luteinizing hormone-releasing hormone release from a rodent hypothalamic neuronal cell line (Beak et al., 1998); both are associated with increased intracellular cAMP concentration that is inhibited by GLP-1 antagonists. Furthermore, central administration of GLP-1 increases plasma levels of luteinizing hormone in rats (Beak et al., 1998). However, *GLP-1R(-/-)* mice show normal hypothalamic *CRH* gene expression and plasma levels of corticosterone, thyroid hormone, testosterone, estradiol, and progesterone under usual conditions, but they exhibit increased corticosterone responses to stress (MacLusky et al., 2000), which seems at variance with the human data mentioned above (Ryan et al., 1998).

VIII. The Development of Therapies for Diabetes Based on the Incretin Actions

A. Exenatide

DPP4 cleaves peptides with an alanine, proline, or hydroxyproline in the penultimate N-terminal position, and therefore various modifications of GLP-1 at His⁷, Ala⁸, or Glu⁹ have been investigated for insulinotropic action. Additional mid-chain modifications of the GLP-1 peptide to prevent neutral endopeptidase (NEP) hydrolysis are also being investigated to provide longer biological activity. As of now, GLP-1R agonists and GLP-1 analogs resistant to DPP4 degradation and NEP hydrolysis are at various stages of preclinical or clinical development. Exenatide (synthetic Ex-4) is the only GLP-1R agonist approved by regulatory agencies as an adjunct therapy in the general population suffering from T2DM and not otherwise achieving satisfactory glycemic control. It is a 39-amino acid peptide produced in the salivary glands of the Gila monster lizard (*Heloderma suspectum*) (Eng et al., 1992). It has 53% amino acid homology to full-length GLP-1, and it binds with greater affinity than GLP-1 to the GLP-1R in GLP-1 receptor-expressing cells (Göke et al., 1993; Thorens et al., 1993). There seems to be no specific Ex-4 receptor in the Gila monster. Ex-4 is not a substrate for DPP4 because it has a Gly⁸ in place of an Ala⁸. In addition, some of the target bonds for NEP are absent in Ex-4 and its secondary and tertiary structures may also prevent NEP action, thereby leading to a prolonged half-life. Exenatide, a peptide, must be injected subcutaneously. It was shown to be eliminated by the kidneys through glomerular filtration in a nonhuman study (Simonsen et al., 2006). In human studies, the mean half-life ranged from 3.3 to 4 h (Kolterman et al., 2005). It is detected in the plasma up to 15 h after subcutaneous injection and its biological effect remains for up to at least 8 h after dosing (Kolterman et al., 2005). It has ~5000-fold greater potency in lowering blood glucose than GLP-1 (Parkes et al., 2001). In addition, it has all of the same effects in the pancreas, GI tract, and brain as GLP-1.

1. Relevant Clinical Studies—In a short-term study, i.v. infusion of exenatide was shown to stimulate insulin secretion in a glucose-dependent manner and to delay gastric emptying in both nondiabetic subjects as well as T2DM (Egan et al., 2002). Exenatide has also been shown to restore first-phase insulin secretion in response to i.v. glucose in patients with T2DM, hence improving the ability of β cells to respond to rapid glycemic changes (Fehse et al., 2005).

The first three large clinical trials of exenatide, which lead to its approval by regulatory agencies, were randomized, placebo-controlled, blinded 30-week studies that examined the effects of exenatide in subjects with T2DM not achieving adequate glycemic control on

metformin and/or sulfonylurea (Buse et al., 2004; DeFronzo et al., 2005; Kendall et al., 2005). In these trials, exenatide was given as twice daily (b.i.d.) subcutaneous injection of 5 μ g for 4 weeks followed by 5 or 10 μ g b.i.d. for the remainder 26 weeks. In all three studies, by 30 weeks exenatide had significantly reduced HbA_{1c} in the treatment groups. With sulfonylurea/10 μ g exenatide b.i.d., HbA_{1c} was lowered by 0.86%, and with sulfonylurea/5 μ g exenatide b.i.d., HbA_{1c} was lowered by 0.46%. In the metformin/exenatide 10 μ g b.i.d. arm, 41% of patients achieved HbA_{1c} \leq 7% and had a mean weight loss of 2.8 kg. In the sulfonylurea/exenatide 10 μ g b.i.d. arm, 41% of patients also achieved HbA_{1c} of \leq 7% and had a mean weight loss of 1.6 kg, and with the triple combination of metformin/sulfonylurea/exenatide 10 μ g b.i.d., 34% of patients achieved HbA_{1c} of \leq 7% and had a mean weight loss of 1.6 kg. In all three trials, nausea was more common during the initial weeks of therapy and then declined. The dropout rate in the three trials as a result of nausea was 2 to 4% in the exenatide arms. Nausea was not correlated with weight loss; subjects who never reported nausea also experienced some weight loss. Adding exenatide to metformin also did not increase the risk of hypoglycemia. As sulfonylureas close K_{ATP} channels on β cells, leading to depolarization followed by increased insulin secretion, one would expect that an exenatide/sulfonylurea combination would place patients at risk for hypoglycemic episodes and, indeed, adding exenatide to sulfonylurea increased the incidence of mild to moderate hypoglycemia to 36% in the 10 μ g arm and 14% in the 5 μ g arm compared with a hypoglycemic rate of 3% in the placebo arm. However, no cases of severe hypoglycemia were reported. Likewise, when exenatide was added to metformin/sulfonylurea, the incidence of mild to moderate hypoglycemia was 28% (10 μ g), 19% (5 μ g), and 13% (placebo), with one case of severe hypoglycemia reported in the 5 μ g arm. Anti-exenatide antibodies were detected in 41 to 49% of patients in the treatment arms but were not associated with loss of glycemic control (Buse et al., 2004; DeFronzo et al., 2005; Heine et al., 2005; Kendall et al., 2005; Nauck et al., 2007a; Zinman et al., 2007). Patients from the above three trials and their open-label extensions were folded into one open-ended, open-label trial. Results from its 3-year follow-up showed that with exenatide (10 μ g b.i.d.), a reduction in HbA_{1c} of 1.0% was sustained, with 46% achieving HbA_{1c} of \leq 7%, and reduction in body weight was progressive with weight loss of 5.3 kg at the end of 3 years of treatment (Klonoff et al., 2008).

Another trial studied the efficacy of exenatide (10 μ g b.i.d.) added to the TZDs rosiglitazone (\geq 4 mg/day) or pioglitazone (\geq 30 mg/day), alone or combined with metformin for 16 weeks. Compared with addition of placebo, addition of exenatide to a TZD in the presence or absence of metformin reduced HbA_{1c} by 1%, mean FPG by 30 mg/dl, and body weight by 1.5 kg. However, only 71% of subjects given exenatide compared with 86% of subjects in the placebo group completed the study. Many of the dropouts in the exenatide treatment groups (14%) were because of GI symptoms from exenatide (Zinman et al., 2007).

Exenatide has also been compared with insulin therapy as add-on to oral agents. In a 26-week multicenter, open-label, randomized, controlled trial, subjects with T2DM whose glycemia was not adequately controlled with metformin and/or sulfonylurea were randomized to either addition of 10 μ g b.i.d. exenatide or insulin glargine daily (titrating to FPG of <100 mg/dl) to their ongoing oral regimen. At the end of 26 weeks, both groups achieved similar improvement in glycemic control with HbA_{1c} reductions of 1.1% in both groups. There were subtle differences in the actual patterns of reductions in blood glucose in the groups. The exenatide group had better PPG control than the glargine group, but the glargine group had lower FPG levels, and an average weight loss of 2.3 kg was seen with exenatide treatment, compared with an average weight gain of 1.8 kg with glargine. Whereas those with longer durations of nausea tended to lose more weight, patients who did not experience nausea during the trial also lost weight, similar to the first three studies discussed above. The dropout rate was 19.4% with exenatide (6% due to nausea) and 9.7% with glargine (Heine et al., 2005).

Last, exenatide was also compared with biphasic insulin aspart (30% rapid-acting insulin aspart) in a 52-week, randomized, open-label trial in patients with T2DM whose glycemia was not sufficiently controlled with metformin and sulfonylurea (Nauck et al., 2007a). When comparing the exenatide group to the biphasic insulin group, reduction in HbA_{1c} was 1 versus 0.9% (difference not significant) and reduction in FPG was 1.8 versus 1.7 mM. The exenatide group had weight reduction of 2.5 kg, whereas the biphasic insulin group had a weight increase of 2.9 kg.

A meta-analysis of the randomized controlled trials with exenatide showed that severe hypoglycemia was rare. Mild-to-moderate hypoglycemia was 16 versus 7% (exenatide versus placebo) and more common with coadministration with a sulfonylurea. The most common side effects of exenatide were nausea (57%) and vomiting (17%), nausea usually mild to moderate in nature and being most common during the initial 8 weeks of therapy and declined thereafter. Overall, 4% of patients withdrew from the studies because of gastrointestinal side effects (Amori et al., 2007). Antiexenatide antibodies were present in 41 to 49% of patients in the treatment arms, but 1), their presence did not seem to impact the degree of glycemic control, and 2), increasing titer of antibody also did not seem to be reflected in worsening blood glucose control (Buse et al., 2004; DeFronzo et al., 2005; Heine et al., 2005; Kendall et al., 2005; Nauck et al., 2007a; Zinman et al., 2007). Because exenatide must be injected b.i.d., attempts have been made to extend its length of action. A formulation of exenatide (exenatide LAR) has already been tested in humans and seems to have potential for once weekly subcutaneous injection (Kim et al., 2007a). Future follow-up of exenatide treatment should, in our view, be accompanied by monitoring serum calcitonin and, if possible, bone outcomes due to the information mentioned above (section VII.B.4) on the effects of GLP-1 and Ex-4, and presumably, therefore, exenatide on murine thyroid C cells: to these authors' knowledge, any effect of exenatide on human thyroid C cells has not been reported. [Use of TZDs for treating T2DM is associated with greater bone loss in women (Schwartz et al., 2006).] It is possible that addition of exenatide to TZD treatment might prevent that side effect. It should also be noted that FDA has received information on six cases of pancreatitis, two of those six resulted in death, in patients on exenatide treatment (FDA Update, 8/18/2008). This leads us to speculate that activation of ductal cell GLP-1Rs by exenatide might be a causative factor in this life-threatening condition.

B. Sitagliptin

If pharmacological levels of exogenous GLP-1 result in lowering of blood glucose in type 2 diabetes, then supraphysiological levels of *endogenous* active GLP-1 should also lower blood glucose. No secretagogue of L cells has been specifically developed, although clear headway has been made in uncovering how food products, specifically fat and sweet-tasting foods, in the gut increase L cell secretion, as described above, section III. In addition, non-peptide-based GPR119 receptor agonists have been shown to increase both GLP-1 and GIP secretion, even though the receptors seem to be not present on K cells (Chu et al., 2008). Transgenic mice lacking DPP4 have elevated fasting incretin levels, lower plasma glucose, and higher plasma insulin levels after oral glucose compared with wild-type mice (Marguet et al., 2000). Many drug companies set about developing inhibitors of DPP4 that would be active in the gut in an effort to increase plasma levels of the full GLP-1 molecule, with the goal being reduced blood glucose levels. Sitagliptin is the sole DPP4 inhibitor in use for treatment of T2DM, though there are others in the pipeline. It is a small non-peptide-based orally active molecule that seems to be selective for DPP4 and to not interact with other closely related proteases (Kim et al., 2005a). Oral sitagliptin is rapidly absorbed and achieves peak plasma levels in 1 to 6 h. Its half-life is 8 to 14 h, and its bioavailability, in the presence or absence of food, is approximately 87% (Herman et al., 2005). Approximately 80% of the dose is excreted unchanged by the kidney, and a small amount (15%) of the bioavailable drug is metabolized by CYP3A4 and

CYP2C8 in the liver (Bergman et al., 2007). The maximum recommended dose is 100 mg daily because plasma DPP4 activity is inhibited by at least 80% over a 24-h period with this dose. Furthermore, 200 mg daily did not inhibit DPP4 activity any more than did the 100-mg dose (Raz et al., 2006). Dose size needs to be reduced to 50 mg if creatinine clearance is <50 ml/min and to 25 mg if creatinine clearance is <30 ml/min (Bergman et al., 2007).

1. Relevant clinical studies—The first clinical trial on sitagliptin was carried out on patients with T2DM who were not adequately controlled by diet and exercise in a randomized, double-blinded, placebo-controlled manner to uncover any possible glucose-lowering effect of sitagliptin monotherapy (Raz et al., 2006). After 18 weeks, the group on sitagliptin monotherapy achieved 0.5% reduction in HbA_{1c}. Those patients with HbA_{1c} < 8% experienced less HbA_{1c} reduction compared with those with HbA_{1c} > 8%, meaning that the higher the average blood glucose level, the greater the glucose-lowering effect.

Five randomized, double-blind, placebo-controlled trials of 24 weeks' duration of sitagliptin in patients with T2DM were then carried out in an attempt to define the patient population that might have most benefit from it (Aschner et al., 2006; Charbonnel et al., 2006; Rosenstock et al., 2006; Goldstein et al., 2007; Hermansen et al., 2007). These were: 1) sitagliptin monotherapy; 2) comparison of sitagliptin monotherapy, metformin monotherapy, and initial combination therapy of sitagliptin and metformin; 3) sitagliptin added to ongoing pioglitazone; 4) sitagliptin added to ongoing metformin; and, finally, 5) sitagliptin added to ongoing sulfonylurea and/or metformin. The reduction in HbA_{1c} was 0.6 to 0.7% with sitagliptin monotherapy (100 mg/day), 1.1% with metformin (2000 mg/day) alone, and 1.9% with sitagliptin/metformin (100 mg/2000 mg), whereas 20 to 41, 38, and 66% of patients, respectively, achieved HbA_{1c} < 7%. When sitagliptin (100 mg) was added to ongoing pioglitazone, HbA_{1c} was reduced by 0.7%, whereas 45% of patients achieved HbA_{1c} < 7%. When sitagliptin (100 mg) was added to ongoing sulfonylurea alone or to a combination of sulfonylurea/metformin, HbA_{1c} levels were reduced by only 0.3% and 0.6%, respectively, whereas only 11 and 23% of patients, respectively, achieved HbA_{1c} < 7% at the end of 24 weeks. Some of these trials were then extended to 54 weeks after completing the placebo-controlled phase, but the results are not yet available in manuscript form.

Another 52-week trial, looking at the efficacy and safety of sitagliptin versus glipizide added to ongoing metformin therapy, showed a reduction in HbA_{1c} of 0.7% in both the sitagliptin and the glipizide group. However, the maximal efficacy in HbA_{1c} reduction was observed at 24 to 30 weeks, with a gradual rise in HbA_{1c} from week 30 to 52, which raises the issue of declining sitagliptin efficacy (Nauck et al., 2007b).

A factor common to all DPP4 inhibitors is that their use actually leads to reduced incretin secretion (El-Ouaghlidi et al., 2007). This could be as much as 72% reduction in total GLP-1 secretion after an oral glucose tolerance test. Clearly, this severely limits the degree to which the active, endogenous level of incretins can actually be enhanced and therefore limits their glucose-lowering effects.

Sitagliptin is reported to be weight-neutral in all the clinical trials reported so far. A study is currently in the recruiting phase to investigate whether sitagliptin would be useful as an adjunct to exogenous insulin in patients with or without metformin treatment.

Pooled analysis of 5141 patients in clinical trials for ≤ 2 years showed that sitagliptin alone or added to other combinations (metformin, pioglitazone, sulfonylurea, or a sulfonylurea/metformin combination) was well tolerated, and hypoglycemia occurred only in the setting of combination therapy with sulfonylureas (Stein et al., 2007). The adverse events reported that were somewhat higher with sitagliptin compared with nonexposed groups were headaches,

nasopharyngitis, contact dermatitis, and arthralgias. In addition, urinary tract infections (3.1 versus 2.6% for comparator groups) are more common (Amori et al., 2007) and may reflect a lack of DPP4 activity required for immunosurveillance. Because urinary tract infections are also more common in diabetic subjects than in the general population, the increasing incidence with sitagliptin might be clinically relevant. In view of the expression of DPP4 in plasma and on cell membranes, especially on lymphocytes, ongoing interest in sitagliptin's long-term safety profile is needed. With DPP4 inhibition, metabolites of several peptides may not be formed over time, and their absence may have adverse effects that are not easily predicted. In addition, several parent compounds may accumulate, again with unpredictable effects. Longitudinal studies of the use of DPP4 for any evidence of a negative impact on cardiovascular disease as a result of nonformation of GLP-1 (9–36) amide as well as surveillance for any effects in the liver will be needed. Like exenatide, it is possible that sitagliptin may be beneficial in osteopenia because the increased GLP-1 levels might influence thyroid C cell secretion.

C. Therapies under Development

1. Vildagliptin—Vildagliptin (LAF237) is a selective, reversible, orally active competitive inhibitor of DPP4 under development by Novartis (Villhauer et al., 2003; Barlocco, 2004). It is rapidly absorbed and achieves peak plasma levels in 1 to 2 h (He et al., 2007c, 2008). Its half-life of 2 h is shorter than that of sitagliptin. Its bioavailability is approximately 85% (He et al., 2007b), and its pharmacokinetics seems unaffected by food (Sunkara et al., 2007). It inhibits 98% of DPP4 activity 45 min after oral dosing with 100 mg and 60% at 24 h. Approximately 69% of vildagliptin is hydrolyzed in the liver to LAY151, an inactive metabolite, and it, in addition to unaltered vildagliptin, are then excreted by the kidneys. Even though vildagliptin is subject to hydrolysis in the liver, a study suggested that there was no significant difference in exposure to vildagliptin in patients with various degrees of hepatic impairment, although there have been reports of elevated liver enzymes with the 100-mg dose. Although it has been suggested that changes in dosing with vildagliptin are not necessary in patients with liver disease (He et al., 2007a), this is not yet conclusive. Likewise, reductions in dose may not be needed in renal failure because such a large portion of the drug is inactivated in the liver. However, the FDA requested additional data on patients with renal impairment before granting approval of vildagliptin, and Novartis has, at least for now, withdrawn its application from the FDA; it is approved for use by the European Medicines Agency.

2. Liraglutide—Liraglutide, under development by Novo Nordisk, is a long-acting GLP-1 analog with a substitution of Lys³⁴ with Arg³⁴ and an attachment of a C-16 free-fatty acid derivative via a glutamoyl spacer to Lys²⁶ (Fig. 7). The free-fatty acid derivative promotes noncovalent binding of liraglutide to albumin, thereby increasing plasma half-life through protection from renal clearance and slowing the absorption rate from injection site. This is similar to how the Novo Nordisk has prolonged the actions of insulin detemir. As with exenatide, liraglutide needs to be injected subcutaneously. After subcutaneous injection, maximum plasma concentration is reached after 10 to 14 h, and it has a half-life of 11 to 13 h (Knudsen et al., 2000; Agersø et al., 2002).

a. Selected Clinical Trials: A liraglutide/metformin combination was associated with a 0.8% reduction in HbA_{1c} and a 70 mg/dl reduction in FPG compared with metformin alone in a 5-week dose-escalation study. In addition, liraglutide/metformin significantly reduced FPG (by 21.6 mg/dl) and body weight (by 2.9 kg) compared with metformin/glimepiride group; liraglutide/placebo significantly reduced FPG (by 25.2 mg/dl) compared with metformin/placebo group (Nauck et al., 2006).

In a 14-week study of liraglutide versus placebo, liraglutide significantly reduced HbA_{1c} by 1.45, 1.40, and 0.98% in the 1.90-, 1.25-, and 0.65-mg groups, respectively, whereas the

placebo group had an increase of 0.29% in HbA_{1c}. The percentages of patients who achieved HbA_{1c} ≤ 7% were 46, 48, and 38% in the 1.9, 1.25, and 0.65 mg groups, respectively (VilSBøll et al., 2007); only 5% of the placebo group achieved HbA_{1c} ≤ 7%. The results of a 52-week long head-to-head, placebo-controlled, double-blind study of two doses of liraglutide (1.2 and 1.8 mg daily) versus glimepiride, an orally active sulfonylurea, were recently published (Garber et al., 2008). The highest dose of liraglutide (1.8 mg daily) resulted in an HbA_{1c} reduction of 1.14%, compared with 0.51% reduction with 8 mg of glimepiride. It is noteworthy that HbA_{1c} reduction with liraglutide was maximal at 12 weeks and did not subsequently increase. This leads us to speculate that β-cell function did not deteriorate, at least over 1 year, in patients receiving liraglutide. Six patients exited the liraglutide groups because of vomiting. The liraglutide groups lost 2.0 to 2.5 kg body weight, whereas the glimepiride group gained 1.1 kg.

b. Side Effects: The most frequently reported adverse events were nausea and vomiting, especially at the higher doses, and two cases of pancreatitis have been reported (Amori et al., 2007; VilSBøll et al., 2007; Garber et al., 2008b; Madsbad, 2008). There is also no development of antibodies to GLP-1 noted in trials up to 14 weeks (Madsbad et al., 2004; Feinglos et al., 2005; VilSBøll et al., 2007).

IX. Potential Disease-Modifying Effects of Incretin-Based Therapies

Patients with type 2 diabetes do not produce enough insulin to compensate for insulin resistance, which means that blood glucose levels eventually increase. Drugs that improve insulin sensitivity and actions, metformin and the thiazolidinediones (pioglitazone and rosiglitazone), are in use in humans. In addition, drugs that stimulate insulin secretion (that is, insulin secretagogues in which the mechanism(s) of action is not through incretin receptors) have been in use since the 1950s. The natural history, however, is for patients to continue to lose β-cell secretory capacity, resulting in the need for combination therapies and eventually in the need for exogenous injections of insulin. Therefore, the aim of therapy should be not only to lower blood glucose but also to prevent deterioration of β-cell function. Weight loss is the most desirable treatment for T2DM, and more and more this is being attained with the surgical manipulations of gastric/small bowel bypass and gastric restriction methods such as banding. However, in the development of agents to lower blood glucose in the diabetic state, we think it needs to be considered whether the agents can also modify the natural history of the disease.

The newer agents discussed above are insulin secretagogues: exenatide by directly activating the GLP-1Rs on β cells and sitagliptin by increasing endogenous levels of full-length GLP-1. Therefore they should be placed in context with the most frequently used and oldest secretagogues, the sulfonylureas. The sulfonylureas increase insulin secretion by blocking the K_{ATP} channels, which, as described above, leads to depolarization of the β-cell membrane and influx of Ca²⁺ through voltage-gated channels, leading to exocytosis of the insulin-laden granules. Unlike exenatide/GLP-1, however, sulfonylureas do not have any pleiotropic effects on β cells, and some studies, such as the University Group Diabetes Project study (Goldner et al., 1971) seem to indicate that, over time, they actually worsen β-cell function. In the ADOPT (A Diabetes Outcome Progression Trial) study, the sulfonylurea glyburide was compared with metformin and rosiglitazone as treatments for T2DM. Glyburide had results superior to those of the other two drugs in the first year of treatment, but after 5 years, the group receiving it had the highest blood sugars (Kahn et al., 2006). Thus, it may have been associated with worsening β-cell function. But sulfonylureas are associated with weight gain, and the ADOPT study patients were no exception. Therefore, we feel that an alternative explanation for the worsening blood glucose might be that insulin resistance increased, leading to an even greater requirement for insulin and greater stress on β cells, over the 5 years of the study. In favor of this latter explanation are the data from the recently published ADVANCE study (Gerstein et al.,

2008). In that study, >90% of patients received gliclazide, another sulfonylurea, as part of an intensive treatment strategy aimed at lowering HbA_{1c} to 6.5%. Only 1.6% of patients in the standard treatment group received gliclazide. After 5 years, HbA_{1c} levels were maintained at 6.5% in the intensively treated group; interestingly, however, the patients did not gain weight. Therefore, it seems that when weight gain does not occur with sulfonylurea treatment, β -cell function deterioration also does not occur. We now have 3-year data from patients taking exenatide in conjunction with either sulfonylurea or metformin (Klonoff et al., 2008). A 1.1% reduction on HbA_{1c} was sustained for the 3 years of follow-up, and a progressive weight loss of 5.3 kg was observed. It seems therefore reasonable to conclude that exenatide is a disease-modifying treatment, because it modified the behavior that led to the T2DM state and blood glucose control was sustained; i.e., there was no appearance of worsening of β -cell function. In the ADVANCE study, patients on sulfonylureas did not fair worse than patients not taking sulfonylureas, again illustrating that sulfonylureas, per se, are unlikely to be damaging β cells. It seems that loss of β -cell function can be prevented with insulin secretagogues provided weight gain does not occur, and this also applies to sulfonylurea-only treatment. Sitagliptin therapy has not been evaluated for longer than 52 weeks. In a comparison study of sitagliptin or glipizide added on to metformin, blood glucose was similar with both treatments over 52 weeks, and blood glucose started to rise equally with both drugs (Nauck et al., 2007b).

In summary, although animal studies, many of which have been outlined above, demonstrate that exenatide is associated with β -cell proliferation and preservation, there is no direct evidence for this in humans. On balance, it seems that any insulin secretagogue may be used in T2DM, provided weight gain can be prevented, and clearly exenatide has an advantage in this regard. Much further research is needed to elucidate if incretin-based therapies are also disease-modifying therapies and if they provide any advantage over existing therapies.

X. Concluding Remarks

Incretin-based therapies are now in use to lower blood glucose levels in T2DM. Their development arose from our understanding and extensive research into incretin physiology and metabolism. Exenatide, as adjuvant therapy in T2DM, leads to a sustained HbA_{1c} reduction of 1.0% and seems to improve β -cell function. It also leads to weight loss, at least over a three-year period. Long-acting forms of once-weekly injection, such as exenatide LAR, are under development. Daily liraglutide therapy had similar effects with respect to blood glucose control, β -cell function, and weight loss as twice-daily exenatide, and its effects were sustained for at least 1 year: no further long-term data are available. Both liraglutide and exenatide must be injected subcutaneously.

The DPP4 inhibitors have a big advantage in that they are effective when administered orally, but they do not have as potent an effect on blood glucose control as either exenatide or liraglutide. Their effect is limited by the undesirable fact that they diminish total incretin secretion after at least a nutritive stimulus to incretin secretion. Both DPP4 inhibitors discussed in this review are weight neutral, and their effects on other DPP4 substrates need further research.

Continued monitoring for extrapancreatic effects of both groups of compounds is necessary. Whether the reported cases of pancreatitis with exenatide and liraglutide are representative of an increased risk is not yet clear but must be taken into account by any physician about to prescribe this class of compounds, especially if the patients have had pancreatitis in the past or perhaps suffer from gallstones. We do not yet know whether these new agents will have significant, long-term disease-modifying effects.

DPP4 inhibitors would almost certainly be more useful in T2DM management if used in addition to agents (incretinotropics) that would increase incretin secretion. Metformin actually increases incretin secretion (Chia and Egan, 2008), and this factor probably explains why the addition of DPP4 inhibitors to metformin therapy has more of an effect on reductions in HbA_{1c} than would be expected from simply adding together the effects of each as a monotherapy: metformin prevents the negative feedback of DPP4 inhibition on incretin secretion. New incretinotropic agents would need to be non-nutrient-containing, and likely candidate agents are agonists of taste receptors and GPR119.

A better understanding of the effects of GLP-1 and GLP-1 mimetics on β -cell function/mass in humans and the mechanism of action by which they lower glucagon secretion is needed. A drug that would lower fasting plasma glucagon levels into the normal range would be a major advancement in therapy. Further research on the actions of GIPRs in adipose tissue might prove fruitful, especially if TZD/GIPR interactions are occurring.

Incretin-based therapies give new options for lowering blood glucose and should be placed alongside, and added to, older options. No data are yet available on whether these new agents affect hard endpoints such as cardiovascular disease, morbidity, and mortality.

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REFERENCES

- 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. [PubMed: 15862798]
- Acitores A, González N, Sancho V, Valverde I, Villanueva-Peñacarrillo ML. Cell signalling of glucagon-like peptide-1 action in rat skeletal muscle. *J Endocrinol* 2004;180:389–398. [PubMed: 15012593]
- Agersø H, Jensen LB, Elbrønd B, Rolan P, Zdravkovic M. The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men. *Diabetologia* 2002;45:195–202. [PubMed: 11935150]
- Ahrén B. Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. *Am J Physiol Regul Integr Comp Physiol* 2004;286:R269–F272. [PubMed: 14707011]
- Alcántara AI, Morales M, Delgado E, López-Delgado MI, Clemente F, Luque MA, Malaisse WJ, Valverde I, Villanueva-Peñacarrillo ML. Exendin-4 agonist and exendin(9–39)amide antagonist of the GLP-1(7–36)amide effects in liver and muscle. *Arch Biochem Biophys* 1997;341:1–7. [PubMed: 9143346]
- Amiranoff B, Vauclin-Jacques N, Laburthe M. Functional GIP receptors in a hamster pancreatic beta cell line. In 111: specific binding and biological effects. *Biochem Biophys Res Commun* 1984;123:671–676. [PubMed: 6091638]
- Amiranoff B, Vauclin-Jacques N, Laburthe M. Interaction of gastric inhibitory polypeptide (GIP) with the insulin-secreting pancreatic beta cell line, In 111: characteristics of GIP binding sites. *Life Sci* 1985;36:807–813. [PubMed: 2983172]
- Amori RE, Lau J, Pittas AG. Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. *Jama* 2007;298:194–206. [PubMed: 17622601]
- Anini Y, Brubaker PL. Muscarinic receptors control glucagon-like peptide 1 secretion by human endocrine L cells. *Endocrinology* 2003a;144:3244–3250. [PubMed: 12810581]
- Anini Y, Brubaker PL. Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes* 2003b;52:252–259. [PubMed: 12540594]

- 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. [PubMed: 12021207]
- Aschner P, Kipnes MS, Lunceford JK, Sanchez M, Mickel C, Williams-Herman DE. Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. *Diabetes Care* 2006;29:2632–2637. [PubMed: 17130196]
- Ayachi SE, Borie F, Magous R, Sasaki K, le Nguyen D, Bali JP, Millat B, Jarrousse C. Contraction induced by glicentin on smooth muscle cells from the human colon is abolished by exendin (9–39). *Neurogastroenterol Motil* 2005;17:302–309. [PubMed: 15787950]
- Baggio L, Kieffer TJ, Drucker DJ. Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, regulates fasting glycemia and nonenteral glucose clearance in mice. *Endocrinology* 2000;141:3703–3709. [PubMed: 11014225]
- Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007;132:2131–2157. [PubMed: 17498508]
- Baggio LL, Huang Q, Brown TJ, Drucker DJ. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology* 2004a;127:546–558. [PubMed: 15300587]
- 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* 2004b;53:2492–2500. [PubMed: 15331566]
- Baggio LL, Huang Q, Cao X, Drucker DJ. An albumin-exendin-4 conjugate engages central and peripheral circuits regulating murine energy and glucose homeostasis. *Gastroenterology* 2008;134:1137–1147. [PubMed: 18313669]
- 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* 2004c;53:S205–S214. [PubMed: 15561912]
- Balkan B, Li X. Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. *Am J Physiol Regul Integr Comp Physiol* 2000;279:R1449–R1454. [PubMed: 11004015]
- Balks HJ, Holst JJ, von zur Mühlen 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. [PubMed: 9062483]
- Ban K, Noyan-Ashraf MH, Hofer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation* 2008;117:2340–2350. [PubMed: 18427132]
- Barlocco D. LAF-237 (Novartis). *Curr Opin Investig Drugs* 2004;5:1094–1100.
- Barragán JM, Eng J, Rodríguez R, Blázquez 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. [PubMed: 10567003]
- Barragán JM, Rodríguez RE, Blázquez E. Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7–36) amide in rats. *Am J Physiol* 1994;266:E459–E466. [PubMed: 8166268]
- Bayliss WM, Starling EH. The mechanism of pancreatic secretion. *J Physiol* 1902;28:325–353. [PubMed: 16992627]
- Beak SA, Heath MM, Small CJ, Morgan DG, Ghatei MA, Taylor AD, Buckingham JC, Bloom SR, Smith DM. Glucagon-like peptide-1 stimulates luteinizing hormone-releasing hormone secretion in a rodent hypothalamic neuronal cell line. *J Clin Invest* 1998;101:1334–1341. [PubMed: 9502775]
- Beak SA, Small CJ, Ilovaiskaia I, Hurley JD, Ghatei MA, Bloom SR, Smith DM. Glucagon-like peptide-1 (GLP-1) releases thyrotropin (TSH): characterization of binding sites for GLP-1 on alpha-TSH cells. *Endocrinology* 1996;137:4130–4138. [PubMed: 8828468]
- Bell GI. The glucagon superfamily: precursor structure and gene organization. *Peptides* 1986;7:27–36. [PubMed: 3092195]

- Bell GI, Santerre RF, Mullenbach GT. Hamster proglucagon contains the sequence of glucagon and two related peptides. *Nature* 1983;302:716–718. [PubMed: 6835407]
- Bergman A, Ebel D, Liu F, Stone J, Wang A, Zeng W, Chen L, Dilzer S, Lasseter K, Herman G, et al. Absolute bioavailability of sitagliptin, an oral dipeptidyl peptidase-4 inhibitor, in healthy volunteers. *Biopharm Drug Dispos* 2007;28:315–322. [PubMed: 17575559]
- Besterman HS, Bloom SR, Sarson DL, Blackburn AM, Johnston DI, Patel HR, Stewart JS, Modigliani R, Guerin S, Mallinson CN. Gut-hormone profile in coeliac disease. *Lancet* 1978;1:785–788. [PubMed: 85811]
- Besterman HS, Cook GC, Sarson DL, Christofides ND, Bryant MG, Gregor M, Bloom SR. Gut hormones in tropical malabsorption. *Br Med J* 1979;2:1252–1255. [PubMed: 519400]
- Betz A, Thakur P, Junge HJ, Ashery U, Rhee JS, Scheuss V, Rosenmund C, Rettig J, Brose N. Functional interaction of the active zone proteins Munc13–1 and RIM1 in synaptic vesicle priming. *Neuron* 2001;30:183–196. [PubMed: 11343654]
- Blandino-Rosano M, Perez-Arana G, Mellado-Gil JM, Segundo C, Aguilar-Diosdado M. Anti-proliferative effect of pro-inflammatory cytokines in cultured beta cells is associated with extracellular signal-regulated kinase 1/2 pathway inhibition: protective role of glucagon-like peptide-1. *J Mol Endocrinol* 2008;41:35–44. [PubMed: 18487229]
- Bollag RJ, Zhong Q, Ding KH, Phillips P, Zhong L, Qin F, Cranford J, Mulloy AL, Cameron R, Isaacs CM. Glucose-dependent insulinotropic peptide is an integrative hormone with osteotropic effects. *Mol Cell Endocrinol* 2001;177:35–41. [PubMed: 11377818]
- Bollag RJ, Zhong Q, Phillips P, Min L, Zhong L, Cameron R, Mulloy AL, Rasmussen H, Qin F, Ding KH, et al. Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors. *Endocrinology* 2000;141:1228–1235. [PubMed: 10698200]
- 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. [PubMed: 15616022]
- Bose AK, Mocanu MM, Carr RD, Yellon DM. Myocardial ischaemiareperfusion injury is attenuated by intact glucagon like peptide-1 (GLP-1) in the in vitro rat heart and may involve the p70s6K pathway. *Cardiovasc Ther* 2007;21:253–256. [PubMed: 17541736]
- Boyer DF, Fujitani Y, Gannon M, Powers AC, Stein RW, Wright CV. Complementation rescue of Pdx1 null phenotype demonstrates distinct roles of proximal and distal cis-regulatory sequences in pancreatic and duodenal expression. *Dev Biol* 2006;298:616–631. [PubMed: 16962573]
- Boylan MO, Jepeal LI, Jarboe LA, Wolfe MM. Cell-specific expression of the glucose-dependent insulinotropic polypeptide gene in a mouse neuroendocrine tumor cell line. *J Biol Chem* 1997;272:17438–17443. [PubMed: 9211887]
- Boylan MO, Jepeal LI, Wolfe MM. Structure of the rat glucose-dependent insulinotropic polypeptide receptor gene. *Peptides* 1999;20:219–228. [PubMed: 10422878]
- Boylan MO, Jepeal LI, Wolfe MM. Sp1/Sp3 binding is associated with cell-specific expression of the glucose-dependent insulinotropic polypeptide receptor gene. *Am J Physiol Endocrinol Metab* 2006;290:E1287–E1295. [PubMed: 16403775]
- Brown JC, Dryburgh JR. A gastric inhibitory polypeptide. II. The complete amino acid sequence. *Can J Biochem* 1971;49:867–872. [PubMed: 5120249]
- Brown JC, Dryburgh JR, Ross SA, Dupré J. Identification and actions of gastric inhibitory polypeptide. *Recent Prog Horm Res* 1975;31:487–532. [PubMed: 128084]128084
- Brubaker PL. Regulation of intestinal proglucagon-derived peptide secretion by intestinal regulatory peptides. *Endocrinology* 1991;128:3175–3182. [PubMed: 1674688]
- 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. [PubMed: 9751489]
- Buchan AM, Polak JM, Capella C, Solcia E, Pearse AG. Electronimmunocytochemical evidence for the K cell localization of gastric inhibitory polypeptide (GIP) in man. *Histochemistry* 1978;56:37–44. [PubMed: 350814]
- Buffa R, Polak JM, Pearse AG, Solcia E, Grimelius L, Capella C. Identification of the intestinal cell storing gastric inhibitory peptide. *Histochemistry* 1975;43:249–255. [PubMed: 1097380]

- Buggy JJ, Heurich RO, MacDougall M, Kelley KA, Livingston JN, Yoo-Warren H, Rossomando AJ. Role of the glucagon receptor COOH-terminal domain in glucagon-mediated signaling and receptor internalization. *Diabetes* 1997;46:1400–1405. [PubMed: 9287038]
- Bugrim AE. Regulation of Ca^{2+} release by cAMP-dependent protein kinase. A mechanism for agonist-specific calcium signaling? *Cell Calcium* 1999;25:219–226. [PubMed: 10378083]
- Bullock BP, Heller RS, Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology* 1996;137:2968–2978. [PubMed: 8770921]
- 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. [PubMed: 11473030]
- Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care* 2004;27:2628–2635. [PubMed: 15504997]
- Buteau J, El-Assaad W, Rhodes CJ, Rosenberg L, Joly E, Prentki M. Glucagon-like peptide-1 prevents beta cell glucolipotoxicity. *Diabetologia* 2004;47:806–815. [PubMed: 15095038]
- 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. [PubMed: 12502502]
- Buteau J, Foisy S, Rhodes CJ, Carpenter L, Biden TJ, Prentki M. Protein kinase Czeta activation mediates glucagon-like peptide-1-induced pancreatic beta-cell proliferation. *Diabetes* 2001;50:2237–2243. [PubMed: 11574404]
- Buteau J, Roduit R, Susini S, Prentki M. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. *Diabetologia* 1999;42:856–864. [PubMed: 10440129]
- 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. [PubMed: 16644672]
- Campos RV, Lee YC, Drucker DJ. Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology* 1994;134:2156–2164. [PubMed: 8156917]
- Cani PD, Holst JJ, Drucker DJ, Delzenne NM, Thorens B, Burcelin R, Knauf C. GLUT2 and the incretin receptors are involved in glucose-induced incretin secretion. *Mol Cell Endocrinol* 2007;276:18–23. [PubMed: 17681422]
- 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. [PubMed: 17130197]
- Chelikani PK, Haver AC, Reidelberger RD. Intravenous infusion of glucagon-like peptide-1 potently inhibits food intake, sham feeding, and gastric emptying in rats. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R1695–R1706. [PubMed: 15718384]
- Chelikani PK, Haver AC, Reidelberger RD. Ghrelin attenuates the inhibitory effects of glucagon-like peptide-1 and peptide YY(3–36) on food intake and gastric emptying in rats. *Diabetes* 2006;55:3038–3046. [PubMed: 17065340]
- Chepurny OG, Hussain MA, Holz GG. Exendin-4 as a stimulator of rat insulin I gene promoter activity via bZIP/CRE interactions sensitive to serine/threonine protein kinase inhibitor Ro 31–8220. *Endocrinology* 2002;143:2303–2313. [PubMed: 12021195]
- Chia CW, Egan JM. Incretin-based therapies in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2008;93:3703–3716. [PubMed: 18628530]
- Chisholm C, Greenberg GR. Somatostatin-28 regulates GLP-1 secretion via somatostatin receptor subtype 5 in rat intestinal cultures. *Am J Physiol Endocrinol Metab* 2002;283:E311–E317. [PubMed: 12110536]
- Cho GJ, Ryu S, Kim YH, Kim YS, Cheon EW, Park JM, Kim HJ, Kang SS, Choi WS. Upregulation of glucose-dependent insulinotropic polypeptide and its receptor in the retina of streptozotocin-induced diabetic rats. *Curr Eye Res* 2002;25:381–388. [PubMed: 12789546]

- Christophe J. Pancreatic tumoral cell line AR42J: an amphicrine model. *Am J Physiol* 1994;266:G963–G971. [PubMed: 7517639]
- Chu ZL, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, Pedraza M, Mondala H, Gao H, Bagnol D, et al. A role for intestinal endocrine cell-expressed g protein-coupled receptor 119 in glycemic control by enhancing glucagon-like Peptide-1 and glucose-dependent insulinotropic Peptide release. *Endocrinology* 2008;149:2038–2047. [PubMed: 18202141]
- Crespel A, De Boisvilliers F, Gros L, Kervran A. Effects of glucagon and glucagon-like peptide-1-(7–36) amide on C cells from rat thyroid and medullary thyroid carcinoma CA-77 cell line. *Endocrinology* 1996;137:3674–3680. [PubMed: 8756532]
- 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. [PubMed: 627329]
- 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. [PubMed: 8725855]
- Crockett SE, Mazzaferri EL, Cataland S. Gastric inhibitory polypeptide (GIP) in maturityonset diabetes mellitus. *Diabetes* 1976;25:931–935. [PubMed: 976601]
- Dakin CL, Small CJ, Batterham RL, Neary NM, Cohen MA, Patterson M, Gbatei MA, Bloom SR. Peripheral oxyntomodulin reduces food intake and body weight gain in rats. *Endocrinology* 2004;145:2687–2695. [PubMed: 15001546]
- D'Alessio DA, Vogel R, Prigeon R, Laschansky E, Koerker D, Eng J, Ensinnck JW. Elimination of the action of glucagon-like peptide 1 causes an impairment of glucose tolerance after nutrient ingestion by healthy baboons. *J Clin Invest* 1996;97:133–138. [PubMed: 8550824]
- Deacon CF, Nauck MA, Meier J, Hücking 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. [PubMed: 11061504]
- DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care* 2005;28:1092–1100. [PubMed: 15855572]
- De León DD, Crutchlow MF, Ham JY, Stoffers DA. Role of glucagon-like peptide-1 in the pathogenesis and treatment of diabetes mellitus. *Int J Biochem Cell Biol* 2006;38:845–859. [PubMed: 16202636]
- de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, Bos JL. Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* 1998;396:474–477. [PubMed: 9853756]
- Dillon JS, Lu M, Bowen S, Homan LL. The recombinant rat glucagon-like peptide-1 receptor, expressed in an alpha-cell line, is coupled to adenylyl cyclase activation and intracellular calcium release. *Exp Clin Endocrinol Diabetes* 2005;113:182–189. [PubMed: 15789279]
- Dillon JS, Tanizawa Y, Wheeler MB, Leng XH, Ligon BB, Rabin DU, Yoo-Warren H, Permutt MA, Boyd AE 3rd. Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. *Endocrinology* 1993;133:1907–1910. [PubMed: 8404634]
- Ding KH, Zhong Q, Xie D, Chen HX, Della-Fera MA, Bollag RJ, Bollag WB, Gujral R, Kang B, Sridhar S, et al. Effects of glucose-dependent insulinotropic peptide on behavior. *Peptides* 2006;27:2750–2755. [PubMed: 16822587]
- 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. [PubMed: 9075801]
- Doyle ME, Egan JM. Pharmacological agents that directly modulate insulin secretion. *Pharmacol Rev* 2003;55:105–131. [PubMed: 12615955]
- Doyle ME, Egan JM. Mechanisms of action of glucagon-like peptide 1 in the pancreas. *Pharmacol Ther* 2007;113:546–593. [PubMed: 17306374]
- Drucker DJ, Asa S. Glucagon gene expression in vertebrate brain. *J Biol Chem* 1988;263:13475–13478. [PubMed: 2901414]

- 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. [PubMed: 7535893]
- Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006;368:1696–1705. [PubMed: 17098089]
- 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. [PubMed: 3033647]
- Druey KM, Blumer KJ, Kang VH, Kehrl JH. Inhibition of G-protein-mediated MAP kinase activation by a new mammalian gene family. *Nature* 1996;379:742–746. [PubMed: 8602223]
- 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. [PubMed: 7789625]
- Dupre J, Caussignac Y, McDonald TJ, Van Vliet S. Stimulation of glucagon secretion by gastric inhibitory polypeptide in patients with hepatic cirrhosis and hyperglucagonemia. *J Clin Endocrinol Metab* 1991;72:125–129. [PubMed: 1986010]
- Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J Clin Endocrinol Metab* 1973;37:826–828. [PubMed: 4749457]
- Ebert R, Creutzfeldt W. Reversal of impaired GIP and insulin secretion in patients with pancreatogenic steatorrhea following enzyme substitution. *Diabetologia* 1980;19:198–204. [PubMed: 6997121]
- Ebert R, Creutzfeldt W. Influence of gastric inhibitory polypeptide anti-serum on glucose-induced insulin secretion in rats. *Endocrinology* 1982;111:1601–1606. [PubMed: 6751797]
- Ebert R, Creutzfeldt W, Brown JC, Frerichs H, Arnold R. Response of gastric inhibitory polypeptide (GIP) to test meal in chronic pancreatitis—relationship to endocrine and exocrine insufficiency. *Diabetologia* 1976;12:609–612. [PubMed: 1001849]
- Edfalk S, Steneberg P, Edlund H. Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. *Diabetes* 2008;57:2280–2287. [PubMed: 18519800]
- Edwards CM, Todd JF, Mahmoudi M, Wang Z, Wang RM, Ghatei MA, Bloom SR. Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9–39. *Diabetes* 1999;48:86–93. [PubMed: 9892226]
- 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. [PubMed: 2483103]
- Egan JM, Bulotta A, Hui H, Perfetti R. GLP-1 receptor agonists are growth and differentiation factors for pancreatic islet beta cells. *Diabetes Metab Res Rev* 2003;19:115–123. [PubMed: 12673779]
- Egan JM, Clocquet AR, Elahi D. The insulinotropic effect of acute exendin-4 administered to humans: comparison of nondiabetic state to type 2 diabetes. *J Clin Endocrinol Metab* 2002;87:1282–1290. [PubMed: 11889200]
- Egan JM, Margolskee RF. Taste cells of the gut and gastrointestinal chemosensation. *Mol Interv* 2008;8:78–81. [PubMed: 18403652]
- Egan JM, Montrose-Rafizadeh C, Wang Y, Bernier M, Roth J. Glucagon-like peptide-1(7–36) amide (GLP-1) enhances insulin-stimulated glucose metabolism in 3T3-L1 adipocytes: one of several potential extrapancreatic sites of GLP-1 action. *Endocrinology* 1994;135:2070–2075. [PubMed: 7956929]
- Ehshes JA, Casilla VR, Doty T, Pospisilik JA, Winter KD, Demuth HU, Pederson RA, McIntosh CH. Glucose-dependent insulinotropic polypeptide promotes beta-(INS-1) cell survival via cyclic adenosine monophosphate-mediated caspase-3 inhibition and regulation of p38 mitogen-activated protein kinase. *Endocrinology* 2003;144:4433–4445. [PubMed: 12960055]
- Ehshes 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. [PubMed: 11323439]
- Ehshes 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. [PubMed: 12138104]

- Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Göke B. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur J Clin Invest* 1992;22:283–291. [PubMed: 1499644]
- Elahi D, McAloon-Dyke M, Fukagawa NK, Meneilly GS, Sclater AL, Minaker KL, Habener JF, Andersen DK. The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7–37) in normal and diabetic subjects. *Regul Pept* 1994;51:63–74. [PubMed: 8036284]
- Eliasson L, Renström E, Ding WG, Proks P, Rorsman P. Rapid ATP-dependent priming of secretory granules precedes Ca^{2+} -induced exocytosis in mouse pancreatic B-cells. *J Physiol* 1997;503:399–412. [PubMed: 9306281]
- 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. [PubMed: 7852887]
- El-Ouaghli A, Rehring E, Holst JJ, Schweizer A, Foley J, Holmes D, Nauck MA. The dipeptidyl peptidase 4 inhibitor vildagliptin does not accentuate glibenclamide-induced hypoglycemia but reduces glucose-induced glucagon-like peptide 1 and gastric inhibitory polypeptide secretion. *J Clin Endocrinol Metab* 2007;92:4165–4171. [PubMed: 17698900]
- Elrick H, Stimmler L, Hlad CJ Jr, Arai Y. Plasma insulin response to oral and intravenous glucose administration. *J Clin Endocrinol Metab* 1964;24:1076–1082. [PubMed: 14228531]
- Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from *Heloderma suspectum* venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem* 1992;267:7402–7405. [PubMed: 1313797]
- Falko JM, Crockett SE, Cataland S, Mazzaferri EL. Gastric inhibitory polypeptide (GIP) stimulated by fat ingestion in man. *J Clin Endocrinol Metab* 1975;41:260–265. [PubMed: 1159045]
- Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noshmehr H, Bertolotto C, Di Mario U, Harlan DM, Perfetti R. Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 2003;144:5149–5158. [PubMed: 12960095]
- Farilla L, Hui H, Bertolotto C, Kang E, Bulotta A, Di Mario U, Perfetti R. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. *Endocrinology* 2002;143:4397–4408. [PubMed: 12399437]
- Fehmann HC, Göke R, Göke B. Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocr Rev* 1995;16:390–410. [PubMed: 7671853]
- 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. [PubMed: 1672112]
- Fehmann HC, Jiang J, Schweinfurth J, Dörsch K, Wheeler MB, Boyd AE 3rd, Göke B. Ligand-specificity of the rat GLP-I receptor recombinantly expressed in Chinese hamster ovary (CHO-) cells. *Z Gastroenterol* 1994;32:203–207. [PubMed: 8017094]
- Fehse F, Trautmann M, Holst JJ, Halseth AE, Nanayakkara N, Nielsen LL, Fineman MS, Kim DD, Nauck MA. Exenatide augments first- and second-phase insulin secretion in response to intravenous glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 2005;90:5991–5997. [PubMed: 16144950]
- Feinglos MN, Saad MF, Pi-Sunyer FX, An B, Santiago O. Effects of liraglutide (NN2211), a long-acting GLP-1 analogue, on glycaemic control and bodyweight in subjects with Type 2 diabetes. *Diabet Med* 2005;22:1016–1023. [PubMed: 16026367]
- Ferdaoussi M, Abdelli S, Yang JY, Cornu M, Niederhauser G, Favre D, Widmann C, Regazzi R, Thorens B, Waeber G, et al. Exendin-4 protects beta-cells from interleukin-1 beta-induced apoptosis by interfering with the c-Jun NH2-terminal kinase pathway. *Diabetes* 2008;57:1205–1215. [PubMed: 18252896]

- Flamez D, Gilon P, Moens K, Van Breusegem A, Delmeire D, Scrocchi LA, Henquin JC, Drucker DJ, Schuit F. Altered cAMP and Ca²⁺ signaling in mouse pancreatic islets with glucagon-like peptide-1 receptor null phenotype. *Diabetes* 1999;48:1979–1986. [PubMed: 10512362]
- Flamez D, Van Breusegem A, Scrocchi LA, Quartier E, Pipeleers D, Drucker DJ, Schuit F. Mouse pancreatic beta-cells exhibit preserved glucose competence after disruption of the glucagon-like peptide-1 receptor gene. *Diabetes* 1998;47:646–652. [PubMed: 9568699]
- Flatt PR, Bailey CJ, Kwasowski P, Swanston-Flatt SK, Marks V. Abnormalities of GIP in spontaneous syndromes of obesity and diabetes in mice. *Diabetes* 1983;32:433–435. [PubMed: 6341126]
- Flatt PR, Kwasowski P, Bailey CJ. Stimulation of gastric inhibitory polypeptide release in ob/ob mice by oral administration of sugars and their analogues. *J Nutr* 1989;119:1300–1303. [PubMed: 2795243]
- Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 1998;101:515–520. [PubMed: 9449682]
- Franklin I, Gromada J, Gjinovci A, Theander S, Wollheim CB. Beta-cell secretory products activate alpha-cell ATP-dependent potassium channels to inhibit glucagon release. *Diabetes* 2005;54:1808–1815. [PubMed: 15919803]
- Fraser ID, Tavalin SJ, Lester LB, Langeberg LK, Westphal AM, Dean RA, Marrion NV, Scott JD. A novel lipid-anchored A-kinase Anchoring Protein facilitates cAMP-responsive membrane events. *EMBO J* 1998;17:2261–2272. [PubMed: 9545239]
- Friedrichsen BN, Neubauer N, Lee YC, Gram VK, Blume N, Petersen JS, Nielsen JH, Møldrup A. Stimulation of pancreatic beta-cell replication by incretins involves transcriptional induction of cyclin D1 via multiple signalling pathways. *J Endocrinol* 2006;188:481–492. [PubMed: 16522728]
- Fujimoto K, Shibasaki T, Yokoi N, Kashima Y, Matsumoto M, Sasaki T, Tajima N, Iwanaga T, Seino S. Piccolo, a Ca²⁺ sensor in pancreatic beta-cells. Involvement of cAMP-GEFII. Rim2. Piccolo complex in cAMP-dependent exocytosis. *J Biol Chem* 2002;277:50497–50502. [PubMed: 12401793]
- Fujita Y, Chui JW, King DS, Zhang T, Seufert J, Pownall S, Cheung AT, Kieffer TJ. Pax6 and Pdx1 are required for production of glucose-dependent insulinotropic polypeptide in proglucagon-expressing L cells. *Am J Physiol Endocrinol Metab* 2008;295:E648–E657. [PubMed: 18593849]
- Furuta M, Yano H, Zhou A, Rouillé Y, Holst JJ, Carroll R, Ravazzola M, Orci L, Furuta H, Steiner DF. Defective prohormone processing and altered pancreatic islet morphology in mice lacking active SPC2. *Proc Natl Acad Sci USA* 1997;94:6646–6651. [PubMed: 9192619]
- Galera C, Clemente F, Alcantara A, Trapote MA, Perea A, Lopez-Delgado MI, Villanueva-Penacarrillo ML, Valverde I. Inositolphosphoglycans and diacylglycerol are possible mediators in the glycogenic effect of GLP-1(7–36)amide in BC3H-1 myocytes. *Cell Biochem Funct* 1996;14:43–48. [PubMed: 8907253]
- Gameiro A, Reimann F, Habib AM, O'Malley D, Williams L, Simpson AK, Gribble FM. The neurotransmitters glycine and GABA stimulate glucagon-like peptide-1 release from the GLUTag cell line. *J Physiol* 2005;569:761–772. [PubMed: 16223757]
- Gao H, Wang X, Zhang Z, Yang Y, Yang J, Li X, Ning G. GLP-1 amplifies insulin signaling by up-regulation of IRbeta, IRS-1 and Glut4 in 3T3–L1 adipocytes. *Endocrine* 2007;32:90–95. [PubMed: 17992607]
- Garber A, Henry R, Ratner R, Garcia-Hernandez PA, Rodriguez-Pattzi H, Olvera-Alvarez I, Hale PM, Zdravkovic M, Bode B. the LEAD-3 (Mono) Study Group. Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. *Lancet*. 2008in press
- Gardiner SM, March JE, Kemp PA, Bennett T. Autonomic nervous system-dependent and -independent cardiovascular effects of exendin-4 infusion in conscious rats. *Br J Pharmacol* 2008;154:60–71. [PubMed: 18311183]
- 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. [PubMed: 16046312]

- Gault VA, O'Harte FP, Harriott P, Flatt PR. Characterization of the cellular and metabolic effects of a novel enzyme-resistant antagonist of glucose-dependent insulinotropic polypeptide. *Biochem Biophys Res Commun* 2002;290:1420–1426. [PubMed: 11820780]
- Gault VA, O'Harte FP, Harriott P, Mooney MH, Green BD, Flatt PR. Effects of the novel (Pro3)GIP antagonist and exendin(9–39)amide on GIP- and GLP-1-induced cyclic AMP generation, insulin secretion and postprandial insulin release in obese diabetic (ob/ob) mice: evidence that GIP is the major physiological incretin. *Diabetologia* 2003;46:222–230. [PubMed: 12627321]
- Gelling RW, Wheeler MB, Xue J, Gyomory S, Nian C, Pederson RA, McIntosh CH. Localization of the domains involved in ligand binding and activation of the glucose-dependent insulinotropic polypeptide receptor. *Endocrinology* 1997;138:2640–2643. [PubMed: 9165060]
- Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH Jr, et al. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008;358:2545–2559. [PubMed: 18539917]
- Göke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, Göke B. Exendin-4 is a high potency agonist and truncated exendin-(9–39)-amide an antagonist at the glucagon-like peptide 1-(7–36)-amide receptor of insulin-secreting beta-cells. *J Biol Chem* 1993;268:19650–19655. [PubMed: 8396143]
- Göke R, Just R, Lankat-Buttgereit B, Göke B. Glycosylation of the GLP-1 receptor is a prerequisite for regular receptor function. *Peptides* 1994;15:675–681. [PubMed: 7937345]
- Göke R, Larsen PJ, Mikkelsen JD, Sheikh SP. Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites. *Eur J Neurosci* 1995a;7:2294–2300.
- Göke R, Larsen PJ, Mikkelsen JD, Sheikh SP. Identification of specific binding sites for glucagon-like peptide-1 on the posterior lobe of the rat pituitary. *Neuroendocrinology* 1995b;62:130–134.
- Goldner MG, Knatterud GL, Prout TE. Effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. 3. Clinical implications of UGDP results. *JAMA* 1971;218:1400–1410. [PubMed: 4941698]
- Goldstein BJ, Feinglos MN, Luncford JK, Johnson J, Williams-Herman DE. Effect of initial combination therapy with sitagliptin, a dipeptidyl peptidase-4 inhibitor, and metformin on glycemic control in patients with type 2 diabetes. *Diabetes Care* 2007;30:1979–1987. [PubMed: 17485570]
- Goldstone AP, Mercer JG, Gunn I, Moar KM, Edwards CM, Rossi M, Howard JK, Rasheed S, Turton MD, Small C, et al. Leptin interacts with glucagon-like peptide-1 neurons to reduce food intake and body weight in rodents. *FEBS Lett* 1997;415:134–138. [PubMed: 9350983]
- Goldstone AP, Morgan I, Mercer JG, Morgan DG, Moar KM, Ghatei MA, Bloom SR. Effect of leptin on hypothalamic GLP-1 peptide and brain-stem pre-proglucagon mRNA. *Biochem Biophys Res Commun* 2000;269:331–335. [PubMed: 10708552]
- Göncz E, Strowski MZ, Grötzing C, Nowak KW, Kaczmarek P, Sassek M, Mergler S, El-Zayat BF, Theodoropoulou M, Stalla GK, et al. Orexin-A inhibits glucagon secretion and gene expression through a Foxo1-dependent pathway. *Endocrinology* 2008;149:1618–1626. [PubMed: 18162514]
- González N, Acitores A, Sancho V, Valverde I, Villanueva-Peñacarrillo ML. Effect of GLP-1 on glucose transport and its cell signalling in human myocytes. *Regul Pept* 2005;126:203–211. [PubMed: 15664668]
- Graziano MP, Hey PJ, Borkowski D, Chicchi GG, Strader CD. Cloning and functional expression of a human glucagon-like peptide-1 receptor. *Biochem Biophys Res Commun* 1993;196:141–146. [PubMed: 8216285]
- Gromada J, Holst JJ, Rorsman P. Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. *Pflugers Arch* 1998;435:583–594. [PubMed: 9479010]
- 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. [PubMed: 12746281]
- Gutniak M, Orskov C, Holst JJ, Ahrén B, Efendic S. Antidiabetogenic effect of glucagon-like peptide-1 (7–36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 1992;326:1316–1322. [PubMed: 1348845]
- Gutniak MK, Larsson H, Sanders SW, Juneskans O, Holst JJ, Ahrén B. GLP-1 tablet in type 2 diabetes in fasting and postprandial conditions. *Diabetes Care* 1997;20:1874–1879. [PubMed: 9405910]

- Habener JF, Kemp DM, Thomas MK. Minireview: transcriptional regulation in pancreatic development. *Endocrinology* 2005;146:1025–1034. [PubMed: 15604203]
- Hadjiyanni I, Baggio LL, Poussier P, Drucker DJ. Exendin-4 modulates diabetes onset in nonobese diabetic mice. *Endocrinology* 2008;149:1338–1349. [PubMed: 18063685]
- Hällbrink 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. [PubMed: 11257510]
- Hampton SM, Morgan LM, Tredger JA, Cramb R, Marks V. Insulin and C-peptide levels after oral and intravenous glucose. Contribution of enteroinsular axis to insulin secretion. *Diabetes* 1986;35:612–616. [PubMed: 3514335]
- 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. [PubMed: 10537167]
- Hansen L, Hartmann B, Bisgaard T, Mineo H, Jørgensen PN, Holst JJ. Somatostatin restrains the secretion of glucagon-like peptide-1 and -2 from isolated perfused porcine ileum. *Am J Physiol Endocrinol Metab* 2000;278:E1010–E1018. [PubMed: 10827002]
- 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. [PubMed: 15111503]
- Hausdorff WP, Caron MG, Lefkowitz RJ. Turning off the signal: desensitization of beta-adrenergic receptor function. *Faseb J* 1990;4:2881–2889. [PubMed: 2165947]
- He YL, Sabo R, Campestrini J, Wang Y, Ligueros-Saylan M, Lasseter KC, Dilzer SC, Howard D, Dole WP. The influence of hepatic impairment on the pharmacokinetics of the dipeptidyl peptidase IV (DPP-4) inhibitor vildagliptin. *Eur J Clin Pharmacol* 2007a;63:677–686. [PubMed: 17486328]
- He YL, Sabo R, Campestrini J, Wang Y, Riviere GJ, Nielsen JC, Rosenberg M, Ligueros-Saylan M, Howard D, Dole WP. The effect of age, gender, and body mass index on the pharmacokinetics and pharmacodynamics of vildagliptin in healthy volunteers. *Br J Clin Pharmacol* 2008;65:338–346. [PubMed: 17961192]
- He YL, Sadler BM, Sabo R, Balez S, Wang Y, Campestrini J, Laurent A, Ligueros-Saylan M, Howard D. The absolute oral bioavailability and population-based pharmacokinetic modelling of a novel dipeptidylpeptidase-IV inhibitor, vildagliptin, in healthy volunteers. *Clin Pharmacokinet* 2007b;46:787–802. [PubMed: 17713976]
- He YL, Serra D, Wang Y, Campestrini J, Riviere GJ, Deacon CF, Holst JJ, Schwartz S, Nielsen JC, Ligueros-Saylan M. Pharmacokinetics and pharmacodynamics of vildagliptin in patients with type 2 diabetes mellitus. *Clin Pharmacokinet* 2007c;46:577–588. [PubMed: 17596103]
- Heine RJ, Van Gaal LF, Johns D, Mihm MJ, Widel 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. [PubMed: 16230722]
- Heit JJ, Apelqvist AA, Gu X, Winslow MM, Neilson JR, Crabtree GR, Kim SK. Calcineurin/NFAT signalling regulates pancreatic beta-cell growth and function. *Nature* 2006;443:345–349. [PubMed: 16988714]
- Heller RS, Aponte GW. Intra-islet regulation of hormone secretion by glucagon-like peptide-1-(7–36) amide. *Am J Physiol* 1995;269:G852–G860. [PubMed: 8572216]
- 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. [PubMed: 9133545]
- Herbach N, Goeke B, Schneider M, Hermanns W, Wolf E, Wanke R. Overexpression of a dominant negative GIP receptor in transgenic mice results in disturbed postnatal pancreatic islet and beta-cell development. *Regul Pept* 2005;125:103–117. [PubMed: 15582721]
- Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, Snyder K, Hilliard D, Tanen M, Tanaka W, et al. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. *Clin Pharmacol Ther* 2005;78:675–688. [PubMed: 16338283]

- Hermansen K, Kipnes M, Luo E, Fanurik D, Khatami H, Stein P. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor, sitagliptin, in patients with type 2 diabetes mellitus inadequately controlled on glimepiride alone or on glimepiride and metformin. *Diabetes Obes Metab* 2007;9:733–745. [PubMed: 17593236]
- Herrmann C, Göke R, Richter G, Fehmann HC, Arnold R, Göke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 1995;56:117–126. [PubMed: 7750665]
- Herzig S, Fuzesi L, Knepel W. Heterodimeric Pbx-Prep1 homeodomain protein binding to the glucagon gene restricting transcription in a cell type-dependent manner. *J Biol Chem* 2000;275:27989–27999. [PubMed: 10869353]
- Higashimoto Y, Opara EC, Liddle RA. Dietary regulation of glucose-dependent insulinotropic peptide (GIP) gene expression in rat small intestine. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1995;110:207–214. [PubMed: 7599968]
- Higashimoto Y, Simchock J, Liddle RA. Molecular cloning of rat glucose-dependent insulinotropic peptide (GIP). *Biochim Biophys Acta* 1992;1132:72–74. [PubMed: 1380834]
- Hill ME, Asa SL, Drucker DJ. Essential requirement for Pax6 in control of enteroendocrine proglucagon gene transcription. *Mol Endocrinol* 1999;13:1474–1486. [PubMed: 10478839]
- Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, Sugimoto Y, Miyazaki S, Tsujimoto G. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med* 2005;11:90–94. [PubMed: 15619630]
- Holst JJ. Enteroglucagon. *Annu Rev Physiol* 1997;59:257–271. [PubMed: 9074764]
- Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev* 2007;87:1409–1439. [PubMed: 17928588]
- Holst JJ, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol Endocrinol Metab* 2004;287:E199–E206. [PubMed: 15271645]
- Holst JJ, Gromada J, Nauck MA. The pathogenesis of NIDDM involves a defective expression of the GIP receptor. *Diabetologia* 1997;40:984–986. [PubMed: 9267997]
- Holz GG, Chepurny OG. Diabetes outfoxed by GLP-1? *Sci STKE* 2005;2005:pe2. [PubMed: 15671479]
- Holz GG 4th, Kühtreiber 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. [PubMed: 8381211]
- Holz GG 4th, Leech CA, Habener JF. Activation of a cAMP-regulated Ca²⁺-signaling pathway in pancreatic beta-cells by the insulinotropic hormone glucagon-like peptide-1. *J Biol Chem* 1995;270:17749–17757. [PubMed: 7543091]
- 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. [PubMed: 12639928]
- Hui H, Wright C, Perfetti R. Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells. *Diabetes* 2001;50:785–796. [PubMed: 11289043]
- Huisamen B, Genade S, Lochner A. Signalling pathways activated by glucagon-like peptide-1 (7–36) amide in the rat heart and their role in protection against ischaemia. *Cardiovasc J Afr* 2008;19:77–83. [PubMed: 18516352]
- Hulme JT, Lin TW, Westenbroek RE, Scheuer T, Catterall WA. Beta-adrenergic regulation requires direct anchoring of PKA to cardiac CaV1.2 channels via a leucine zipper interaction with A kinase-anchoring protein 15. *Proc Natl Acad Sci USA* 2003;100:13093–13098. [PubMed: 14569017]
- Hupe-Sodmann K, McGregor GP, Bridenbaugh R, Göke R, Göke B, Thole H, Zimmermann B, Voigt K. Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1(7–36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Regul Pept* 1995;58:149–156. [PubMed: 8577927]
- Idris I, Patiag D, Gray S, Donnelly R. Exendin-4 increases insulin sensitivity via a PI-3-kinase-dependent mechanism: contrasting effects of GLP-1. *Biochem Pharmacol* 2002;63:993–996. [PubMed: 11911852]

- Imeryüz N, Yeğen BC, Bozkurt A, Coşkun T, Villanueva-Peñacarrillo ML, Ulusoy NB. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am J Physiol* 1997;273:G920–G927. [PubMed: 9357836]
- Irwin DM, Satkunarajah M, Wen Y, Brubaker PL, Pederson RA, Wheeler MB. The *Xenopus* proglucagon gene encodes novel GLP-1-like peptides with insulinotropic properties. *Proc Natl Acad Sci USA* 1997;94:7915–7920. [PubMed: 9223287]
- Jackson RS, Creemers JW, Farooqi IS, Raffin-Sanson ML, Varro A, Dockray GJ, Holst JJ, Brubaker PL, Corvol P, Polonsky KS, et al. Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. *J Clin Invest* 2003;112:1550–1560. [PubMed: 14617756]
- Jang HJ, Kokrashvili Z, Theodorakis MJ, Carlson OD, Kim BJ, Zhou J, Kim HH, Xu X, Chan SL, Juhaszova M, et al. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci USA* 2007;104:15069–15074. [PubMed: 17724330]
- Jepeal LI, Boylan MO, Michael Wolfe M. GATA-4 upregulates glucose-dependent insulinotropic polypeptide expression in cells of pancreatic and intestinal lineage. *Mol Cell Endocrinol* 2008;287:20–29. [PubMed: 18343025]
- Jepeal LI, Boylan MO, Wolfe MM. Cell-specific expression of the glucose-dependent insulinotropic polypeptide gene functions through a GATA and an ISL-1 motif in a mouse neuroendocrine tumor cell line. *Regul Pept* 2003;113:139–147. [PubMed: 12686473]
- 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. [PubMed: 15486225]
- Jetton TL, Liang Y, Pettepher CC, Zimmerman EC, Cox FG, Horvath K, Matschinsky FM, Magnuson MA. Analysis of upstream glucokinase promoter activity in transgenic mice and identification of glucokinase in rare neuroendocrine cells in the brain and gut. *J Biol Chem* 1994;269:3641–3654. [PubMed: 8106409]
- Jhala US, Canettieri G, Sreanion 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. [PubMed: 12842910]
- Jin SL, Han VK, Simmons JG, Towle AC, Lauder JM, Lund PK. Distribution of glucagonlike peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. *J Comp Neurol* 1988;271:519–532. [PubMed: 3385016]
- Jin T. Mechanisms underlying proglucagon gene expression. *J Endocrinol* 2008;198:17–28. [PubMed: 18577568]
- 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. [PubMed: 8544839]
- Jones IR, Owens DR, Luzio S, Williams S, Hayes TM. The glucose dependent insulinotropic polypeptide response to oral glucose and mixed meals is increased in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1989;32:668–677. [PubMed: 2676668]
- Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006;355:2427–2443. [PubMed: 17145742]
- Kang G, Chepurny OG, Holz GG. cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca^{2+} -induced Ca^{2+} release in INS-1 pancreatic beta-cells. *J Physiol* 2001;536:375–385. [PubMed: 11600673]
- Kang G, Chepurny OG, Malester B, Rindler MJ, Rehmann H, Bos JL, Schwede F, Coetzee WA, Holz GG. cAMP sensor Epac as a determinant of ATP-sensitive potassium channel activity in human pancreatic beta cells and rat INS-1 cells. *J Physiol* 2006a;573:595–609. [PubMed: 16613879]
- Kang G, Joseph JW, Chepurny OG, Monaco M, Wheeler MB, Bos JL, Schwede F, Genieser HG, Holz GG. Epac-selective cAMP analog 8-pCPT-2'-O-Me-cAMP as a stimulus for Ca^{2+} -induced Ca^{2+} release and exocytosis in pancreatic beta-cells. *J Biol Chem* 2003;278:8279–8285. [PubMed: 12496249]

- Kang L, He Z, Xu P, Fan J, Betz A, Brose N, Xu T. Munc13-1 is required for the sustained release of insulin from pancreatic beta cells. *Cell Metab* 2006b;3:463-468. [PubMed: 16697276]
- Kaplan AM, Vigna SR. Gastric inhibitory polypeptide (GIP) binding sites in rat brain. *Peptides* 1994;15:297-302. [PubMed: 8008635]
- 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. [PubMed: 11598134]
- Kato I, Takasawa S, Akabane A, Tanaka O, Abe H, Takamura T, Suzuki Y, Nata K, Yonekura H, Yoshimoto T. Regulatory role of CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase) in insulin secretion by glucose in pancreatic beta cells. Enhanced insulin secretion in CD38-expressing transgenic mice. *J Biol Chem* 1995;270:30045-30050. [PubMed: 8530408]
- Kato I, Yamamoto Y, Fujimura M, Noguchi N, Takasawa S, Okamoto H. CD38 disruption impairs glucose-induced increases in cyclic ADP-ribose, $[Ca^{2+}]_i$, and insulin secretion. *J Biol Chem* 1999;274:1869-1872. [PubMed: 9890936]
- Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE, Graybiel AM. A family of cAMP-binding proteins that directly activate Rap1. *Science* 1998;282:2275-2279. [PubMed: 9856955]
- Kemp DM, Habener JF. Insulinotropic hormone glucagon-like peptide 1 (GLP-1) activation of insulin gene promoter inhibited by p38 mitogen-activated protein kinase. *Endocrinology* 2001;142:1179-1187. [PubMed: 11181533]
- Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. *Diabetes Care* 2005;28:1083-1091. [PubMed: 15855571]
- Kieffer TJ, Huang Z, McIntosh CH, Buchan AM, Brown JC, Pederson RA. Gastric inhibitory polypeptide release from a tumor-derived cell line. *Am J Physiol* 1995;269:E316-E322. [PubMed: 7653549]
- 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. [PubMed: 7628397]
- Kim BJ, Park KH, Yim CY, Takasawa S, Okamoto H, Im MJ, Kim UH. Generation of nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose by glucagon-like peptide-1 evokes Ca^{2+} signal that is essential for insulin secretion in mouse pancreatic islets. *Diabetes* 2008a;57:868-878. [PubMed: 18184929]
- Kim D, MacConell L, Zhuang D, Kothare PA, Trautmann M, Fineman M, Taylor K. Effects of once-weekly dosing of a long-acting release formulation of exenatide on glucose control and body weight in subjects with type 2 diabetes. *Diabetes Care* 2007a;30:1487-1493. [PubMed: 17353504]
- Kim D, Wang L, Beconi M, Eiermann GJ, Fisher MH, He H, Hickey GJ, Kowalchick JE, Leitng B, Lyons K, et al. (2*R*)-4-oxo-4-[3-(Trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 2005a;48:141-151. [PubMed: 15634008]
- Kim MJ, Kang JH, Park YG, Ryu GR, Ko SH, Jeong IK, Koh KH, Rhie DJ, Yoon SH, Hahn SJ, et al. Exendin-4 induction of cyclin D1 expression in INS-1 beta-cells: involvement of cAMP-responsive element. *J Endocrinol* 2006;188:623-633. [PubMed: 16522741]
- 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* 2005b;280:28692-28700. [PubMed: 15955806]
- Kim SJ, Nian C, McIntosh CH. Activation of lipoprotein lipase by glucose-dependent insulinotropic polypeptide in adipocytes. A role for a protein kinase B, LKB1, and AMP-activated protein kinase cascade. *J Biol Chem* 2007b;282:8557-8567. [PubMed: 17244606]
- Kim SJ, Nian C, McIntosh CH. Resistin is a key mediator of glucose-dependent insulinotropic polypeptide (GIP) stimulation of lipoprotein lipase (LPL) activity in adipocytes. *J Biol Chem* 2007c;282:34139-34147. [PubMed: 17890220]
- Kim SJ, Nian C, Widenmaier S, McIntosh CH. Glucose-dependent insulinotropic polypeptide-mediated up-regulation of beta-cell antiapoptotic Bcl-2 gene expression is coordinated by cyclic AMP

(cAMP) response element binding protein (CREB) and cAMP-responsive CREB coactivator 2. *Mol Cell Biol* 2008b;28:1644–1656. [PubMed: 18086876]

- Kim SJ, Winter K, Nian C, Tsuneoka M, Koda Y, McIntosh CH. Glucose-dependent insulinotropic polypeptide (GIP) stimulation of pancreatic beta-cell survival is dependent upon phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) signaling, inactivation of the forkhead transcription factor Foxo1, and down-regulation of bax expression. *J Biol Chem* 2005c;280:22297–22307. [PubMed: 15817464]
- Kinzig KP, D'Alessio DA, Herman JP, Sakai RR, Vahl TP, Figueiredo HF, Murphy EK, Seeley RJ, Figueredo HF. The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J Clin Invest* 2002;110:1839–1847. [PubMed: 12488434]
- Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH 3rd, Wright CV, White MF, Arden KC, Accili D. The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J Clin Invest* 2002;110:1839–1847. [PubMed: 12488434]
- Klinger S, Poussin C, Debril MB, Dolci W, Halban PA, Thorens B. Increasing GLP-1-induced beta-cell proliferation by silencing the negative regulators of signaling cAMP response element modulator-alpha and DUSP14. *Diabetes* 2008;57:584–593. [PubMed: 18025410]
- Klonoff DC, Buse JB, Nielsen LL, Guan X, Bowls CL, Holcombe JH, Wintle ME, Maggs DG. Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years. *Curr Med Res Opin* 2008;24:275–286. [PubMed: 18053320]
- Knauf C, Cani PD, Kim DH, Iglesias MA, Chabo C, Waget A, Colom A, Rastrelli S, Delzenne NM, Drucker DJ, et al. Role of central nervous system glucagon-like Peptide-1 receptors in enteric glucose sensing. *Diabetes* 2008;57:2603–2612. [PubMed: 18519802]
- Knauf C, Cani PD, Perrin C, Iglesias MA, Maury JF, Bernard E, Benhamed F, Grémeaux T, Drucker DJ, Kahn CR, et al. Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. *J Clin Invest* 2005;115:3554–3563. [PubMed: 16322793]
- Knepel W, Chafitz J, Habener JF. Transcriptional activation of the rat glucagon gene by the cyclic AMP-responsive element in pancreatic islet cells. *Mol Cell Biol* 1990;10:6799–6804. [PubMed: 2147227]
- Knoch KP, Bergert H, Borgonovo B, Saeger HD, Altkrüger A, Verkade P, Solimena M. Polypyrimidine tract-binding protein promotes insulin secretory granule biogenesis. *Nat Cell Biol* 2004;6:207–214. [PubMed: 15039777]
- Knoch KP, Meisterfeld R, Kersting S, Bergert H, Altkrüger A, Wegbrod C, Jäger M, Saeger HD, Solimena M. cAMP-dependent phosphorylation of PTB1 promotes the expression of insulin secretory granule proteins in beta cells. *Cell Metab* 2006;3:123–134. [PubMed: 16459313]
- Knop FK, Vilsbøll T, Højberg PV, Larsen S, Madsbad S, Vølund A, Holst JJ, Krarup T. Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? *Diabetes* 2007;56:1951–1959. [PubMed: 17513701]
- Knudsen LB, Nielsen PF, Huusfeldt PO, Johansen NL, Madsen K, Pedersen FZ, Thøgersen H, Wilken M, Agersø H. Potent derivatives of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily administration. *J Med Chem* 2000;43:1664–1669. [PubMed: 10794683]
- Koelle MR, Horvitz HR. EGL-10 regulates G protein signaling in the *C. elegans* nervous system and shares a conserved domain with many mammalian proteins. *Cell* 1996;84:115–125. [PubMed: 8548815]
- Kolligs F, Fehmann HC, Göke R, Göke B. Reduction of the incretin effect in rats by the glucagon-like peptide 1 receptor antagonist exendin (9–39) amide. *Diabetes* 1995;44:16–19. [PubMed: 7813808]
- Kolterman OG, Kim DD, Shen L, Ruggles JA, Nielsen LL, Fineman MS, Baron AD. Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus. *Am J Health Syst Pharm* 2005;62:173–181. [PubMed: 15700891]
- Komatsu R, Matsuyama T, Namba M, Watanabe N, Itoh H, Kono N, Tarui S. Glucagonostatic and insulinotropic action of glucagonlike peptide I-(7–36)-amide. *Diabetes* 1989;38:902–905. [PubMed: 2661287]
- Kreymann B, Williams G, Ghatti MA, Bloom SR. Glucagon-like peptide-1 7–36: a physiological incretin in man. *Lancet* 1987;2:1300–1304. [PubMed: 2890903]

- Kumar KG, Byerley LO, Volafova J, Drucker DJ, Churchill GA, Li R, York B, Zuberi A, Richards BK. Genetic variation in Glp1r expression influences the rate of gastric emptying in mice. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R362–R371. [PubMed: 18077508]
- Kwan EP, Xie L, Sheu L, Nolan CJ, Prentki M, Betz A, Brose N, Gaisano HY. Munc13-1 deficiency reduces insulin secretion and causes abnormal glucose tolerance. *Diabetes* 2006;55:1421–1429. [PubMed: 16644700]
- Kwan EP, Xie L, Sheu L, Ohtsuka T, Gaisano HY. Interaction between Munc13–1 and RIM is critical for glucagon-like peptide-1 mediated rescue of exocytotic defects in Munc13–1 deficient pancreatic beta-cells. *Diabetes* 2007;56:2579–2588. [PubMed: 17639022]
- La Barre J. Sur les possibilités d'un traitement du diabète par l'incrétine. *Bull Acad R Med Belg* 1932;12:620–634.
- Lamari Y, Boissard C, Moukhtar MS, Jullienne A, Rosselin G, Garel JM. Expression of glucagon-like peptide 1 receptor in a murine C cell line: regulation of calcitonin gene by glucagon-like peptide 1. *FEBS Lett* 1996;393:248–252. [PubMed: 8814299]
- Larsen PJ, Holst JJ. Glucagon-related peptide 1 (GLP-1): hormone and neurotransmitter. *Regul Pept* 2005;128:97–107. [PubMed: 15780429]
- Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience* 1997;77:257–270. [PubMed: 9044391]
- 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. [PubMed: 9322962]
- Larsson LI, St-Onge L, Hougaard DM, Sosa-Pineda B, Gruss P. Pax 4 and 6 regulate gastrointestinal endocrine cell development. *Mech Dev* 1998;79:153–159. [PubMed: 10349628]
- Larsson LI, Madsen OD, Serup P, Jonsson J, Edlund H. Pancreatic-duodenal homeobox 1 -role in gastric endocrine patterning. *Mech Dev* 1996;60:175–184. [PubMed: 9025070]
- Lawrence MC, Bhatt HS, Easom RA. NFAT regulates insulin gene promoter activity in response to synergistic pathways induced by glucose and glucagon-like peptide-1. *Diabetes* 2002;51:691–698. [PubMed: 11872668]
- Layer PH, Chan AT, Go VL, Zinsmeister AR, DiMaggio EP. Adrenergic modulation of interdigestive pancreatic secretion in humans. *Gastroenterology* 1992;103:990–993. [PubMed: 1499947]
- Le Lay J, Stein R. Involvement of PDX-1 in activation of human insulin gene transcription. *J Endocrinol* 2006;188:287–294. [PubMed: 16461554]
- Lee YC, Asa SL, Drucker DJ. Glucagon gene 5'-flanking sequences direct expression of simian virus 40 large T antigen to the intestine, producing carcinoma of the large bowel in transgenic mice. *J Biol Chem* 1992;267:10705–10708. [PubMed: 1587847]
- Lee YS, Shin S, Shigihara T, Hahm E, Liu MJ, Han J, Yoon JW, Jun HS. Glucagon-like peptide-1 gene therapy in obese diabetic mice results in long-term cure of diabetes by improving insulin sensitivity and reducing hepatic gluconeogenesis. *Diabetes* 2007;56:1671–1679. [PubMed: 17369525]
- Lester LB, Faux MC, Nauert JB, Scott JD. Targeted protein kinase A and PP-2B regulate insulin secretion through reversible phosphorylation. *Endocrinology* 2001;142:1218–1227. [PubMed: 11181538]
- Lester LB, Langeberg LK, Scott JD. Anchoring of protein kinase A facilitates hormone-mediated insulin secretion. *Proc Natl Acad Sci USA* 1997;94:14942–14947. [PubMed: 9405718]
- 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. [PubMed: 11014226]
- 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. [PubMed: 15677506]
- 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. [PubMed: 12409292]
- Light PE, Manning Fox JE, Riedel MJ, Wheeler MB. Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. *Mol Endocrinol* 2002;16:2135–2144. [PubMed: 12198249]

- Lim GE, Brubaker PL. Glucagon-like peptide 1 secretion by the L-cell. *Diabetes* 2006;55:S70–S77.
- Liu H, Hu Y, Simpson RW, Dear AE. Glucagon-like peptide-1 attenuates tumour necrosis factor-alpha-mediated induction of plasminogen activator inhibitor-1 expression. *J Endocrinol* 2008;196:57–65. [PubMed: 18180317]
- Liu Z, Habener JF. Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic beta cell proliferation. *J Biol Chem* 2008;283:8723–8735. [PubMed: 18216022]
- Loder MK, da Silva Xavier G, McDonald A, Rutter GA. TCF7L2 controls insulin gene expression and insulin secretion in mature pancreatic beta-cells. *Biochem Soc Trans* 2008;36:357–359. [PubMed: 18481957]
- Lonart G, Schoch S, Kaeser PS, Larkin CJ, Südhof TC, Linden DJ. Phosphorylation of RIM1alpha by PKA triggers presynaptic long-term potentiation at cerebellar parallel fiber synapses. *Cell* 2003;115:49–60. [PubMed: 14532002]
- Lotfi S, Li Z, Sun J, Zuo Y, Lam PP, Kang Y, Rahimi M, Islam D, Wang P, Gaisano HY, et al. Role of the exchange protein directly activated by cyclic adenosine 5'-monophosphate (Epac) pathway in regulating proglucagon gene expression in intestinal endocrine L cells. *Endocrinology* 2006;147:3727–3736. [PubMed: 16644915]
- Lupi R, Mancarella R, Del Guerra S, Bugliani M, Del Prato S, Boggi U, Mosca F, Filipponi F, Marchetti P. Effects of exendin-4 on islets from type 2 diabetes patients. *Diabetes Obes Metab* 2008;10:515–519. [PubMed: 18201204]
- Luque MA, González N, Márquez L, Acitores A, Redondo A, Morales M, Valverde I, Villanueva-Peñacarrillo ML. Glucagon-like peptide-1 (GLP-1) and glucose metabolism in human myocytes. *J Endocrinol* 2002;173:465–473. [PubMed: 12065236]
- Lynn FC, Pamir 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. [PubMed: 11334402]
- Lynn FC, Thompson SA, Pospisilik JA, Ehse JA, Hinke SA, Pamir N, McIntosh CH, Pederson RA. A novel pathway for regulation of glucose-dependent insulinotropic polypeptide (GIP) receptor expression in beta cells. *Faseb J* 2003;17:91–93. [PubMed: 12475913]
- Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, Sjögren M, Ling C, Eriksson KF, Lethagen AL, et al. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *J Clin Invest* 2007;117:2155–2163. [PubMed: 17671651]
- MacDonald PE, Sewing S, Wang J, Joseph JW, Smukler SR, Sakellaropoulos G, Wang J, Saleh MC, Chan CB, Tsushima RG, et al. Inhibition of Kv2.1 voltage-dependent K⁺ channels in pancreatic beta-cells enhances glucose-dependent insulin secretion. *J Biol Chem* 2002;277:44938–44945. [PubMed: 12270920]
- MacDonald PE, Wang X, Xia F, El-kholy W, Targonsky ED, Tsushima RG, Wheeler MB. Antagonism of rat beta-cell voltage-dependent K⁺ currents by exendin 4 requires dual activation of the cAMP/protein kinase A and phosphatidylinositol 3-kinase signaling pathways. *J Biol Chem* 2003;278:52446–52453. [PubMed: 14565957]
- 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. [PubMed: 10650957]
- Madsbad S. Treatment of type 2 diabetes with incretin-based therapies. *Lancet*. 2008
- Madsbad S, Schmitz O, Ransam J, Jakobsen G, Matthews DR. Improved glycemic control with no weight increase in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog liraglutide (NN2211): a 12-week, double-blind, randomized, controlled trial. *Diabetes Care* 2004;27:1335–1342. [PubMed: 15161785]
- Maletti M, Portha B, Carlquist M, Kergoat M, Laburthe M, Marie JC, Rosselin G. Evidence for and characterization of specific high affinity binding sites for the gastric inhibitory polypeptide in pancreatic beta-cells. *Endocrinology* 1984;115:1324–1331. [PubMed: 6383787]
- Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, Ribet U, Watanabe T, Drucker DJ, Wagtmann N. Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Natl Acad Sci USA* 2000;97:6874–6879. [PubMed: 10823914]

- Márquez L, Trapote MA, Luque MA, Valverde I, Villanueva-Peñacarrillo ML. Inositolphosphoglycans possibly mediate the effects of glucagon-like peptide-1(7–36)amide on rat liver and adipose tissue. *Cell Biochem Funct* 1998;16:51–56. [PubMed: 9580153]
- Mashima H, Ohnishi H, Wakabayashi K, Mine T, Miyagawa J, Hanafusa T, Seno M, Yamada H, Kojima I. Betacellulin and activin A coordinately convert amylase-secreting pancreatic AR42J cells into insulin-secreting cells. *J Clin Invest* 1996;97:1647–1654. [PubMed: 8601630]
- Matschinsky F, Liang Y, Kesavan P, Wang L, Froguel P, Velho G, Cohen D, Permutt MA, Tanizawa Y, Jetton TL. Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. *J Clin Invest* 1993;92:2092–2098. [PubMed: 8227324]
- McClellan PL, Irwin N, Cassidy RS, Holst JJ, Gault VA, Flatt PR. GIP receptor antagonism reverses obesity, insulin resistance, and associated metabolic disturbances induced in mice by prolonged consumption of high-fat diet. *Am J Physiol Endocrinol Metab* 2007;293:E1746–E1755. [PubMed: 17848629]
- McIntosh CH, Wheeler MB, Gelling RW, Brown JC, Pederson RA. GIP receptors and signal-transduction mechanisms. *Acta Physiol Scand* 1996;157:361–365. [PubMed: 8830895]
- McIntyre N, Holdsworth CD, Turner DS. New interpretation of oral glucose tolerance. *Lancet* 1964;2:20–21. [PubMed: 14149200]
- McKinnon CM, Ravier MA, Rutter GA. FoxO1 is required for the regulation of preproglucagon gene expression by insulin in pancreatic alphaTC1–9 cells. *J Biol Chem* 2006;281:39358–39369. [PubMed: 17062568]
- Meeran K, O'Shea D, Edwards CM, Turton MD, Heath MM, Gunn I, Abusnana S, Rossi M, Small CJ, Goldstone AP, et al. 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. [PubMed: 9886831]
- Meier JJ, Gallwitz B, Askenas M, Vollmer K, Deacon CF, Holst JJ, Schmidt WE, Nauck MA. Secretion of incretin hormones and the insulinotropic effect of gastric inhibitory polypeptide in women with a history of gestational diabetes. *Diabetologia* 2005;48:1872–1881. [PubMed: 16010522]
- 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* 2003a;88:2719–2725. [PubMed: 1278879]
- Meier JJ, Gallwitz B, Siepmann N, Holst JJ, Deacon CF, Schmidt WE, Nauck MA. Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia. *Diabetologia* 2003b;46:798–801. [PubMed: 12764578]
- Meier JJ, Goetze O, Anstipp J, Hagemann D, Holst JJ, Schmidt WE, Gallwitz B, Nauck MA. Gastric inhibitory polypeptide does not inhibit gastric emptying in humans. *Am J Physiol Endocrinol Metab* 2004;286:E621–E625. [PubMed: 14678954]
- Meier JJ, Hücking K, Holst JJ, Deacon CF, Schmiegel WH, Nauck MA. Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. *Diabetes* 2001;50:2497–2504. [PubMed: 11679427]
- Meneilly GS, Elahi D. Effect of glucagon-like peptide-1 on the beta cell response to glucose-dependent insulinotropic polypeptide in elderly patients with diabetes mellitus. *J Am Geriatr Soc* 2006;54:554–555. [PubMed: 16551338]
- Meneilly GS, Greig N, Tildesley H, Habener JF, Egan JM, Elahi D. Effects of 3 months of continuous subcutaneous administration of glucagon-like peptide 1 in elderly patients with type 2 diabetes. *Diabetes Care* 2003;26:2835–2841. [PubMed: 14514588]
- Messenger B, Clifford MN, Morgan LM. Glucose-dependent insulinotropic polypeptide and insulin-like immunoreactivity in saliva following sham-fed and swallowed meals. *J Endocrinol* 2003;177:407–412. [PubMed: 12773121]
- Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou H, Fujimoto S, Oku A, Tsuda K, Toyokuni S, et al. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med* 2002;8:738–742. [PubMed: 12068290]
- Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, Kubota A, Fujimoto S, Kajikawa M, Kuroe A, et al. 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. [PubMed: 10611300]
- Moens K, Heimberg H, Flamez D, Huypens P, Quartier E, Ling Z, Pipeleers D, Gremlich S, Thorens B, Schuit F. Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. *Diabetes* 1996;45:257–261. [PubMed: 8549871]
- 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. [PubMed: 3528148]
- 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. [PubMed: 10067836]
- Montrose-Rafizadeh C, Egan JM, Roth J. Incretin hormones regulate glucose-dependent insulin secretion in RIN 1046–38 cells: mechanisms of action. *Endocrinology* 1994;135:589–594. [PubMed: 8033807]
- Moore B. On the treatment of diabetes mellitus by acid extract of duodenal mucous membrane. *Biochem J* 1906;1:28–38. [PubMed: 16742013]
- Morales M, López-Delgado MI, Alcántara A, Luque MA, Clemente F, Márquez L, Puente J, Viñambres C, Malaisse WJ, Villanueva-Peñacarrillo ML, et al. Preserved GLP-I effects on glycogen synthase activity and glucose metabolism in isolated hepatocytes and skeletal muscle from diabetic rats. *Diabetes* 1997;46:1264–1269. [PubMed: 9231649]
- 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. [PubMed: 12832109]
- Nagell CF, Wettergren A, Ørskov C, Holst JJ. Inhibitory effect of GLP-1 on gastric motility persists after vagal deafferentation in pigs. *Scand J Gastroenterol* 2006;41:667–672. [PubMed: 16716964]
- Nakabayashi H, Nishizawa M, Nakagawa A, Takeda R, Nijima A. Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. *Am J Physiol* 1996;271:E808–E813. [PubMed: 8944665]
- Nakagawa A, Satake H, Nakabayashi H, Nishizawa M, Furuya K, Nakano S, Kigoshi T, Nakayama K, Uchida K. Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. *Auton Neurosci* 2004;110:36–43. [PubMed: 14766323]
- Nakajima T, Nakajima E, Fukiage C, Azuma M, Shearer TR. Differential gene expression in the lens epithelial cells from selenite injected rats. *Exp Eye Res* 2002;74:231–236. [PubMed: 11950233]
- Nakazaki M, Crane A, Hu M, Seghers V, Ullrich S, Aguilar-Bryan L, Bryan J. cAMP-activated protein kinase-independent potentiation of insulin secretion by cAMP is impaired in SUR1 null islets. *Diabetes* 2002;51:3440–3449. [PubMed: 12453898]
- Näslund E, Barkeling B, King N, Gutniak M, Blundell JE, Holst JJ, Rössner S, Hellström PM. Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord* 1999;23:304–311. [PubMed: 10193877]
- Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF. Insulinotropic action of glucagonlike peptide-I-(7–37) in diabetic and nondiabetic subjects. *Diabetes Care* 1992;15:270–276. [PubMed: 1547685]
- Nauck M, Schmidt WE, Ebert R, Strietzel J, Cantor P, Hoffmann G, Creutzfeldt W. Insulinotropic properties of synthetic human gastric inhibitory polypeptide in man: interactions with glucose, phenylalanine, and cholecystokinin-8. *J Clin Endocrinol Metab* 1989;69:654–662. [PubMed: 2668324]
- Nauck M, Stöckmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1986;29:46–52. [PubMed: 3514343]
- 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. [PubMed: 1459356]

- 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* 2007a;50:259–267. [PubMed: 17160407]
- Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R, Hübner 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. [PubMed: 11889194]
- 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* 1993a;91:301–307. [PubMed: 8423228]
- Nauck MA, Holst JJ, Willms B. Glucagon-like peptide 1 and its potential in the treatment of non-insulin-dependent diabetes mellitus. *Horm Metab Res* 1997;29:411–416. [PubMed: 9370106]
- 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. [PubMed: 17039422]
- Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 1993b;36:741–744. [PubMed: 8405741]
- Nauck MA, Meininger G, Sheng D, Terranella L, Stein PP. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor, sitagliptin, compared with the sulfonylurea, glipizide, in patients with type 2 diabetes inadequately controlled on metformin alone: a randomized, double-blind, non-inferiority trial. *Diabetes Obes Metab* 2007b;9:194–205. [PubMed: 17300595]
- Neill JD, Duck LW, Sellers JC, Musgrove LC, Scheschonka A, Druey KM, Kehrl JH. Potential role for a regulator of G protein signaling (RGS3) in gonadotropin-releasing hormone (GnRH) stimulated desensitization. *Endocrinology* 1997;138:843–846. [PubMed: 9003025]
- Newgard CB. Cellular engineering and gene therapy strategies for insulin replacement in diabetes. *Diabetes* 1994;43:341–350. [PubMed: 8314006]
- Ni Z, Anini Y, Fang X, Mills G, Brubaker PL, Jin T. Transcriptional activation of the proglucagon gene by lithium and beta-catenin in intestinal endocrine L cells. *J Biol Chem* 2003;278:1380–1387. [PubMed: 12421827]
- Nian M, Drucker DJ, Irwin D. Divergent regulation of human and rat proglucagon gene promoters in vivo. *Am J Physiol* 1999;277:G829–G837. [PubMed: 10516149]
- Nikolaidis LA, Doverspike A, Hentosz T, Zourelis L, Shen YT, Elahi D, Shannon RP. Glucagon-like peptide-1 limits myocardial stunning following brief coronary occlusion and reperfusion in conscious canines. *J Pharmacol Exp Ther* 2005;312:303–308. [PubMed: 15356213]
- 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* 2004a;110:955–961. [PubMed: 15313949]
- 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* 2004b;109:962–965. [PubMed: 14981009]
- Nishizawa M, Nakabayashi H, Uchida K, Nakagawa A, Nijima A. The hepatic vagal nerve is receptive to incretin hormone glucagon-like peptide-1, but not to glucose-dependent insulinotropic polypeptide, in the portal vein. *J Auton Nerv Syst* 1996;61:149–154. [PubMed: 8946334]
- Norata GD, Tibolla G, Seccomandi PM, Poletti A, Catapano AL. Dihydrotestosterone decreases tumor necrosis factor-alpha and lipopolysaccharide-induced inflammatory response in human endothelial cells. *J Clin Endocrinol Metab* 2006;91:546–554. [PubMed: 16317058]
- Novak U, Wilks A, Buell G, McEwen S. Identical mRNA for preproglucagon in pancreas and gut. *Eur J Biochem* 1987;164:553–558. [PubMed: 3569278]
- Nyberg J, Anderson MF, Meister B, Alborn AM, Ström AK, Brederlau A, Illerskog AC, Nilsson O, Kieffer TJ, Hietala MA, et al. Glucose-dependent insulinotropic polypeptide is expressed in adult

- hippocampus and induces progenitor cell proliferation. *J Neurosci* 2005;25:1816–1825. [PubMed: 15716418]
- Nyberg J, Jacobsson C, Anderson MF, Eriksson PS. Immunohistochemical distribution of glucose-dependent insulinotropic polypeptide in the adult rat brain. *J Neurosci Res* 2007;85:2099–2119. [PubMed: 17510976]
- Nyström T, Gutniak MK, Zhang Q, Zhang F, Holst JJ, Ahrén B, Sjöholm A. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab* 2004;287:E1209–E1215. [PubMed: 15353407]
- Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 1996;122:983–995. [PubMed: 8631275]
- Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO J* 1993;12:4251–4259. [PubMed: 7901001]
- Orskov C, Holst JJ, Knuhtsen S, Baldissera FG, Poulsen SS, Nielsen OV. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. *Endocrinology* 1986;119:1467–1475. [PubMed: 3530719]
- Orskov C, Poulsen SS. Glucagonlike peptide-I-(7–36)-amide receptors only in islets of Langerhans. Autoradiographic survey of extracerebral tissues in rats. *Diabetes* 1991;40:1292–1296. [PubMed: 1657666]
- Orskov C, Poulsen SS, Møller M, Holst JJ. Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. *Diabetes* 1996;45:832–835. [PubMed: 8635662]
- Otonkoski T, Hayek A. Constitution of a biphasic insulin response to glucose in human fetal pancreatic beta-cells with glucagon-like peptide 1. *J Clin Endocrinol Metab* 1995;80:3779–3783. [PubMed: 8530635]
- Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, Ueno H, Sunaga Y, Yano H, Matsuura Y, Iwanaga T, et al. cAMP-GEFII is a direct target of cAMP in regulated exocytosis. *Nat Cell Biol* 2000;2:805–811. [PubMed: 11056535]
- Pamir N, Lynn FC, Buchan AM, Ehse J, Hinke SA, Pospisilik JA, Miyawaki K, Yamada Y, Seino Y, McIntosh CH, et al. Glucose-dependent insulinotropic polypeptide receptor null mice exhibit compensatory changes in the enteroinsular axis. *Am J Physiol Endocrinol Metab* 2003;284:E931–E939. [PubMed: 12540373]
- 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. [PubMed: 16272563]
- Parkes D, Jodka C, Smith P, Nayak S, Rinehart L, Gingerich R, Chen K, Young A. Pharmacokinetic actions of exendin-4 in the rat: comparison with glucagon-like peptide-1. *Drug Dev Res* 2001;53:260–267.
- Pederson RA, Brown JC. Inhibition of histamine-, pentagastrin-, and insulin-stimulated canine gastric secretion by pure “gastric inhibitory polypeptide”. *Gastroenterology* 1972;62:393–400. [PubMed: 4551806]
- Pederson RA, Brown JC. Interaction of gastric inhibitory polypeptide, glucose, and arginine on insulin and glucagon secretion from the perfused rat pancreas. *Endocrinology* 1978;103:610–615. [PubMed: 744105]
- Pederson RA, Satkunarajah M, McIntosh CH, Scrocchi LA, Flamez D, Schuit F, Drucker DJ, Wheeler MB. Enhanced glucose-dependent insulinotropic polypeptide secretion and insulinotropic action in glucagon-like peptide 1 receptor $-/-$ mice. *Diabetes* 1998;47:1046–1052. [PubMed: 9648827]
- Pederson RA, Schubert HE, Brown JC. Gastric inhibitory polypeptide. Its physiologic release and insulinotropic action in the dog. *Diabetes* 1975;24:1050–1056. [PubMed: 1193309]
- Perfetti R, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 2000;141:4600–4605. [PubMed: 11108273]
- Perley MJ, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. *J Clin Invest* 1967;46:1954–1962. [PubMed: 6074000]

- Persson K, Gingerich RL, Nayak S, Wada K, Wada E, Ahrén B. Reduced GLP-1 and insulin responses and glucose intolerance after gastric glucose in GRP receptor-deleted mice. *Am J Physiol Endocrinol Metab* 2000;279:E956–E962. [PubMed: 11052949]
- Philippe J. Glucagon gene transcription is negatively regulated by insulin in a hamster islet cell line. *J Clin Invest* 1989;84:672–677. [PubMed: 2668337]
- Philippe J. Insulin regulation of the glucagon gene is mediated by an insulin-responsive DNA element. *Proc Natl Acad Sci USA* 1991;88:7224–7227. [PubMed: 1651499]
- Philippe J, Drucker DJ, Knepel W, Jepeal L, Misulovin Z, Habener JF. Alpha-cell-specific expression of the glucagon gene is conferred to the glucagon promoter element by the interactions of DNA-binding proteins. *Mol Cell Biol* 1988;8:4877–4888. [PubMed: 3062372]
- Pipeleers DG, Schuit FC, Van Schravendijk CF, Van de Winkel M. Interplay of nutrients and hormones in the regulation of glucagon release. *Endocrinology* 1985;117:817–823. [PubMed: 2862020]
- Piteau S, Olver A, Kim SJ, Winter K, Pospisilik JA, Lynn F, Manhart S, Demuth HU, Speck M, Pederson RA, et al. Reversal of islet GIP receptor down-regulation and resistance to GIP by reducing hyperglycemia in the Zucker rat. *Biochem Biophys Res Commun* 2007;362:1007–1012. [PubMed: 17803965]
- 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 anaesthetised pig. *Diabetologia* 2005;48:1882–1890. [PubMed: 16025254]
- Preitner F, Ibberson M, Franklin I, Binnert C, Pende M, Gjinovci A, Hansotia T, Drucker DJ, Wollheim C, Burcelin R, et al. Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors. *J Clin Invest* 2004;113:635–645. [PubMed: 14966573]
- Premont RT, Inglese J, Lefkowitz RJ. Protein kinases that phosphorylate activated G protein-coupled receptors. *Faseb J* 1995;9:175–182. [PubMed: 7781920]
- Prigeon RL, Quddusi S, Paty B, D'Alessio DA. Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. *Am J Physiol Endocrinol Metab* 2003;285:E701–E707. [PubMed: 12773303]
- Qin X, Shen H, Liu M, Yang Q, Zheng S, Sabo M, D'Alessio DA, Tso P. GLP-1 reduces intestinal lymph flow, triglyceride absorption, and apolipoprotein production in rats. *Am J Physiol Gastrointest Liver Physiol* 2005;288:G943–G949. [PubMed: 15677555]
- Rachman J, Barrow BA, Levy JC, Turner RC. Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM. *Diabetologia* 1997;40:205–211. [PubMed: 9049482]
- Ranta F, Avram D, Berchtold S, Düfer M, Drews G, Lang F, Ullrich S. Dexamethasone induces cell death in insulin-secreting cells, an effect reversed by exendin-4. *Diabetes* 2006;55:1380–1390. [PubMed: 16644695]
- 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. [PubMed: 17001471]
- Redondo A, Trigo MV, Acitores A, Valverde I, Villanueva-Peñacarrillo ML. Cell signalling of the GLP-1 action in rat liver. *Mol Cell Endocrinol* 2003;204:43–50. [PubMed: 12850280]
- Reneke JE, Blumer KJ, Courchesne WE, Thorner J. The carboxy-terminal segment of the yeast alpha-factor receptor is a regulatory domain. *Cell* 1988;55:221–234. [PubMed: 2844413]
- Richter G, Feddersen O, Wagner U, Barth P, Göke R, Göke B. GLP-1 stimulates secretion of macromolecules from airways and relaxes pulmonary artery. *Am J Physiol* 1993;265:L374–L381. [PubMed: 8238371]
- Rinaman L. Interoceptive stress activates glucagon-like peptide-1 neurons that project to the hypothalamus. *Am J Physiol* 1999;277:R582–R590. [PubMed: 10444567]
- Rizos CV, Liberopoulos EN, Mikhailidis DP, Elisaf MS. Pleiotropic effects of thiazolidinediones. *Expert Opin Pharmacother* 2008;9:1087–1108. [PubMed: 18422468]
- Roberge JN, Brubaker PL. Secretion of proglucagon-derived peptides in response to intestinal luminal nutrients. *Endocrinology* 1991;128:3169–3174. [PubMed: 2036983]

- Roberge JN, Gronau KA, Brubaker PL. Gastrin-releasing peptide is a novel mediator of proximal nutrient-induced proglucagon-derived peptide secretion from the distal gut. *Endocrinology* 1996;137:2383–2388. [PubMed: 8641190]
- Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology* 1999;140:1687–1694. [PubMed: 10098504]
- 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. [PubMed: 17157112]
- Ross SA, Brown JC, Dupré J. Hypersecretion of gastric inhibitory polypeptide following oral glucose in diabetes mellitus. *Diabetes* 1977;26:525–529. [PubMed: 324834]
- Ross SA, Dupre J. Effects of ingestion of triglyceride or galactose on secretion of gastric inhibitory polypeptide and on responses to intravenous glucose in normal and diabetic subjects. *Diabetes* 1978;27:327–333. [PubMed: 640238]
- 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. [PubMed: 7730317]
- Rouillé 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. [PubMed: 7592866]
- Rouillé Y, Westermarck G, Martin SK, Steiner DF. Proglucagon is processed to glucagon by prohormone convertase PC2 in alpha TC1–6 cells. *Proc Natl Acad Sci U S A* 1994;91:3242–3246. [PubMed: 8159732]
- Ruiz-Grande C, Alarcón C, Alcántara A, Castilla C, López Novoa JM, Villanueva-Peñacarrillo ML, Valverde I. Renal catabolism of truncated glucagon-like peptide 1. *Horm Metab Res* 1993;25:612–616. [PubMed: 8119664]
- 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. [PubMed: 9661618]
- Sagar SM, Sharp FR, Curran T. Expression of c-fos protein in brain: metabolic mapping at the cellular level. *Science* 1988;240:1328–1331. [PubMed: 3131879]
- Salehi A, Meidute Abaraviciene S, Jimenez-Feltstrom J, Ostenson CG, Efendic S, Lundquist I. Excessive islet NO generation in type 2 diabetic GK rats coincides with abnormal hormone secretion and is counteracted by GLP-1. *PLoS ONE* 2008;3:e2165. [PubMed: 18478125]
- Sancho V, Nuche B, Arnés L, Cancelas J, González N, Díaz-Miguel M, Martín-Duce A, Valverde I, Villanueva-Peñacarrillo ML. The action of GLP-1 and exendins upon glucose transport in normal human adipocytes, and on kinase activity as compared with morbidly obese patients. *Int J Mol Med* 2007;19:961–966. [PubMed: 17487430]
- Sancho V, Trigo MV, González N, Valverde I, Malaisse WJ, Villanueva-Peñacarrillo ML. Effects of glucagon-like peptide-1 and exendins on kinase activity, glucose transport and lipid metabolism in adipocytes from normal and type-2 diabetic rats. *J Mol Endocrinol* 2005;35:27–38. [PubMed: 16087719]
- Sander M, Neubüser 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. [PubMed: 9224716]
- Sandhu H, Wiesenthal SR, MacDonald PE, McCall RH, Tchipashvili V, Rashid S, Satkunarajah M, Irwin DM, Shi ZQ, Brubaker PL, et al. Glucagon-like peptide 1 increases insulin sensitivity in depancreatized dogs. *Diabetes* 1999;48:1045–1053. [PubMed: 10331409]
- Sandoval DA, Bagnol D, Woods SC, D'Alessio DA, Seeley RJ. Arcuate GLP-1 receptors regulate glucose homeostasis but not food intake. *Diabetes* 2008;57:2046–2054. [PubMed: 18487451]
- Sarkar S, Fekete C, Légrádi G, Lechan RM. Glucagon like peptide-1 (7–36) amide (GLP-1) nerve terminals densely innervate corticotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. *Brain Res* 2003;985:163–168. [PubMed: 12967720]

- Schauder P, Brown JC, Frerichs H, Creutzfeldt W. Gastric inhibitory polypeptide: effect on glucose-induced insulin release from isolated rat pancreatic islets in vitro. *Diabetologia* 1975;11:483–484. [PubMed: 1103369]
- Schinner S, Barthel A, Dellas C, Grzeskowiak R, Sharma SK, Oetjen E, Blume R, Knepel W. Protein kinase B activity is sufficient to mimic the effect of insulin on glucagon gene transcription. *J Biol Chem* 2005;280:7369–7376. [PubMed: 15590659]
- Schirra J, Katschinski M, Weidmann C, Schäfer T, Wank U, Arnold R, Göke B. Gastric emptying and release of incretin hormones after glucose ingestion in humans. *J Clin Invest* 1996;97:92–103. [PubMed: 8550855]
- Schirra J, Nicolaus M, Roggel R, Katschinski M, Storr M, Woerle HJ, Göke B. Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. *Gut* 2006;55:243–251. [PubMed: 15985560]
- Schmid R, Schusdziarra V, Aulehner R, Weigert N, Classen M. Comparison of GLP-1 (7–36amide) and GIP on release of somatostatin-like immunoreactivity and insulin from the isolated rat pancreas. *Z Gastroenterol* 1990;28:280–284. [PubMed: 2238756]
- Schmidt WE, Siegel EG, Creutzfeldt W. Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. *Diabetologia* 1985;28:704–707. [PubMed: 3905480]
- Schwartz AV, Sellmeyer DE, Vittinghoff E, Palermo L, Lecka-Czernik B, Feingold KR, Strotmeyer ES, Resnick HE, Carbone L, Beamer BA, et al. Thiazolidinedione use and bone loss in older diabetic adults. *J Clin Endocrinol Metab* 2006;91:3349–3354. [PubMed: 16608888]
- Scopsi L, Gullo M, Rilke F, Martin S, Steiner DF. Proprotein convertases (PC1/PC3 and PC2) in normal and neoplastic human tissues: their use as markers of neuroendocrine differentiation. *J Clin Endocrinol Metab* 1995;80:294–301. [PubMed: 7829629]
- Scrocchi LA, Brown TJ, McClusky 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. [PubMed: 8898756]
- Scrocchi LA, Marshall BA, Cook SM, Brubaker PL, Drucker DJ. Identification of glucagon-like peptide 1 (GLP-1) actions essential for glucose homeostasis in mice with disruption of GLP-1 receptor signaling. *Diabetes* 1998;47:632–639. [PubMed: 9568697]
- Shibasaki T, Sunaga Y, Seino S. Integration of ATP, cAMP, and Ca²⁺ signals in insulin granule exocytosis. *Diabetes* 2004;53:S59–S62. [PubMed: 15561922]
- Shin YK, Martin B, Golden E, Dotson CD, Maudsley S, Kim W, Jang HJ, Mattson MP, Drucker DJ, Egan JM, et al. Modulation of taste sensitivity by GLP-1 signaling. *J Neurochem* 2008;106:455–463. [PubMed: 18397368]
- Shiota C, Larsson O, Shelton KD, Shiota M, Efanov AM, Hoy M, Lindner J, Kooptiwut S, Juntti-Berggren L, Gromada J, et al. Sulfonylurea receptor type 1 knock-out mice have intact feeding-stimulated insulin secretion despite marked impairment in their response to glucose. *J Biol Chem* 2002;277:37176–37183. [PubMed: 12149271]
- Shughrue PJ, Lane MV, Merchenthaler I. Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. *Endocrinology* 1996;137:5159–5162. [PubMed: 8895391]
- Simonsen L, Holst JJ, Deacon CF. Exendin-4, but not glucagon-like peptide-1, is cleared exclusively by glomerular filtration in anaesthetised pigs. *Diabetologia* 2006;49:706–712. [PubMed: 16447056]
- Skoglund G, Hussain MA, Holz GG. Glucagon-like peptide 1 stimulates insulin gene promoter activity by protein kinase A-independent activation of the rat insulin I gene cAMP response element. *Diabetes* 2000;49:1156–1164. [PubMed: 10909973]
- Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. *J Card Fail* 2006;12:694–699. [PubMed: 17174230]
- Someya Y, Inagaki N, Maekawa T, Seino Y, Ishii S. Two 3',5'-cyclicadenosine monophosphate response elements in the promoter region of the human gastric inhibitory polypeptide gene. *FEBS Lett* 1993;317:67–73. [PubMed: 8428636]
- Sondhi S, Castellano JM, Chong VZ, Rogoza RM, Skoblenick KJ, Dyck BA, Gabriele J, Thomas N, Ki K, Pristupa ZB, et al. cDNA array reveals increased expression of glucose-dependent insulinotropic

polypeptide following chronic clozapine treatment: role in atypical antipsychotic drug-induced adverse metabolic effects. *Pharmacogenomics J* 2006;6:131–140. [PubMed: 16402076]

- Song DH, Getty-Kaushik L, Tseng E, Simon J, Corkey BE, Wolfe MM. Glucose-dependent insulinotropic polypeptide enhances adipocyte development and glucose uptake in part through Akt activation. *Gastroenterology* 2007;133:1796–1805. [PubMed: 18054552]
- Sonne DP, Engstrøm T, Treiman M. Protective effects of GLP-1 analogues exendin-4 and GLP-1(9–36) amide against ischemia-reperfusion injury in rat heart. *Regul Pept* 2008;146:243–249. [PubMed: 17976835]
- Sonoda N, Imamura T, Yoshizaki T, Babendure JL, Lu JC, Olefsky JM. Beta-Arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic beta cells. *Proc Natl Acad Sci USA* 2008;105:6614–6619. [PubMed: 18445652]
- Starling EH. The Cronian Lectures on the chemical correlation of the functions of the body. *Lancet* 1905;2:339–341. 423–425, 501–503, 579–583.
- Stein PP, Williams-Herman D, Khatami H, Meininger G, Round E, Sheng D, Sanchez M, Lunceford J, Kaufman KD, Amatrua JM. Sitagliptin, a selective DPP-4 Inhibitor, is well tolerated in patients with type 2 diabetes: pooled analysis of 5141 patients in clinical trials for up to 2 years (Abstract). *Diabetes* 2007;56:A142.
- Stoffel M, Espinosa R 3rd, Le Beau MM, Bell GI. Human glucagon-like peptide-1 receptor gene. Localization to chromosome band 6p21 by fluorescence in situ hybridization and linkage of a highly polymorphic simple tandem repeat DNA polymorphism to other markers on chromosome 6. *Diabetes* 1993;42:1215–1218. [PubMed: 8392011]
- Stoffers DA, Desai BM, DeLeon DD, Simmons RA. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes* 2003;52:734–740. [PubMed: 12606515]
- Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, Egan JM. Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* 2000;49:741–748. [PubMed: 10905482]
- Sunkara G, Sabo R, Wang Y, He YL, Campestrini J, Rosenberg M, Howard D, Dole WP. Dose proportionality and the effect of food on vildagliptin, a novel dipeptidyl peptidase IV inhibitor, in healthy volunteers. *J Clin Pharmacol* 2007;47:1152–1158. [PubMed: 17656620]
- Syme CA, Zhang L, Bisello A. Caveolin-1 regulates cellular trafficking and function of the glucagon-like Peptide 1 receptor. *Mol Endocrinol* 2006;20:3400–3411. [PubMed: 16931572]
- Takeda J, Seino Y, Tanaka K, Fukumoto H, Kayano T, Takahashi H, Mitani T, Kurono M, Suzuki T, Tobe T. Sequence of an intestinal cDNA encoding human gastric inhibitory polypeptide precursor. *Proc Natl Acad Sci USA* 1987;84:7005–7008. [PubMed: 2890159]
- 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. [PubMed: 15932924]
- Tang-Christensen M, Larsen PJ, Göke R, Fink-Jensen A, Jessop DS, Møller 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. [PubMed: 8897973]
- Tei E, Mehta S, Tulachan SS, Yew H, Hembree M, Preuett B, Snyder CL, Yamataka A, Miyano T, Gittes GK. Synergistic endocrine induction by GLP-1 and TGF-beta in the developing pancreas. *Pancreas* 2005;31:138–141. [PubMed: 16025000]
- Tews D, Werner U, Eckel J. Enhanced protection against cytokine- and fatty acid-induced apoptosis in pancreatic beta cells by combined treatment with glucagon-like peptide-1 receptor agonists and insulin analogues. *Horm Metab Res* 2008;40:172–180. [PubMed: 18348079]
- 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 Endocrinol Metab* 2006;290:E550–E559. [PubMed: 16219666]
- Theodorakis MJ, Carlson O, Muller DC, Egan JM. Elevated plasma glucose-dependent insulinotropic polypeptide associates with hyperinsulinemia in impaired glucose tolerance. *Diabetes Care* 2004;27:1692–1698. [PubMed: 15220248]

- Thor P, Laskiewicz J, Konturek JW, Konturek SJ, Creutzfeldt W. Role of GIP and insulin in glucose-induced changes in intestinal motility patterns. *Am J Physiol* 1987;252:G8–G12. [PubMed: 3101511]
- Thorens B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci USA* 1992;89:8641–8645. [PubMed: 1326760]
- Thorens B, Porret A, Bühler L, Deng SP, Morel P, Widmann C. Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9–39) an antagonist of the receptor. *Diabetes* 1993;42:1678–1682. [PubMed: 8405712]
- Thrainsdottir I, Malmberg K, Olsson A, Gutniak M, Rydén L. Initial experience with GLP-1 treatment on metabolic control and myocardial function in patients with type 2 diabetes mellitus and heart failure. *Diab Vasc Dis Res* 2004;1:40–43. [PubMed: 16305055]
- Tibaduiza EC, Chen C, Beinborn M. A small molecule ligand of the glucagon-like peptide 1 receptor targets its amino-terminal hormone binding domain. *J Biol Chem* 2001;276:37787–37793. [PubMed: 11498540]
- Tillmar L, Carlsson C, Welsh N. Control of insulin mRNA stability in rat pancreatic islets. Regulatory role of a 3'-untranslated region pyrimidine-rich sequence. *J Biol Chem* 2002;277:1099–1106. [PubMed: 11696543]
- Todd JF, Bloom SR. Incretins and other peptides in the treatment of diabetes. *Diabet Med* 2007;24:223–232. [PubMed: 17263764]
- Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 2001;86:3717–3723. [PubMed: 11502801]
- Tornehave D, Kristensen P, Rømer J, Knudsen LB, Heller RS. Expression of the GLP-1 receptor in mouse, rat, and human pancreas. *J Histochem Cytochem* 2008;56:841–851. [PubMed: 18541709]
- Tourrel C, Bailbé D, Meile MJ, Kergoat M, Portha B. Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes* 2001;50:1562–1570. [PubMed: 11423477]
- Trapote MA, Clemente F, Galera C, Morales M, Alcántara AI, López-Delgado MI, Villanueva-Peñacarrillo ML, Valverde I. Inositolphosphoglycans are possible mediators of the glucagon-like peptide 1 (7–36)amide action in the liver. *J Endocrinol Invest* 1996;19:114–118. [PubMed: 8778163]
- 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. [PubMed: 12540617]
- Trümper A, Trümper K, Hörsch D. Mechanisms of mitogenic and anti-apoptotic signaling by glucose-dependent insulinotropic polypeptide in beta(INS-1)-cells. *J Endocrinol* 2002;174:233–246. [PubMed: 12176662]
- Trümper A, Trümper K, Trusheim H, Arnold R, Göke B, Hörsch D. Glucose-dependent insulinotropic polypeptide is a growth factor for beta (INS-1) cells by pleiotropic signaling. *Mol Endocrinol* 2001;15:1559–1570. [PubMed: 11518806]
- Tseng CC, Boylan MO, Jarboe LA, Usdin TB, Wolfe MM. Chronic desensitization of the glucose-dependent insulinotropic polypeptide receptor in diabetic rats. *Am J Physiol* 1996a;270:E661–E666. [PubMed: 8928774]
- Tseng CC, Jarboe LA, Landau SB, Williams EK, Wolfe MM. Glucose-dependent insulinotropic peptide: structure of the precursor and tissue-specific expression in rat. *Proc Natl Acad Sci USA* 1993;90:1992–1996. [PubMed: 8446620]
- Tseng CC, Jarboe LA, Wolfe MM. Regulation of glucose-dependent insulinotropic peptide gene expression by a glucose meal. *Am J Physiol* 1994;266:G887–G891. [PubMed: 8203533]
- 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* 1996b;98:2440–2445. [PubMed: 8958204]
- Tseng CC, Zhang XY. Role of regulator of G protein signaling in desensitization of the glucose-dependent insulinotropic peptide receptor. *Endocrinology* 1998;139:4470–4475. [PubMed: 9794454]

- Tsuboi T, da Silva Xavier G, Holz GG, Jouaville LS, Thomas AP, Rutter GA. Glucagon-like peptide-1 mobilizes intracellular Ca^{2+} and stimulates mitochondrial ATP synthesis in pancreatic MIN6 beta-cells. *Biochem J* 2003;369:287–299. [PubMed: 12410638]
- Tsukiyama K, Yamada Y, Yamada C, Harada N, Kawasaki Y, Ogura M, Bessho K, Li M, Amizuka N, Sato M, et al. Gastric inhibitory polypeptide as an endogenous factor promoting new bone formation after food ingestion. *Mol Endocrinol* 2006;20:1644–1651. [PubMed: 16469773]
- Tsunekawa S, Yamamoto N, Tsukamoto K, Itoh Y, Kaneko Y, Kimura T, Ariyoshi Y, Miura Y, Oiso Y, Niki I. Protection of pancreatic beta-cells by exendin-4 may involve the reduction of endoplasmic reticulum stress; in vivo and in vitro studies. *J Endocrinol* 2007;193:65–74. [PubMed: 17400804]
- Turk J, Gross RW, Ramanadham S. Amplification of insulin secretion by lipid messengers. *Diabetes* 1993;42:367–374. [PubMed: 8432406]
- Turk J, Wolf BA, McDaniel ML. The role of phospholipid-derived mediators including arachidonic acid, its metabolites, and inositoltrisphosphate and of intracellular Ca^{2+} in glucose-induced insulin secretion by pancreatic islets. *Prog Lipid Res* 1987;26:125–181. [PubMed: 3116559]
- Turner AJ, Isaac RE, Coates D. The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. *Bioessays* 2001;23:261–269. [PubMed: 11223883]
- Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, et al. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 1996;379:69–72. [PubMed: 8538742]
- 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. [PubMed: 16476726]
- Ugleholdt R, Zhu X, Deacon CF, Ørskov C, Steiner DF, Holst JJ. Impaired intestinal proglucagon processing in mice lacking prohormone convertase 1. *Endocrinology* 2004;145:1349–1355. [PubMed: 14630721]
- 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. [PubMed: 8243312]
- Vaag AA, Holst JJ, Vølund 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. [PubMed: 8921824]
- Vázquez P, Roncero I, Blázquez E, Alvarez E. The cytoplasmic domain close to the transmembrane region of the glucagon-like peptide-1 receptor contains sequence elements that regulate agonist-dependent internalisation. *J Endocrinol* 2005a;186:221–231.
- Vázquez P, Roncero I, Blázquez E, Alvarez E. Substitution of the cysteine 438 residue in the cytoplasmic tail of the glucagon-like peptide-1 receptor alters signal transduction activity. *J Endocrinol* 2005b;185:35–44.
- Verdich C, Flint A, Gutzwiller JP, Näslund E, Beglinger C, Hellström PM, Long SJ, Morgan LM, Holst JJ, Astrup A. A meta-analysis of the effect of glucagon-like peptide-1 (7–36) amide on ad libitum energy intake in humans. *J Clin Endocrinol Metab* 2001;86:4382–4389. [PubMed: 11549680]
- Villanueva-Peñacarrillo ML, Puente J, Redondo A, Clemente F, Valverde I. Effect of GLP-1 treatment on GLUT2 and GLUT4 expression in type 1 and type 2 rat diabetic models. *Endocrine* 2001;15:241–248. [PubMed: 11720253]
- Villhauer EB, Brinkman JA, Naderi GB, Burkey BF, Dunning BE, Prasad K, Mangold BL, Russell ME, Hughes TE. 1-[[[(3-hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(S)-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J Med Chem* 2003;46:2774–2789. [PubMed: 12801240]
- Vilsbøll T, Agersø 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* 2003a;88:220–224.
- Vilsbøll T, Agersø 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. [PubMed: 16934887]

- VilSBøll 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. [PubMed: 11246881]
- VilSBøll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese Type II diabetic patients. *Diabetologia* 2002;45:1111–1119. [PubMed: 12189441]
- VilSBøll T, Krarup T, Madsbad S, Holst JJ. Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. *Regul Pept* 2003b;114:115–121.
- VilSBøll T, Krarup T, Sonne J, Madsbad S, Volund A, Juul AG, Holst JJ. Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2003c;88:2706–2713.
- VilSBøll T, Zdravkovic M, Le-Thi T, Krarup T, Schmitz O, Courrèges JP, Verhoeven R, Bugánová I, Madsbad S. Liraglutide, a long-acting human glucagon-like peptide-1 analog, given as monotherapy significantly improves glycemic control and lowers body weight without risk of hypoglycemia in patients with type 2 diabetes. *Diabetes Care* 2007;30:1608–1610. [PubMed: 17372153]
- Vollmer K, Holst JJ, Baller B, Ellrichmann M, Nauck MA, Schmidt WE, Meier JJ. Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance. *Diabetes* 2008;57:678–687. [PubMed: 18057091]
- von Eggelkraut-Gottanka R, Beck-Sickinger AG. Biosynthesis of peptide hormones derived from precursor sequences. *Curr Med Chem* 2004;11:2651–2665. [PubMed: 15544467]
- 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. [PubMed: 12242459]
- Wang X, Cahill CM, Piñeyro 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. [PubMed: 10499550]
- Wang X, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 causes pancreatic duodenal homeobox-1 protein translocation from the cytoplasm to the nucleus of pancreatic beta-cells by a cyclic adenosine monophosphate/protein kinase A-dependent mechanism. *Endocrinology* 2001;142:1820–1827. [PubMed: 11316746]
- Wang Y, Kole HK, Montrose-Rafizadeh C, Perfetti R, Bernier M, Egan JM. Regulation of glucose transporters and hexose uptake in 3T3-L1 adipocytes: glucagon-like peptide-1 and insulin interactions. *J Mol Endocrinol* 1997a;19:241–248. [PubMed: 9460645]
- 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. [PubMed: 8822268]
- Wang Y, Okamoto M, Schmitz F, Hofmann K, Südhof TC. Rim is a putative Rab3 effector in regulating synaptic-vesicle fusion. *Nature* 1997b;388:593–598. [PubMed: 9252191]
- Wang Z, Wang RM, Owji AA, Smith DM, Ghatei MA, Bloom SR. Glucagon-like peptide-1 is a physiological incretin in rat. *J Clin Invest* 1995;95:417–421. [PubMed: 7814643]
- Wei Y, Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett* 1995;358:219–224. [PubMed: 7843404]
- Wettergren A, Maina P, Boesby S, Holst JJ. Glucagon-like peptide-1 7–36 amide and peptide YY have additive inhibitory effect on gastric acid secretion in man. *Scand J Gastroenterol* 1997a;32:552–555. [PubMed: 9200286]
- Wettergren A, Petersen H, Orskov C, Christiansen J, Sheikh SP, Holst JJ. Glucagon-like peptide-1 7–36 amide and peptide YY from the L-cell of the ileal mucosa are potent inhibitors of vagally induced gastric acid secretion in man. *Scand J Gastroenterol* 1994;29:501–505. [PubMed: 7915853]
- Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Truncated GLP-1 (proglucagon 78–107-amide) inhibits gastric and pancreatic functions in man. *Dig Dis Sci* 1993;38:665–673. [PubMed: 8462365]

- Wettergren A, Wøjdemann M, Meisner S, Stadil F, Holst JJ. The inhibitory effect of glucagon-like peptide-1 (GLP-1) 7–36 amide on gastric acid secretion in humans depends on an intact vagal innervation. *Gut* 1997b;40:597–601. [PubMed: 9203936]
- 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. [PubMed: 10455124]
- Wheeler MB, Gelling RW, McIntosh CH, Georgiou J, Brown JC, Pederson RA. Functional expression of the rat pancreatic islet glucose-dependent insulinotropic polypeptide receptor: ligand binding and intracellular signaling properties. *Endocrinology* 1995;136:4629–4639. [PubMed: 7664683]
- Wheeler MB, Lu M, Dillon JS, Leng XH, Chen C, Boyd AE 3rd. 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. [PubMed: 8391428]
- White JW, Saunders GF. Structure of the human glucagon gene. *Nucleic Acids Res* 1986;14:4719–4730. [PubMed: 3725587]
- Wideman RD, Covey SD, Webb GC, Drucker DJ, Kieffer TJ. A switch from prohormone convertase (PC)-2 to PC1/3 expression in transplanted alpha-cells is accompanied by differential processing of proglucagon and improved glucose homeostasis in mice. *Diabetes* 2007;56:2744–2752. [PubMed: 17698597]
- Wideman RD, Yu IL, Webber TD, Verchere CB, Johnson JD, Cheung AT, Kieffer TJ. Improving function and survival of pancreatic islets by endogenous production of glucagon-like peptide 1 (GLP-1). *Proc Natl Acad Sci U S A* 2006;103:13468–13473. [PubMed: 16938896]
- Widmann C, Dolci W, Thorens B. Agonist-induced internalization and recycling of the glucagon-like peptide-1 receptor in transfected fibroblasts and in insulinomas. *Biochem J* 1995;310:203–214. [PubMed: 7646446]
- Widmann C, Dolci W, Thorens B. Desensitization and phosphorylation of the glucagon-like peptide-1 (GLP-1) receptor by GLP-1 and 4-phorbol 12-myristate 13-acetate. *Mol Endocrinol* 1996a;10:62–75. [PubMed: 8838146]
- Widmann C, Dolci W, Thorens B. Heterologous desensitization of the glucagon-like peptide-1 receptor by phorbol esters requires phosphorylation of the cytoplasmic tail at four different sites. *J Biol Chem* 1996b;271:19957–19963. [PubMed: 8702711]
- Widmann C, Dolci W, Thorens B. Internalization and homologous desensitization of the GLP-1 receptor depend on phosphorylation of the receptor carboxyl tail at the same three sites. *Mol Endocrinol* 1997;11:1094–1102. [PubMed: 9212057]
- Willms B, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Nauck MA. Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1) (7–36) amide in type 2 (non-insulin-dependent) diabetic patients. *J Clin Endocrinol Metab* 1996;81:327–332. [PubMed: 8550773]
- Winzell MS, Ahrén B. Durable islet effects on insulin secretion and protein kinase A expression following exendin-4 treatment of high-fat diet-fed mice. *J Mol Endocrinol* 2008;40:93–100. [PubMed: 18234911]
- 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. [PubMed: 9700754]
- Wolfe MM, Hocking MP, Maico DG, McGuigan JE. Effects of antibodies to gastric inhibitory peptide on gastric acid secretion and gastrin release in the dog. *Gastroenterology* 1983;84:941–948. [PubMed: 6832569]
- Woods SC, West DB, Stein LJ, McKay LD, Lotter EC, Porte SG, Kenney NJ, Porte D Jr. Peptides and the control of meal size. *Diabetologia* 1981;20:305–313. [PubMed: 7014327]
- Xie D, Cheng H, Hamrick M, Zhong Q, Ding KH, Correa D, Williams S, Mulloy A, Bollag W, Bollag RJ, et al. Glucose-dependent insulinotropic polypeptide receptor knockout mice have altered bone turnover. *Bone* 2005;37:759–769. [PubMed: 16219496]

- Xie D, Zhong Q, Ding KH, Cheng H, Williams S, Correa D, Bollag WB, Bollag RJ, Insogna K, Troiano N, et al. Glucose-dependent insulintropic peptide-overexpressing transgenic mice have increased bone mass. *Bone* 2007a;40:1352–1360. [PubMed: 17321229]
- Xie T, Chen M, Zhang QH, Ma Z, Weinstein LS. Beta cell-specific deficiency of the stimulatory G protein alpha-subunit G α leads to reduced beta cell mass and insulin-deficient diabetes. *Proc Natl Acad Sci U S A* 2007b;104:19601–19606. [PubMed: 18029451]
- Xu G, Kaneto H, Laybutt DR, Duvivier-Kali VF, Trivedi N, Suzuma K, King GL, Weir GC, Bonner-Weir S. Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. *Diabetes* 2007;56:1551–1558. [PubMed: 17360984]
- Yamada C, Yamada Y, Tsukiyama K, Yamada K, Udagawa N, Takahashi N, Tanaka K, Drucker DJ, Seino Y, Inagaki N. The murine glucagon-like peptide-1 receptor is essential for control of bone resorption. *Endocrinology* 2008;149:574–579. [PubMed: 18039776]
- 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. [PubMed: 8575774]
- 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. [PubMed: 12684481]
- Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, et al. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest* 2002;110:43–52. [PubMed: 12093887]
- Yang H, Egan JM, Wang Y, Moyes CD, Roth J, Montrose MH, Montrose-Rafizadeh C. GLP-1 action in L6 myotubes is via a receptor different from the pancreatic GLP-1 receptor. *Am J Physiol* 1998;275:C675–C683. [PubMed: 9730951]
- Yasuda K, Inagaki N, Yamada Y, Kubota A, Seino S, Seino Y. Hamster gastric inhibitory polypeptide receptor expressed in pancreatic islets and clonal insulin-secreting cells: its structure and functional properties. *Biochem Biophys Res Commun* 1994;205:1556–1562. [PubMed: 7811236]
- Yew KH, Hembree M, Prasad K, Preuett B, McFall C, Benjes C, Crowley A, Sharp S, Tulachan S, Mehta S, et al. Cross-talk between bone morphogenetic protein and transforming growth factor-beta signaling is essential for exendin-4-induced insulin-positive differentiation of AR42J cells. *J Biol Chem* 2005;280:32209–32217. [PubMed: 16020542]
- Yew KH, Prasad KL, Preuett BL, Hembree MJ, McFall CR, Benjes CL, Crowley AR, Sharp SL, Li Z, Tulachan SS, et al. Interplay of glucagon-like peptide-1 and transforming growth factor-beta signaling in insulin-positive differentiation of AR42J cells. *Diabetes* 2004;53:2824–2835. [PubMed: 15504962]
- 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. [PubMed: 15525634]
- Yi F, Sun J, Lim GE, Fantus IG, Brubaker PL, Jin T. Cross talk between the insulin and Wnt signaling pathways: evidence from intestinal endocrine L cells. *Endocrinology* 2008;149:2341–2351. [PubMed: 18258680]
- Yip RG, Boylan MO, Kieffer TJ, Wolfe MM. Functional GIP receptors are present on adipocytes. *Endocrinology* 1998;139:4004–4007. [PubMed: 9724057]
- Yip RG, Wolfe MM. GIP biology and fat metabolism. *Life Sci* 2000;66:91–103. [PubMed: 10666005]
- 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. [PubMed: 17084712]
- Zaidi M, Inzerillo AM, Moonga BS, Bevis PJ, Huang CL. Forty years of calcitonin—where are we now? A tribute to the work of Iain Macintyre, FRS. *Bone* 2002;30:655–663. [PubMed: 11996901]

- 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. [PubMed: 11897280]
- 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 postischemic isolated rat hearts. *J Pharmacol Exp Ther* 2006;317:1106–1113. [PubMed: 16489128]
- Zhong Q, Itokawa T, Sridhar S, Ding KH, Xie D, Kang B, Bollag WB, Bollag RJ, Hamrick M, Insogna K, et al. Effects of glucose-dependent insulinotropic peptide on osteoclast function. *Am J Physiol Endocrinol Metab* 2007;292:E543–E548. [PubMed: 17003233]
- Zhou J, Livak MF, Bernier M, Muller DC, Carlson OD, Elahi D, Maudsley S, Egan JM. Ubiquitination is involved in glucose-mediated downregulation of GIP receptors in islets. *Am J Physiol Endocrinol Metab* 2007;293:E538–E547. [PubMed: 17505054]
- Zhou J, Montrose-Rafizadeh C, Janczewski AM, Pineyro MA, Sollott SJ, Wang Y, Egan JM. Glucagon-like peptide-1 does not mediate amylase release from AR42J cells. *J Cell Physiol* 1999a;181:470–478. [PubMed: 10528233]
- Zhou J, Pineyro MA, Wang X, Doyle ME, Egan JM. Exendin-4 differentiation of a human pancreatic duct cell line into endocrine cells: involvement of PDX-1 and HNF3beta transcription factors. *J Cell Physiol* 2002;192:304–314. [PubMed: 12124776]
- Zhou J, Wang X, Pineyro MA, Egan JM. Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. *Diabetes* 1999b;48:2358–2366. [PubMed: 10580424]
- Zhu X, Zhou A, Dey A, Norrbom C, Carroll R, Zhang C, Laurent V, Lindberg I, Ugleholdt R, Holst JJ, et al. Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects. *Proc Natl Acad Sci U S A* 2002;99:10293–10298. [PubMed: 12145326]
- Zinman B, Hoogwerf BJ, Durán García S, Milton DR, Giaconia JM, Kim DD, Trautmann ME, Brodows RG. The effect of adding exenatide to a thiazolidinedione in suboptimally controlled type 2 diabetes: a randomized trial. *Ann Intern Med* 2007;146:477–485. [PubMed: 17404349]

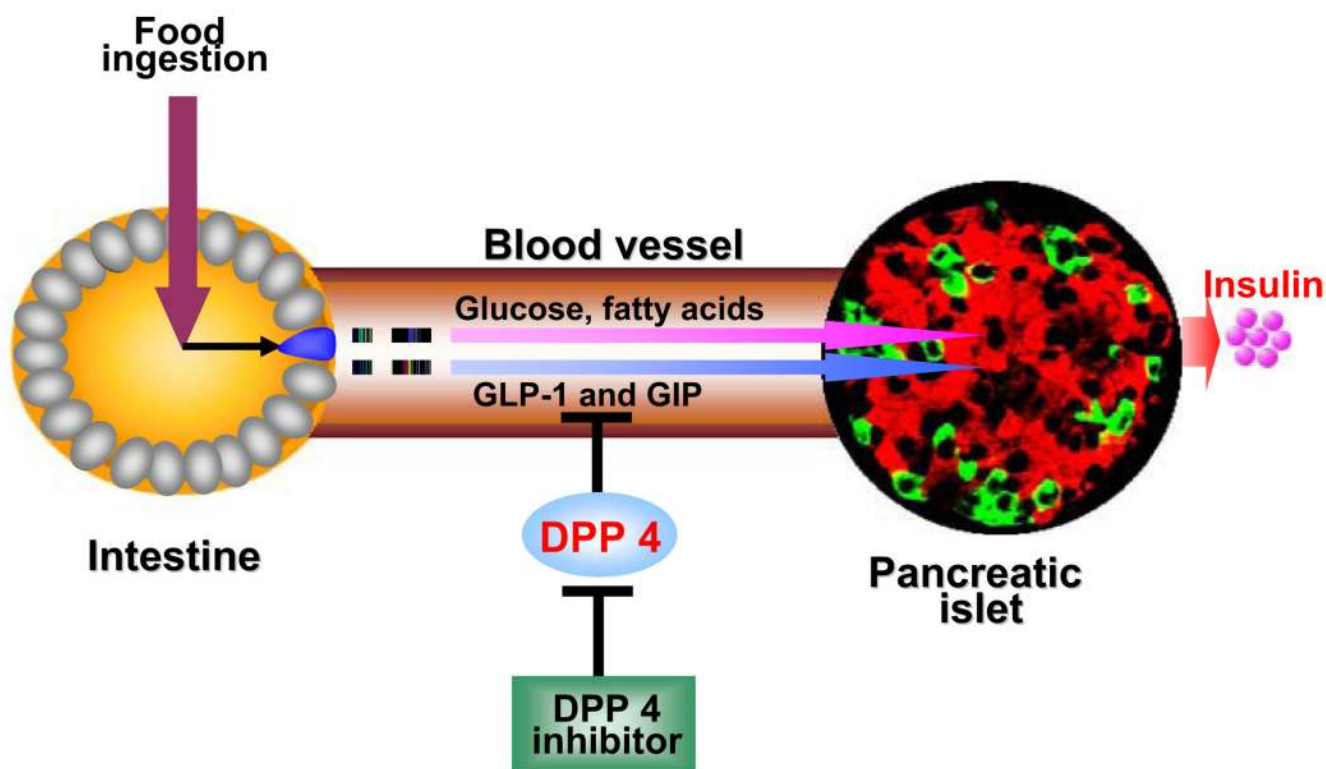


FIG. 1. Schematic representation of incretin secretion and action. GIP and GLP-1 are secreted after food ingestion, and they then stimulate glucose-dependent insulin secretion. Once released, GIP and GLP-1 are subject to degradation by DPP4 on lymphocytes and on endothelial cells of blood vessels. The red cells in the islets are insulin-containing (β) cells and the green cells are glucagon-containing (α) cells.

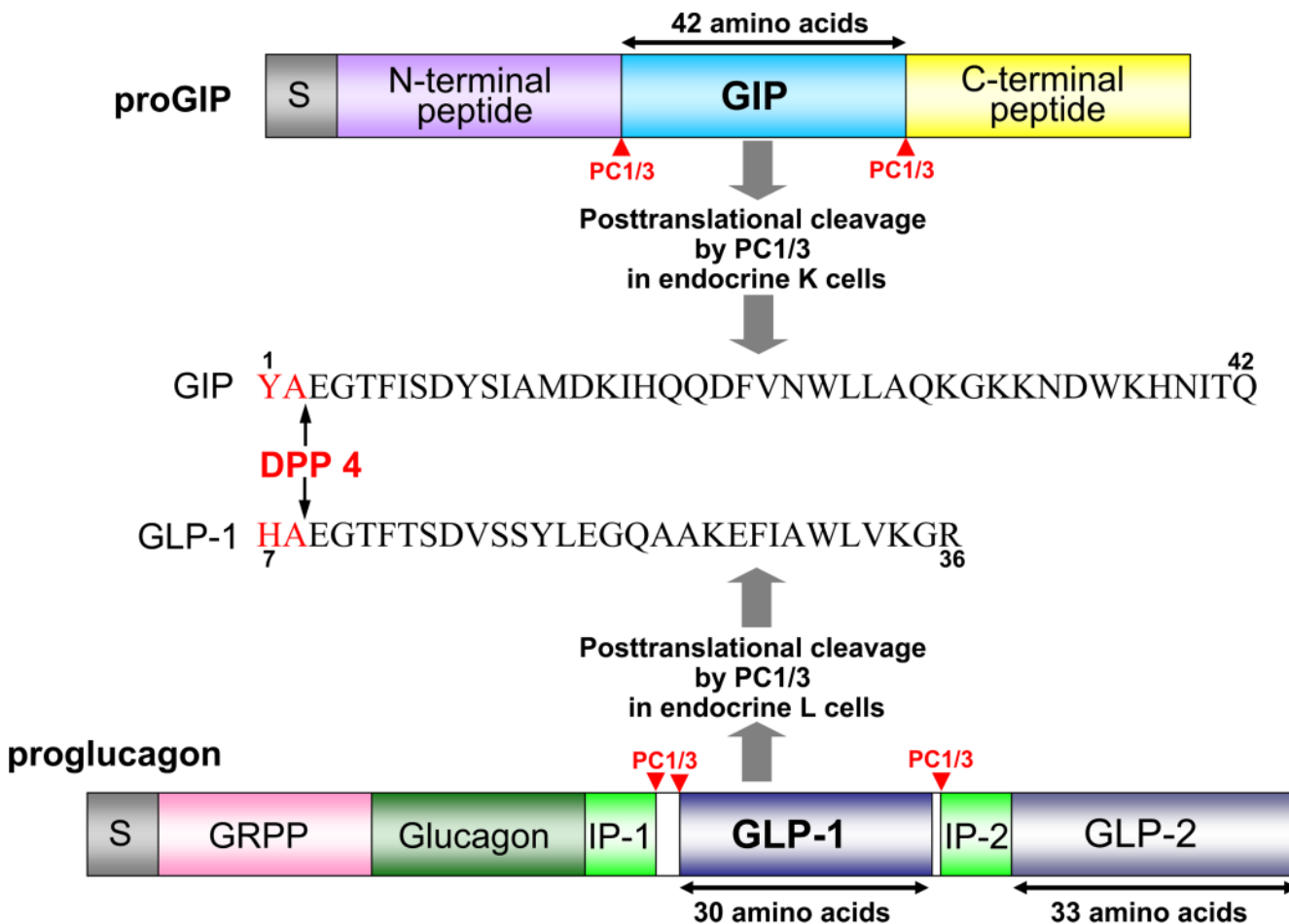
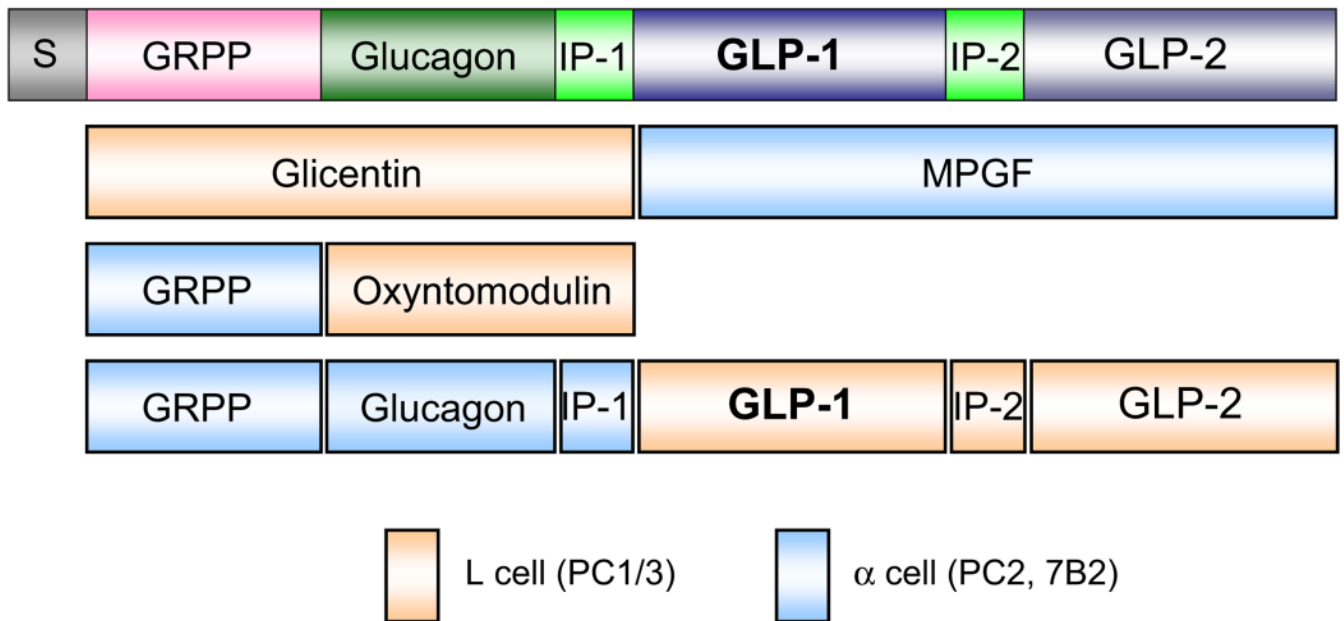


FIG. 2. Schematic representation of proGIP and proglucagon. GIP is a single 42-amino acid peptide derived from the post-translational processing of proGIP by PC1/3 in enteroendocrine K cells. It is the only functional proGIP product in all species examined to date, and there is a greater than 90% amino acid identity between human, rat, murine, porcine, and bovine sequences. GLP-1 is a post-translational cleavage product of the proglucagon gene by PC1/3 in enteroendocrine L cells and GLP-1 (7–36) amide is a major form of circulating biologically active GLP-1 in humans. In mammals, proglucagon is expressed in pancreas, enteroendocrine L cells, brain, and taste cells with an identical mRNA transcript in each tissue. Fish and bird proglucagon mRNA in pancreas and liver, however, have different 3'-ends because of differential splicing upon pancreatic expression. The *Xenopus laevis* proglucagon gene encodes three unique GLP-1-like peptides, each with insulinotropic properties that are capable of activating the human GLP-1R, and one of which seems more potent than human GLP-1 (Irwin et al., 1997).

**FIG. 3.**

Schematic representation of tissue-specific post-translational processing of proglucagon in the intestinal L cell and the pancreatic α cell. PC1/3 is responsible for the processing of proglucagon in the L cell to release GLP-1 (and GLP-2), and PC2, in conjunction with 7B2, is responsible for the pancreatic α cell-specific processing of proglucagon. Processing of proglucagon also occurs in brain. In taste cells of the tongue, both PC1/3 and PC2 (as well as 7B2) are present, and consequently GLP-1, GLP-2, and glucagon are all present.

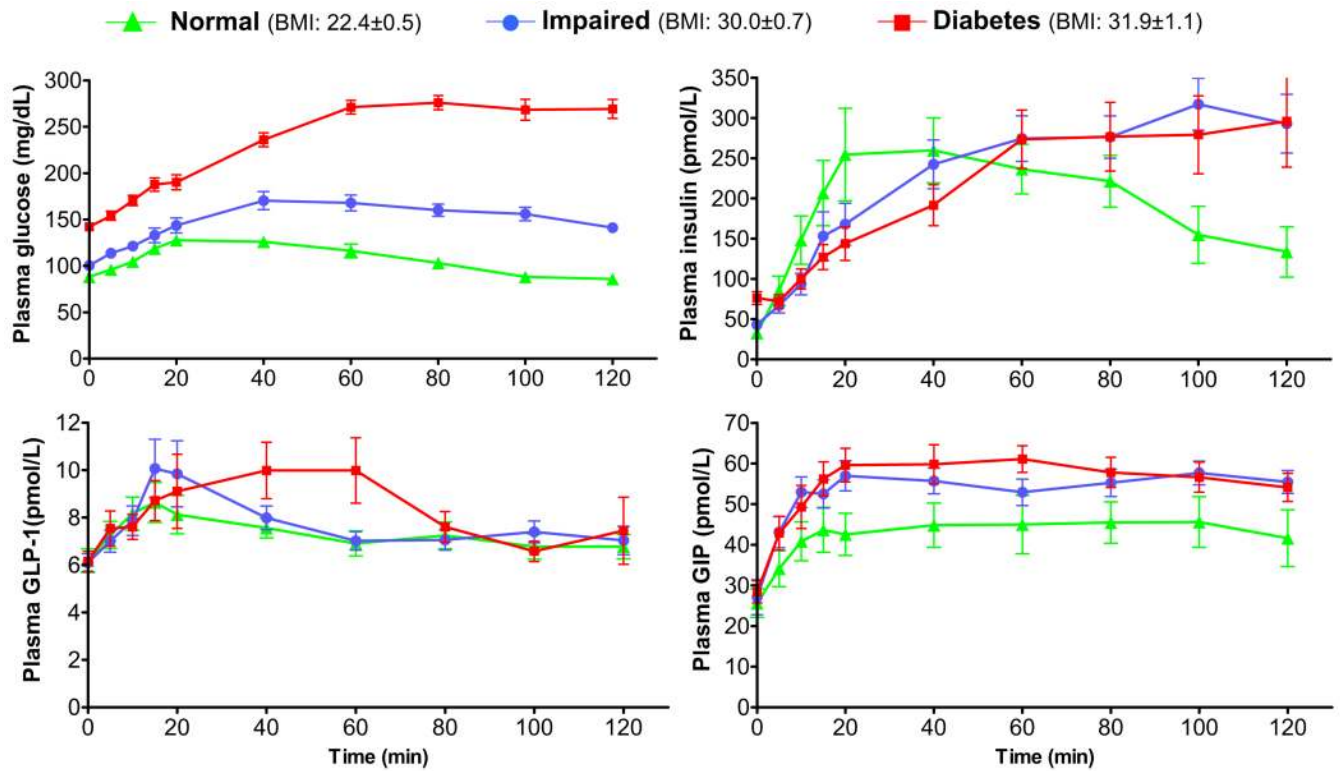


FIG. 4.

Hormone responses to oral glucose (75 g) in nondiabetic (green), glucose intolerant (blue), and newly diagnosed type 2 diabetic (red, T2DM) subjects within Baltimore Longitudinal Study of Aging: the impaired and diabetes subjects were matched for body mass index. These subjects were not taking any glucose-lowering medications. It is evident that glucose-mediated incretin secretion (GIP and GLP-1) is not deficient in newly diagnosed T2DM.

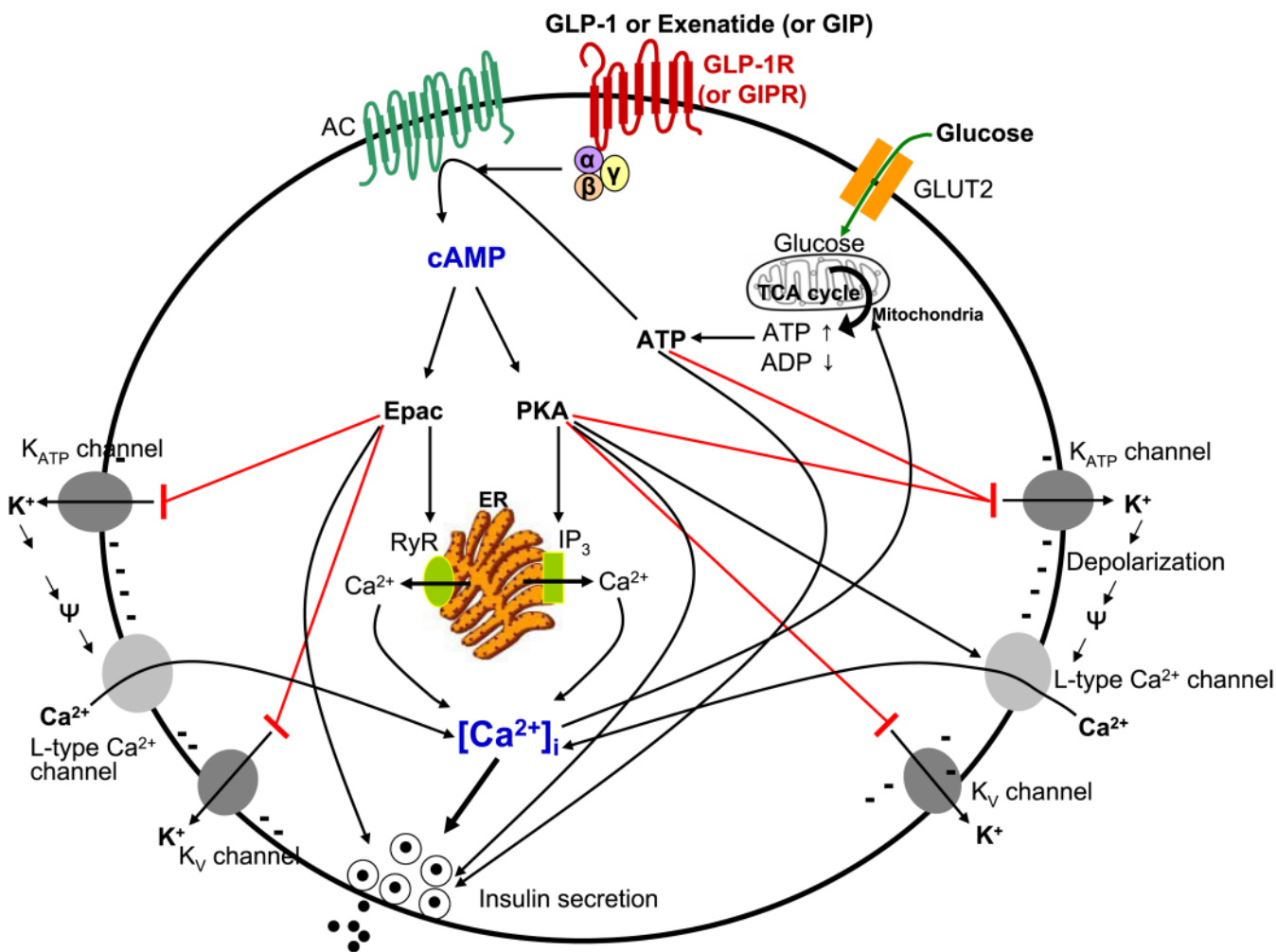


FIG. 5. A schematic representation of the main molecular events during incretin-induced insulin secretion from a β -cell. The binding of incretin ligands or agonists to the incretin receptors results in production of cAMP via adenylyl cyclase (AC) activation and subsequent activation of PKA and the Epac family of cAMP-regulated guanine nucleotide exchange factors (cAMP-GEFs), which leads to elevation of intracellular Ca^{2+} levels via a depolarization of plasma membrane by inhibition of K_{ATP} and K_{V} channels after ATP generation from glucose and consequent opening of voltage-gated L-type Ca^{2+} channels. Intracellular Ca^{2+} levels are further increased via stimulation of IP_3R and RyR on the ER. Long-term GLP-1 treatment also stimulates the expression of GLUT2 transporter and glucokinase (the β -cell glucose sensor), which lead to increased mitochondrial ATP synthesis. In addition, L-type Ca^{2+} channels are phosphorylated by PKA, resulting in an increase of their open probability and thus facilitation of enhanced Ca^{2+} influx. The changes in intracellular Ca^{2+} concentrations lead to fusion of insulin-containing vesicles to the plasma membrane and subsequent rapid exocytosis of insulin from β cells. In addition, the exocytosis of insulin-containing vesicles is directly regulated by PKA and Epac2 via interaction with the regulators of exocytosis and ATP.

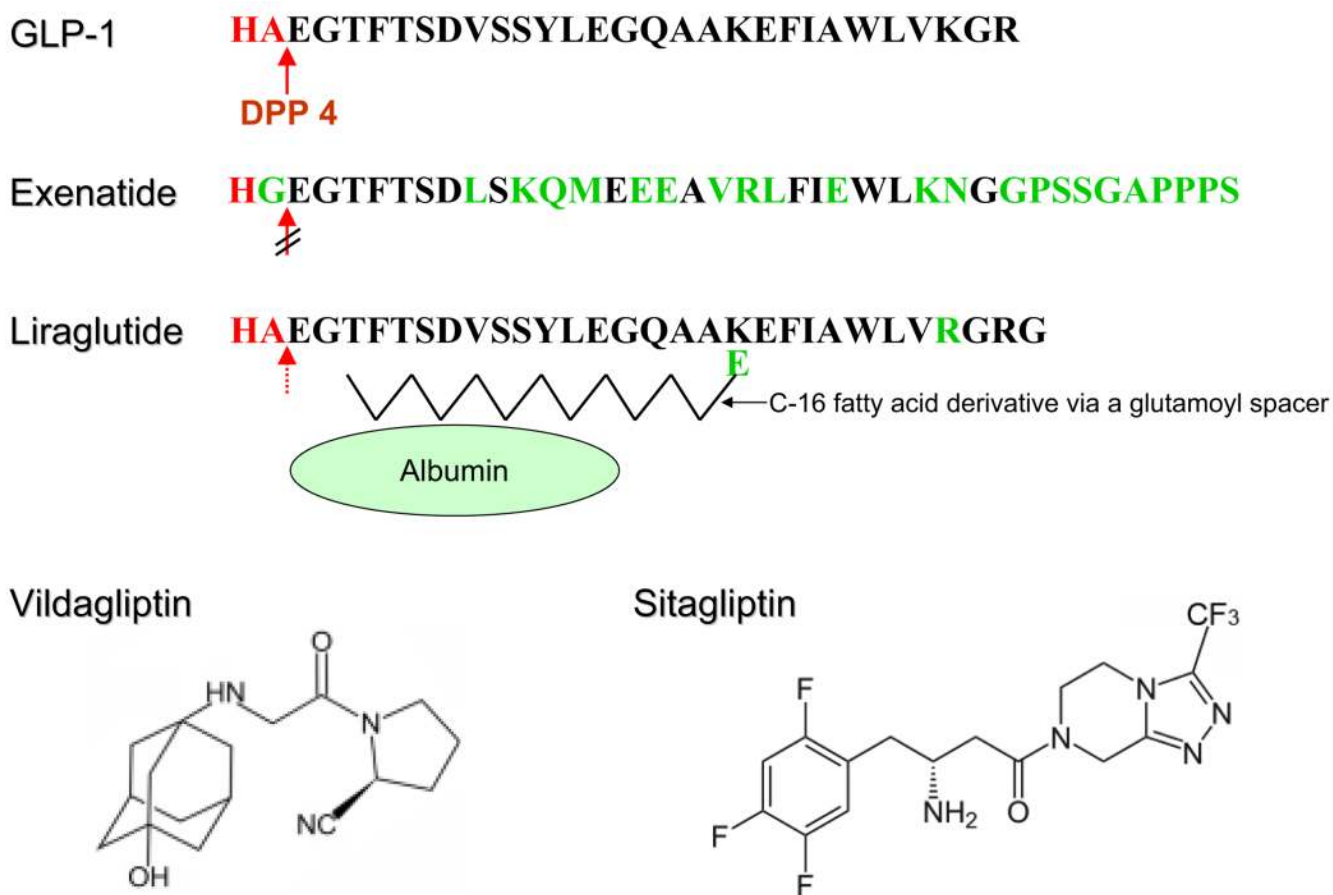


FIG. 7. Structure of native GLP-1, exenatide, liraglutide, sitagliptin, and vildagliptin. Green letters indicate changes introduced in derivatives or occur naturally in exendin-4 (and replicated in the synthetic version, exenatide). The N-terminal dipeptide “HA” of GLP-1 and liraglutide is the site of proteolytic cleavage of DPP4. A broken red arrow indicates absent DPP4 activity, and a dotted red arrow indicates reduced DPP4 activity.

TABLE 1

Time-line to use of incretin-based therapies in the treatment of diabetes

Year	The Development of Incretin-Based Therapies
1869	Islets of Langerhans (nests of cells that appeared different from the surrounding pancreatic tissue) in the pancreas were described.
1901	The role of islets of Langerhans (what ultimately became known as an endocrine function) in diabetes was described.
1902	The role of a substance (called secretin) secreted by gut cells that stimulates the digestive juices for the pancreas (what ultimately became known as exocrine function) was described.
1905	This type of substance, a presumed "chemical messenger," was now called a "hormone."
1906	The role of a gut-derived hormone to treat diabetes was first alluded to.
1921–1922	Extraction of insulin from pancreas and its potential to treat type 1 diabetes was shown.
1932	The term "incretin" was used for the first time to refer to a substance derived from the gut, presumably a hormone, that regulates insulin secretion after eating.
1960	Radioimmunoassay was developed for measurement of plasma insulin levels.
1964–1967	Clinical proof that a gut-derived factor positively modulated insulin secretion.
1971	The first incretin, GIP, was isolated and sequenced.
1985	The second incretin, GLP-1, was described.
1992–1994	Studies show that exogenous GIP does not lower blood glucose in T2DM, but exogenous GLP-1 does so.
2002	Exendin-4, a GLP-1 receptor agonist extracted from Gila monster lizard saliva, was shown to powerfully stimulate insulin secretion in a glucose-dependent manner in subjects with and without T2DM.
2005	Exenatide (synthetic exendin-4) came into clinical use for T2DM.
2006	Sitagliptin, an orally active dipeptidyl peptidase 4 inhibitor, came into use in T2DM.

TABLE 2
Actions of GIP and GLP-1 that affect blood glucose levels

	GIP	GLP-1
Islets		
Insulin secretion	↑	↑
Insulin synthesis	↑	↑
Insulin, glucokinase and GLUT2 expression	↑	↑
Glucagon secretion	↑	↓ (indirect)
Somatostatin secretion	↑	↑ (indirect)
β cell proliferation	↑	↑
β cell apoptosis	↓	↓
Sweet taste modulator	—	Yes
Gastrointestinal tract		
Gastric emptying	—	↓
Gastric acid secretion	↓	↓
Motility	—	↓
Central nervous system		
Food intake	—	↓
Satiety	—	↑
Muscle		
Glucose uptake	—	↑
Liver		
Glucose production	↓ (indirect)	↓ (indirect)

↑, increase; ↓, decrease; —, no effect or not reported.