Chapter 4
The Role of Incretins in Insulin Secretion

Brock E. Schroeder and Orville Kolterman

Overview of Glucose Regulation and Insulin Secretion

The maintenance of the plasma glucose concentration is a critical bodily function. Hyperglycemia is associated with long-term micro- and macrovascular complications, while hypoglycemia can lead to serious injury to the brain, which is dependent on plasma glucose as a fuel source. At any given time the body's plasma glucose concentration is a balance between the relative rates of glucose appearance and disappearance. These rates are regulated by several key organs through the actions of multiple hormonal signals. A brief introduction follows; however, for more detailed information, see Chapter 2.

During fasting and before meals, glucose appearance is regulated largely by glucagon-induced hepatic glucose output. Binding of glucagon to receptors in the liver leads to both glycogenolysis and gluconeogenesis. Glucose disappearance is regulated by peripheral glucose uptake – primarily by the brain, muscle, and splanchnic organs. Together, these processes normally keep plasma glucose regulated between approximately 70 and 100 mg/dl during the fasting state.

During a meal and in the postprandial period, meal-derived glucose is the major determinant of glucose appearance. Glucose absorption in the gut leads to a rise in plasma glucose. This increase in plasma glucose stimulates insulin secretion from β-cells in the pancreas. Meal-induced increases in plasma insulin – 3 to 4-fold within 30–60 min of a meal – stimulate glucose uptake by peripheral tissues, keeping 2-h postprandial plasma glucose concentrations below approximately 140 mg/dl in healthy individuals.

The mechanisms underlying glucose-stimulated insulin secretion from β-cells are complex and involve the integration of signals from multiple internal and external stimuli. Under normal circumstances, glucose elevation induces a biphasic pattern of insulin release.[1,2] Within a few minutes of plasma glucose increases, first-phase insulin release occurs. This phase, which lasts for approximately 10 min, is thought to reflect a "readily releasable" pool of insulin stored within β-cell secretory vesicles. A longer-lasting second-phase of insulin release follows – reflecting release of both stored insulin as well as newly produced insulin – and lasts as long as plasma glucose remains elevated.

These processes describe a general framework of glucose-induced insulin secretion; however, our current understanding of the mechanisms underlying insulin secretion, as mentioned above, involves an integrated and complex regulatory system. A key to this understanding has been the identification of the "incretin" hormones and elucidation of the role they play in the regulation of glucose-dependent insulin release.

O. Kolterman (✉)
Amylin Pharmaceuticals, Inc., San Diego, CA 92121, USA
e-mail: laura.featherstone@amylin.com

L. Poretsky (ed.), *Principles of Diabetes Mellitus*, DOI 10.1007/978-0-387-09841-8_4,

Incretin Hormones: Introduction and History

In the 1960s, several groups first described what has become known as the "incretin effect," based upon observations that glucose administered orally elicits an augmented insulin secretory response compared to an equivalent glucose load administered intravenously (IV).[3,4] Elrick and colleagues[4] first described an experiment in subjects without diabetes in which the mean increase in plasma insulin during the first hour after glucose administration was 37% greater following oral glucose than following IV glucose. This increase occurred despite higher mean blood glucose concentrations in the IV administration group. During the second hour following glucose administration, the elevated plasma insulin concentrations were maintained in the oral glucose group (in fact, plasma insulin increased ~55% compared to the first hour), while plasma insulin returned toward fasting concentrations in the IV administration group.

Perley and Kipnis[3] confirmed these findings, demonstrating that oral glucose administration elicited an approximately 60–70% greater insulin secretory response than an equivalent IV glucose load (see Fig. 4.1a, b).

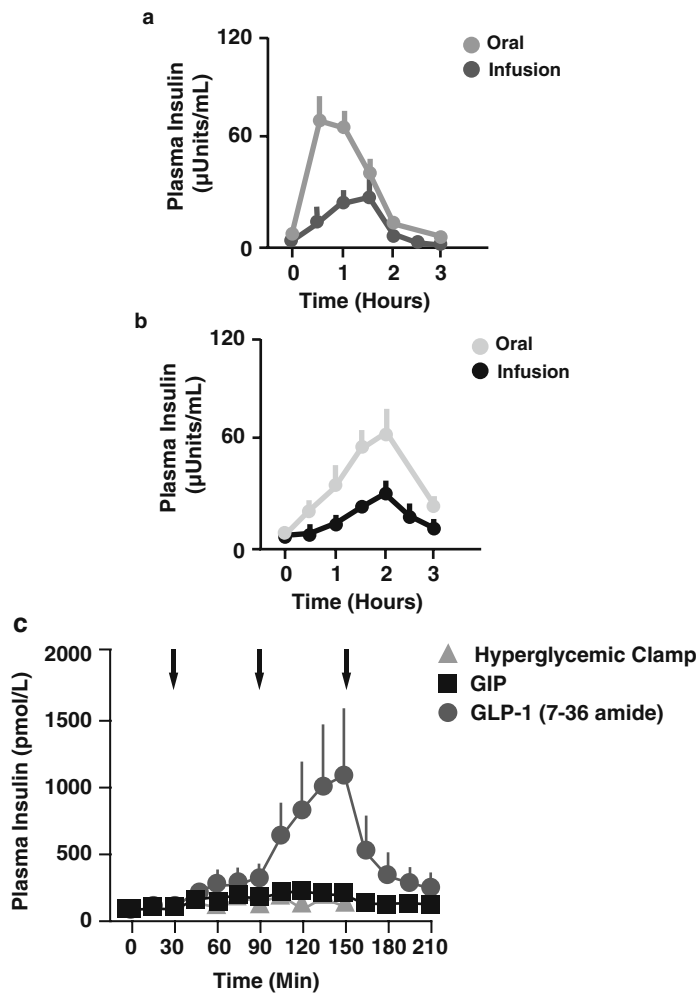

Fig. 4.1 (**a**, **b**) Plasma insulin responses to oral or infused glucose in healthy individuals (**a**) and patients with type 2 diabetes (**b**). Data from Perley and Kipnis[3]. (**c**) Insulinotropic effects of GLP-1, but not GIP, infusion in patients with type 2 diabetes under hyperglycemic clamp conditions. *Arrows* indicate start of low, then high-dose administrations of GLP-1 or GIP, followed by end of administration. Data from Nauck et al.[41]. All data points, Mean ± SE

In addition, they noted that the timing of insulin secretion was also different between the two groups: maximal plasma insulin concentrations were reached earlier (~30–60 min) following oral glucose administration than following IV administration (~90–120 min). Perley and Kipnis also demonstrated for the first time that patients with type 2 diabetes (T2DM) exhibit the incretin effect; however, they noted that patients with diabetes exhibited a decreased insulin response to oral glucose, a concept which will be explored in much greater detail below.

The findings described above indicate that insulin secretion following meals is not accounted for solely by changes in blood glucose concentration. In fact, it has been estimated that approximately 60% of insulin secreted in response to a meal is due to the incretin effect.[5] The discovery of the incretin effect led to a search for mechanisms triggered by oral glucose administration which might play a role in mediating insulin secretion. While a number of factors were initially proposed,[3] currently the incretin effect is attributed largely to two hormones secreted by specialized endocrine cells in the gut: glucose-dependent insulinotropic peptide (GIP, also termed gastric inhibitory polypeptide) and glucagon-like peptide-1 (GLP-1).

Glucose-Dependent Insulinotropic Peptide (GIP)

GIP is a peptide hormone, 42-amino acids in length, processed from a 153-amino acid precursor. It is secreted by the endocrine K-cells of the gut,[6] which are located in highest density in the duodenum and upper intestinal tract. Secretion of GIP increases by approximately 10-fold in response to meal ingestion.[7,8] The insulinotropic effects of GIP are stimulated via activation of specific G protein-coupled receptors on pancreatic β-cells.[9] Following secretion, GIP is rapidly metabolized by the ubiquitous enzyme dipeptidyl peptidase-4 (DPP-4),[10,11] and has a half-life of approximately 7 min.[11]

GIP Function Overview

The insulinotropic properties of GIP were identified first in 1973,[12] and since have been characterized in islet cells, isolated pancreas, and in vivo in healthy humans.[13–17] The insulinotropic effect of GIP is glucose-dependent, and is absent at glucose concentrations under 140 mg/dl.[18] Physiologically, it has been estimated that GIP-dependent insulin secretion accounts for approximately 20–50% of the incretin effect.[19,20] Multiple groups have shown that inhibiting GIP function causes reduced insulin secretion and impaired glucose regulation in animals models.[20,21] Furthermore, mice with genetic deletions of the GIP receptor develop glucose intolerance.[22]

In addition to its incretin effects, a number of other effects of GIP have been identified. These include the following:

(1) GIP has both proliferative and anti-apoptotic effects on β-cells.[23–26] The physiological importance of these findings and potential effects in humans are not known at present.
(2) Evidence for a role of GIP signaling in obesity has come from a variety of studies. GIP receptors are expressed on adipocytes[27] and GIP has been implicated in lipid metabolism in a variety of studies.[28–30] Mice with genetic disruption of the GIP receptor are resistant to diet-induced obesity and have reduced adiposity following high-fat feeding.[22,31] Furthermore, when GIP receptors were disrupted in ob/ob mice (a mouse model of obesity), these mice experienced less weight gain, decreased fat, and increased energy expenditure.[31] While in theory antagonism of GIP signaling may have beneficial effects on obesity, the benefits are likely outweighed by the negative effects on glucose tolerance.
(3) GIP receptors are also expressed on bone and stimulation of this pathway elicits new bone formation.[32] Conversely, young mice lacking GIP receptors have reduced bone size and mass.[33] The potential for clinical application of GIP effects on bone is unknown at present.

Glucagon-Like Peptide-1 (GLP-1)

While early studies conclusively demonstrated that GIP elicited insulin secretion, Ebert and colleagues[34] showed that removal of GIP from the gut did not eliminate the incretin effect. This finding provided strong evidence for the existence of additional gut-derived factors with insulinotropic properties. The second incretin hormone identified is GLP-1, a product of the proglucagon gene (the same precursor gene which codes for glucagon when expressed in the pancreas). GLP-1 is rapidly secreted from the L-cells of the lower gut following meal ingestion.[35] A truncated version of GLP-1 (amino acids 7–36; GLP-$1_{7–36}$) has been shown to be the predominant form of bioactive GLP-1 in circulation following meals.[36–38] Like GIP, GLP-1 is rapidly metabolized by the enzyme DPP-4 following release (resulting in the inactive fragment GLP-$1_{9–36}$),[10,11,39,40] and has a half-life of only 2 min in circulation.[11]

Following a meal, the concentration of GLP-1 rises by about 3-fold.[38] This increase is notably less than that of GIP following a meal; however, GLP-1 has been shown to be the more potent insulinotropic compound.[41] In fact, GLP-1 is one of the most potent insulin-releasing substances known.[42] GLP-1 exerts its activity via interaction with specific GLP-1 receptors on β-cells (despite its name, GLP-1 does not bind to the glucagon receptor). GLP-1 receptors are G protein-coupled receptors which belong to the same family as the GIP receptor[9,43] and, as described below, the intracellular signaling cascade, which follows incretin binding, elicits insulin release.

GLP-1 Function Overview

The insulinotropic effects of GLP-1 have been identified by several groups in both humans[16,44–46] and animal models.[37,47,48] Similar to GIP, GLP-1-induced insulin release is glucose-dependent,[46,49] such that increased insulin secretion only occurs in the presence of elevated glucose concentrations. This characteristic has been important in the development of incretin-based therapeutics, as the glucose dependence greatly reduces the risk of treatment-induced hypoglycemia. In animal experiments, treatment with a GLP-1 receptor-specific antagonist (exendin 9–39) increased both fasting and postprandial glucose concentrations and lowered insulin concentrations following an oral glucose load.[50–52] These studies also showed that GLP-1 signaling is responsible for a considerable proportion (as much as 60%) of the insulin response to an oral glucose load. Lending further support to the physiological role of GLP-1, mice with a genetic deletion of the GLP-1 receptor have diminished circulating insulin and increased plasma glucose following an oral glucose challenge.[53] In humans, administration of the GLP-1-receptor antagonist exendin 9–39 caused an approximately 35% increase in postprandial glucose,[54] suggesting that GLP-1 is essential for normal glucose tolerance. Lastly, GLP-1 has been shown to contribute to first-phase insulin secretion[55] – the robust insulin secretion that occurs during the first 10 min following glucose administration. Because first-phase insulin secretion is characteristically absent in patients with T2DM, the ability of GLP-1 to affect first-phase insulin release is an important therapeutic consideration.

In addition to glucose-dependent insulinotropic effects, GLP-1 is known to have several other important functions which affect glucoregulation. These include the following:

(1) GLP-1 suppresses the secretion of glucagon by pancreatic α-cells in a glucose-dependent manner,[41,56,57] which leads to a reduction in hepatic glucose production. This effect reinforces the insulin-induced suppression of glucagon release that occurs during the fed state, helping to regulate postprandial glucose control.
(2) GLP-1 delays gastric emptying.[58–60] Slowing nutrient entry into the gut moderates plasma glucose increases in the post-meal period. The delay in gastric emptying is thought to be mediated via GLP-1 receptors in the brain, which lead to stimulation of the parasympathetic vagus nerve.[61] In addition, GLP-1 reduces the production of gastric acid, helping to regulate digestion of stomach contents.[59,62]
(3) A number of lines of evidence suggest that GLP-1 plays a role in the central nervous system control of food intake. First, GLP-1 receptors are present in a number of brain regions implicated in the control of food intake including the hypothalamus and area postrema.[63,64] These regions lack a blood–brain barrier, permitting

GLP-1 to access the brain directly from the circulation. In rodents, direct intracerebroventricular injection of GLP-1 produced a dose-dependent reduction in food intake,[53,63–66] while repeated intracerebroventricular administration resulted in long-term reductions in food intake and body weight.[67] Conversely, administration of a GLP-1 antagonist increased food intake and resulted in weight gain.[68] GLP-1 has also been shown to reduce appetite and caloric intake in studies of healthy humans[69] and in patients with T2DM.[70] Moreover, chronic GLP-1 administration is associated with weight loss,[71] an effect which was attributed to reduced appetite in this study.

(4) Lastly, GLP-1 has been shown to have trophic effects on β-cells.[72] In animal studies, GLP-1 administration resulted in islet neogenesis, β-cell proliferation, and an increase in β-cell mass.[73–77] GLP-1 has also been shown to enhance the proliferation of new β-cells from pancreatic progenitor cells.[78–80] Finally, GLP-1 has been reported to inhibit apoptosis of β-cells.[81,82] These results suggest that GLP-1 may be beneficial in patients with T2DM by protecting existing β-cells and/or influencing proliferation of new β-cells; however, effects in humans have not been established.

Incretin-Induced Insulin Secretion: Mechanism of Action

Before considering the mechanisms of action underlying incretin-induced insulin secretion, it is important to understand the basic cellular physiology underlying glucose-induced insulin secretion in β-cells. The details of the regulation of insulin secretion by glucose are reviewed in Chapters 2 and 3 as well by other authors (for example, see review by Henquin[83]). Briefly, glucose enters β-cells via facilitated transport (Glut2 transporters[84]), where it is metabolized, and adenosine triphosphate (ATP) is generated. The ensuing increase in the intracellular ATP/adenosine diphosphate (ADP) ratio causes inhibition of ATP-sensitive potassium channels (K_{ATP}). Potassium efflux through K_{ATP} channels normally keeps the β-cell membrane polarized (negative resting voltage); thus, when K_{ATP} channels are inhibited, the cell membrane is depolarized (moves toward a neutral or positive resting voltage) in the immediate vicinity of the K_{ATP} channels. This depolarization activates voltage-dependent calcium channels (VDCCs), allowing calcium to enter the cell. Calcium entry leads to insulin secretory vesicle exocytosis and insulin release.[85] Under normal circumstances, delayed rectifier voltage-dependent potassium channels (K_v) then open, allowing potassium to leave the cell. This efflux repolarizes the cell membrane and halts the insulin release.

The cellular and molecular mechanisms by which GLP-1 and GIP elicit insulin secretion overlap considerably and include (see Fig. 4.2) the following:

(1) K_{ATP} Channel Modulation. Both GLP-1 and GIP bind to G protein-coupled receptors and activate adenylate cyclase, which catalyzes the conversion of ATP to the cellular second messenger 3′–5′-cyclic adenosine monophosphate (cAMP). These initial steps begin a series of cellular actions by which GLP-1 and GIP are thought to exert insulinotropic effects. The first downstream mechanism involves modulation of K_{ATP} channels. A number of groups have shown that both GLP-1 and GIP cause closure of K_{ATP} channels.[86–90] As described above, inhibition of K_{ATP} channels facilitates membrane depolarization which induces downstream insulin release. The mechanism underlying the effect on K_{ATP} channels is thought to involve cAMP-dependent protein kinase (PKA); inhibition of PKA reverses the effects of both incretin hormones on K_{ATP} channels[86,91] (but see also Suga and colleagues[88]). Furthermore, in mouse models with a genetic mutation causing an absence of K_{ATP} channels, GLP-1- and GIP-induced insulin secretion is diminished.[92,93] These results provide further evidence that K_{ATP} channel modulation represents an important component of incretin-induced insulin secretion.

Interestingly, GLP-1 action at the K_{ATP} channel may play an important role in the glucose-dependence of GLP-1-dependent insulin secretion. In the absence of elevated glucose concentrations, GLP-1 cannot inhibit K_{ATP} channels enough to affect exocytosis. However, when GLP-1 is administered with a sulfonylurea, which directly inhibits K_{ATP} channels in a glucose-independent manner, GLP-1 dependent insulin secretion is augmented.[94,95] This effect – uncoupling the glucose dependence of GLP-1 – has consequences in GLP-1-based therapy which are detailed later in this chapter.

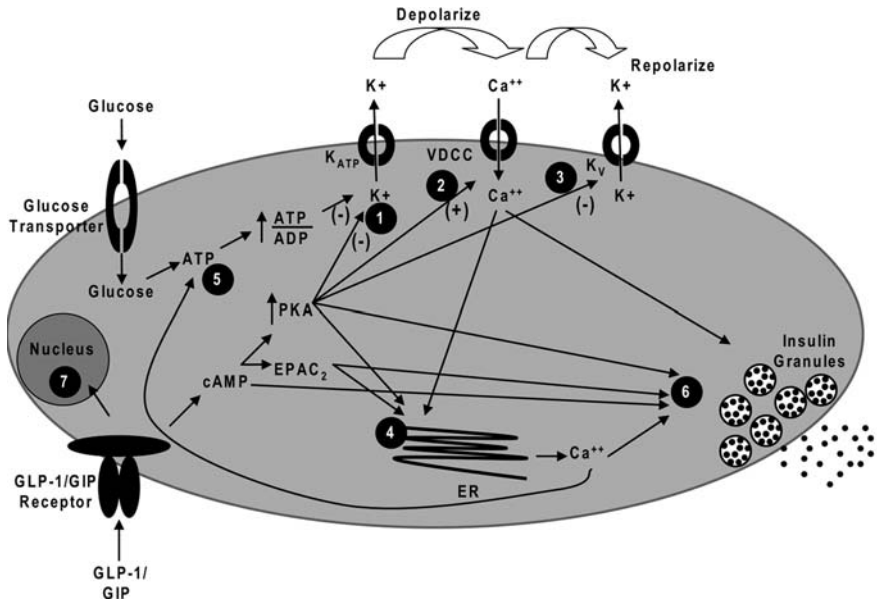

Fig. 4.2 Schematic of intracellular mechanisms of action underlying incretin-induced insulin secretion. GLP-1 receptor activation leads cAMP generation and PKA activation, leading to (1) inhibition of K_{ATP} channels, which depolarizes β-cells leading to increased excitability and downstream insulin release; (2) increased VDCC activity, leading to an increase in intracellular calcium; (3) inhibition of K_v channels, delaying repolarization and extending β-cell excitability. (4) Additional calcium is released from intracellular stores via PKA, $EPAC_2$, and calcium entry through VDCCs. (5) Intracellular calcium increases stimulate mitochondrial ATP production, increasing the ATP/ADP ratio, and leading to additional effects on K_{ATP} channels. (6) Multiple intracellular steps involving PKA, $EPAC_2$, calcium, and cAMP lead to the priming and mobilization of insulin granules for release. (7) Receptor activation leads to new insulin synthesis as well as increases in the transcription of genes involved in insulin synthesis

(2) Calcium Efflux Through VDCCs. Gromada and colleagues[89,90] have demonstrated that GLP-1 and GIP administration also increase VDCC activity, leading to increased calcium entry into β-cells and insulin exocytosis. As with K_{ATP} channel effects, PKA activation appears to underlie the effects on VDCC current changes.[89,90,96]

(3) K_v Channel Modulation. As described above, K_v channels are integral in restoring cell membrane potential following depolarization and thereby limiting calcium entry and further exocytosis of insulin-containing granules. GLP-1 receptor activation has been shown to inhibit K_v channel currents by approximately 40% in rat pancreatic β-cells.[97] GIP has been reported to have similar effects on K_v channel currents.[7] Thus, inhibiting K_v channel currents may lead to prolonged exocytosis. The effects on K_v channels appear to be dependent on PKA signaling as well as the phosphatidylinositol-3 kinase pathway.[98] In addition to effects on K_v channel currents, GIP has also been reported to affect cell surface expression and modulation of K_v channels.[99]

(4) Intracellular Calcium Stores. In addition to the direct and indirect effects that GLP-1 and GIP have on calcium entry into the cell through VDCCs, additional calcium is released from intracellular stores such as the endoplasmic reticulum (ER). This process is thought to be dependent on converging intracellular signals. For example, GLP-1-stimulated PKA[100] and cAMP-regulated guanine nucleotide exchange factor-II (Epac2, also termed cAMP-GEFII)[101] sensitize calcium channels in the ER. Intracellular calcium release is then initiated by the transient increase in calcium entering the cell through VDCCs,[101–105] the net result being a further increase in intracellular calcium as well as a wider spatial distribution of intracellular calcium. GIP has been reported to have similar effects.[7] The entire process, termed "calcium-induced calcium release," is thought to contribute to exocytosis of insulin granules located in subcellular regions not located in the immediate vicinity of the VDCCs.[7,106,107] Thus, calcium-induced calcium release may play a prominent role in the postprandial state, allowing for an even greater incretin-induced insulin secretory response.

(5) Mitochondrial ATP synthesis. In addition to stimulating exocytosis, the increase in calcium-induced intracellular calcium release described above has also been shown to affect mitochondrial ATP production.[108] Amplified ATP production may lead to further effects on K_{ATP} channels in a feed-forward manner.[107]
(6) cAMP-associated Insulin Granule Mobilization. The insulinotropic activity of GLP-1 results in part from calcium influx through VDCCs (described above). However, only a small fraction of insulin-containing granules (less than 1%[109]) belong to what is termed the "readily releasable pool,"[90,110] meaning that they are located close enough to VDCCs that they undergo exocytosis soon after VDCC opening. The remaining insulin-containing granules must be "primed" by series of cellular steps involving cAMP, calcium, and both PKA and Epac2.[90,111,112] These steps involve granule mobilization (via PKA) and increases in the size of granules (via Epac2), both processes which are influenced by GLP-1 and GIP signaling. The increased availability of insulin-containing granules for exocytosis has been estimated to account for as much as 70% of the insulinotropic activity of GLP-1 and GIP.[89,113]
(7) Insulin Biosynthesis. In addition to effecting acute changes in insulin release, both GLP-1 and GIP simulate insulin synthesis and gene transcription in β-cells.[114–116] This process ensures that adequate insulin remains available for secretion. Moreover, GLP-1 has been shown to upregulate the transcription of genes involved in insulin secretion.[117]

Incretins and Type 2 Diabetes Mellitus

It is generally accepted that two key pathophysiological defects contribute to the metabolic irregularities observed in T2DM: first, progressive β-cell dysfunction with associated insulin secretory deficits; and second, peripheral insulin resistance. Both defects play a fundamental role in the chronic progression of hyperglycemia and both are targets of therapeutic intervention. While β-cell loss – in excess of 50% on average at the time of T2DM diagnosis[118] – certainly influences insulin secretion deficits, the discovery and continued research into incretin hormones and the incretin effect has shed light on new pathways that may play a role in the progression of T2DM as well as new therapeutic options. Patients with T2DM have been shown to have a significantly reduced incretin effect.[119] Theoretically, this deficit could be caused by impaired secretion of GIP or GLP-1, accelerated metabolism of the hormones, or defective responsiveness to either.

GIP in Type 2 Diabetes

In contrast to its effects in healthy humans, the role of GIP in patients with T2DM is unclear. Decreased GIP secretion in T2DM has been reported by one group;[120] however, the majority of published studies have reported normal or even increased GIP secretion in T2DM.[121,122] Importantly, a number of groups have reported that the insulinotropic effects of GIP are lost or nearly lost in T2DM[41,123–126] (see Fig. 4.1c), even when GIP is administered at supraphysiological concentrations.[41] These results indicate that patients with T2DM have a defective responsiveness to GIP. Genetic factors may underlie this effect, as first-degree relatives of patients with T2DM have diminished GIP-induced insulin secretion compared to normal patients.[127] While a conclusive explanation regarding the loss of the insulinotropic activity of GIP in T2DM has not been determined, some evidence indicates that GIP receptor downregulation and desensitization may be responsible.[128,129]

GLP-1 in Type 2 Diabetes

Unlike GIP, GLP-1 secretion has been demonstrated to be deficient in patients with T2DM.[121,130] Whether this defect is a primary causative factor in the pathogenesis of diabetes or a secondary effect has not been conclusively determined; however, studies of identical twins in which only one twin has T2DM have demonstrated that GLP-1

secretion is impaired only in the sibling with diabetes.[131] This result suggests that GLP-1 secretion deficits are secondary to the development of T2DM.

While GLP-1 secretion is abnormal in patients with T2DM, cellular responsiveness to GLP-1 is not diminished[41] (see Fig. 4.1c). Thus, unlike with GIP, therapeutic replacement of GLP-1 holds promise for pharmacologic development. A number of proof-of-concept studies demonstrated the therapeutic potential of GLP-1 in patients with T2DM. First, GLP-1 infusion consistently has been shown to induce insulin release.[41,46] Second, GLP-1 maintained its effects on gastric emptying and glucagon release in patients with T2DM.[41,59,132] The glucoregulatory outcomes of GLP-1 infusion have also been investigated. Acute infusion studies (leading to pharmacological plasma concentrations of GLP-1) have demonstrated beneficial effects on both fasting and postprandial blood glucose concentrations.[45,46,132] Longer-term experiments have shown normalized blood glucose, improved hemoglobin A1C (A1C), and body weight loss.[71,133–136]

While early GLP-1 infusion studies conclusively demonstrated the potential for GLP-1-based therapy for T2DM, the pharmacotherapeutic value of GLP-1 is significantly limited by its rapid degradation by the enzyme DPP-4. As described earlier, the half-life of GLP-1 in circulation is approximately 2 min. As a result, the benefits of GLP-1 therapy would only be possible with continuous infusion.

Leveraging the Glucoregulatory Effects of GLP-1

In response to this important clinical challenge, the GLP-1 signaling pathway has been leveraged by two distinct pharmacologic approaches. The first approach involves utilizing peptides that have glucoregulatory effects similar to GLP-1 itself, but are resistant to degradation by DPP-4. These peptides have been termed "incretin mimetics." The second approach involves utilizing a variety of small molecules to inhibit the enzymatic activity of DPP-4, thereby increasing endogenous concentrations of GLP-1. These small molecules have been termed "DPP-4 inhibitors."

Incretin Mimetics

Exenatide

At present, exenatide is the only incretin mimetic which has been approved by the US Food and Drug Administration [FDA] and European Medicines Agency [EMEA]. Liraglutide has been approved by the EMEA, but was under review by the FDA at the time of publication of this book. The vast majority of published clinical data on incretin mimetics have focused on exenatide; consequently, the bulk of the description of incretin mimetics presented here will focus on exenatide.

Exenatide is a synthetic version of exendin-4 (not to be confused with exendin 9-39, a GLP-1 antagonist), a peptide first identified and isolated from the salivary secretions of the Gila Monster (*Heloderma suspectum*).[137] Exenatide shares approximately 50% sequence identity with human GLP-1 and binds to the mammalian GLP-1 receptor;[137–139] however, the unique amino acid sequence renders exenatide resistant to degradation from DPP-4, resulting in detectable concentrations persisting for more than 10 h in the circulation after a single subcutaneous dose.[140]

Exenatide shares many of the same glucoregulatory actions as GLP-1. In both human and animal studies, exenatide enhanced glucose-dependent insulin secretion, suppressed the inappropriate glucagon secretion seen in T2DM in a glucose-dependent manner, and slowed gastric emptying.[141–145] These effects contribute to a lowering of both fasting and postprandial glucose.[140,143] Importantly, though inappropriate glucagon secretion during hyperglycemia is suppressed by exenatide, hypoglycemia-induced glucagon secretion is unimpaired.[146] Like GLP-1, intravenous infusion of exenatide also has been shown to acutely improve β-cell function, as measured by the restoration of first- and second-phase insulin secretion in patients with T2DM following intravenous glucose administration.[147] In this study, exenatide rapidly restored normal glucose-stimulated insulin secretion in patients with T2DM. Both in vivo animal models and human clinical trials have demonstrated that exenatide

reduces food intake and body weight, reproducing the effects of GLP-1 infusion in clinical studies.[145,148–150] Lastly, exenatide has been shown to promote β-cell proliferation and neogenesis in animal models.[75,80,151,152]

The safety and efficacy of exenatide have been investigated in long-term pivotal clinical trials. Patients with T2DM who were inadequately controlled with metformin and/or a sulfonylurea were treated with placebo, 5 or 10 μg exenatide twice daily (BID).[153–156] After 30 weeks of exposure to exenatide, significant changes from baseline in mean A1C and body weight were reported. Exenatide 10 μg was associated with A1C changes from baseline of approximately –1%, with average body weight changes from baseline of –2 to –3 kg.[153–156] In open-label extensions of these placebo-controlled trials, patients received 10 μg exenatide BID for up to 3 years. In the 3-year completer population, mean A1C change from baseline of –1.0% was reported,[157] demonstrating sustained glycemic control. Body weight loss was progressive, with an average change of –5.3 kg in the completer population after 3 years (see Fig. 4.3). In these open-label extension studies, improvements in several cardiovascular (CV) risk factors also were reported after 82 weeks of exenatide treatment. Plasma triglycerides (–39 mg/dl), diastolic blood pressure (–2.7 mmHg), and C-reactive protein (–44%) were all decreased, while plasma HDL cholesterol (+4.6 mg/dl) was increased.[158]

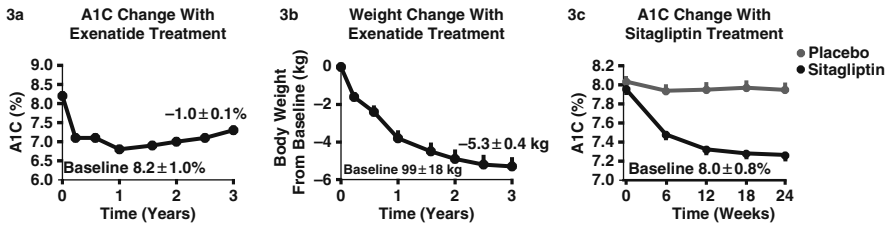

Fig. 4.3 Independent clinical trials of incretin-based therapies. (**a**, **b**) In a 3-year open-label extension of placebo-controlled clinical trials, exenatide treatment led to sustained improvements in A1C (**a**) and progressive weight loss (**b**). Data from Klonoff et al.[157]. (**c**) In an independent trial with a distinct study design, sitagliptin therapy led to improvements in A1C over 24 weeks. Data from Charbonnel et al.[178]. All data points, Mean ± SE, Baselines, Mean ± SD

Exenatide therapy is associated with gastrointestinal side effects. In the three large placebo-controlled trials, nausea, mostly transient, was reported by 41–45% of patients treated with exenatide, compared to approximately 18% in patients receiving placebo.[154–156,159] Most nausea were mild to moderate and declined over the duration of the trial, while severe nausea was uncommon (occurring in less than 5% of subjects).[154–156,159] Importantly, exenatide-associated reductions in body weight have been shown to be independent of nausea.[158]

Because the insulinotropic effects of the GLP-1 pathway are glucose-dependent, exenatide should not have an intrinsic risk for hypoglycemia. Indeed, the risk of hypoglycemia was not increased when exenatide was administered on a background of metformin in patients with T2DM.[155] Moreover, compared to insulin glargine and metformin, the risks of both overall and nocturnal hypoglycemia in patients with T2DM treated with exenatide and metformin were reduced despite similar improvements in A1C.[160] When exenatide was administered to patients also taking a sulfonylurea, however, the risk of mild-to-moderate hypoglycemia was increased.[154,156] This effect is not unexpected, given the aforementioned ability of sulfonylureas to uncouple the glucose-dependence of GLP-1 agonism. Hypoglycemia risk can be mitigated by decreasing the dose of sulfonylurea at the time of exenatide treatment initiation.[156]

A once weekly formulation of exenatide is currently in late-phase development. In a 15-week placebo-controlled study in patients with T2DM, a 2.0 mg/week dose ($n = 15$) exerted a potent effect on hemoglobin A1C (–1.7%) and a robust effect on weight (–3.8 kg).[161] This study suggests that once weekly exenatide may provide 24-h glycemic control with reduction in body weight. Larger long-term Phase 3 clinical trials are currently underway.

Liraglutide

Liraglutide is an acylated analog of GLP-1 currently in Phase 3 of clinical development. By binding to serum albumin, the half-life of liraglutide is increased to approximately 13 h in circulation, allowing for once-daily

injections in patients with T2DM.[162] In trials published to date, liraglutide has been shown to induce glucose-dependent insulin secretion, reduce glucagon secretion, improve fasting and postprandial plasma glucose, and slow gastric emptying in patients with T2DM.[163–168] In multi-dose studies lasting 12–14 weeks, liraglutide has been reported to reduce A1C (–1.5% at highest dose) and lower body weight (–3 kg at highest dose) in patients with T2DM.[169] Adverse events have been reported to be primarily gastrointestinal in nature.[165,167,168,170]

DPP-4 Inhibitors

As described above, GLP-1 undergoes rapid degradation in the circulation by DPP-4, limiting the therapeutic potential of exogenously administered GLP-1. However, the half-life of endogenous GLP-1 (~2 min) can be increased by pharmacologically inhibiting the DPP-4 enzyme.[171] Several DPP-4 inhibitors have been developed for the treatment of patients with T2DM.[172,173] These small molecule agents inhibit the proteolytic cleavage of GLP-1 as well as a number of other peptides that are natural substrates for DPP-4 cleavage. These include GIP as well as a wide range of other peptides including chemokines, glucagon secretin family hormones, pancreatic polypeptide proteins, and neuropeptides. A membrane-bound form of DPP-4, also known as CD26, plays a role in cell signaling and is involved in immune function, ion transport, the regulation of extracellular matrix binding, and cell–cell signaling.[174] The functional effect of inhibiting cleavage of these other peptides in unclear at this time. Two DPP-4 inhibitors, sitagliptin and vildagliptin, have a substantial amount of published clinical data available, and are discussed here.

Sitagliptin

Sitagliptin is the first DPP-4 inhibitor to be approved by regulatory authorities for the treatment of T2DM. Sitagliptin treatment results in an approximately 80% inhibition of DPP-4 activity in the circulation, leading to a 2-fold increase in the plasma concentration of postprandial GLP-1 in healthy human subjects.[175] Following an oral glucose tolerance test in patients with T2DM, sitagliptin increased the active form of GLP-1, as well as insulin and C-peptide, while reducing plasma glucose and glucagon concentrations.[176] Sitagliptin has not been shown to affect gastric emptying or food intake. To date, there are no published data assessing phasic insulin secretion during treatment with DPP-4 inhibitors.

In 24-week clinical trials, patients with T2DM who were unable to achieve adequate glycemic control with metformin, glimepiride, pioglitazone, or diet and exercise experienced significant improvements in A1C (–0.7 to –0.8%, placebo corrected) and fasting plasma glucose with sitagliptin treatment[177–181] (see Fig. 4.3). Body weight was unchanged in these trials. Unlike therapy with incretin mimetics, such as exenatide, which leads to weight loss, administration of DPP-4 inhibitors are not associated with weight reductions. This difference may be explained by the relatively modest increases in postprandial GLP-1 concentrations induced by DPP-4 inhibitors compared with larger pharmacological increases in GLP-1-receptor agonism induced by incretin mimetics. Thus, the relative effect at the GLP-1 receptor may be higher following treatment with incretin mimetics. In a long-term comparator trial, sitagliptin demonstrated non-inferiority versus the sulfonylurea glipizide over 52 weeks in patients with T2DM unable to achieve adequate glycemic control with metformin alone. Sitagliptin treatment was associated with neutral effects on body weight and a lower incidence of hypoglycemia compared to glipizide treatment.[182] In the clinical development of sitagliptin, the most commonly reported adverse events were nasopharyngitis, upper respiratory tract infection, and headache.[178–180] As expected, when sitagliptin is coadministered with a sulfonylurea, the incidence of hypoglycemia is increased.[181]

Vildagliptin

Vildagliptin is a DPP-4 inhibitor in Phase 3 of clinical development. In a 12-week clinical trial in patients with T2DM who were not undergoing treatment with oral antidiabetic agents, patients treated with vildagliptin experienced improvements in hemoglobin A1C, fasting plasma glucose, 4-h postprandial plasma glucose, and insulin concentrations.[173] No significant changes in patient body weight were reported. In a 52-week clinical

trial, patients with T2DM who were not achieving glycemic control with metformin alone reported improvements in glycemic control with vildagliptin treatment.[183] In a similar 24-week clinical study, vildagliptin improved hemoglobin A1C and fasting plasma glucose, in association with neutral effects on body weight.[184] When examined in a monotherapy setting, 24 weeks of vildagliptin treatment was reported to improve A1C and fasting plasma glucose, with neutral effects on body weight;[185] however, vildagliptin failed to demonstrate non-inferiority compared to metformin in this study. The most frequently reported adverse events in vildagliptin clinical studies were headache, upper respiratory tract infection, nasopharyngitis, and symptomatic mild hypoglycemia.[173,185]

Conclusion

The discovery of the incretin effect in the 1960s has led to an enhanced understanding of the importance of gut hormones in normal glucose homeostasis. Notably, the finding that the incretin effect is diminished or absent in T2DM has led to the development of several novel therapeutic options for patients with T2DM. Two distinct classes of medications – incretin mimetics and DPP-4 inhibitors – leverage the incretin pathway to improve blood glucose control. Both classes of compounds have been shown to increase insulin secretion and reduce the paradoxically elevated glucagon concentrations in patients with diabetes. Additional effects demonstrated with incretin mimetics such as exenatide include restoration of first-phase insulin response to IV glucose, slowing of gastric emptying, and reduction of food intake, often resulting in weight loss (Table 4.1). Diabetes treatments based on the multiple pharmacologic effects of incretin hormones can address the multihormonal and multifaceted nature of T2DM and help overcome the clinical barriers present with many traditional therapies.

Table 4.1 Mechanisms of action and clinical results of incretin mimetics and DPP-4 inhibitors

	Incretin mimetics	DPP-4 inhibitors
Mechanism of action		
Increase meal-stimulated insulin secretion	√	√
Restore first-phase insulin response	√	–
Suppression of inappropriate postprandial glucagon secretion	√	√
Slow gastric emptying	√	–
Reduce food intake	√	–
Clinical results		
Improved glycemic control (A1C)	√	√
Improved postprandial glucose control	√	√
Body weight reduction	√	–

References

1. Rorsman P, Renstrom E. Insulin granule dynamics in pancreatic beta cells. *Diabetologia*. 2003;46:1029–1045.
2. Rorsman P, Eliasson L, Renstrom E, Gromada J, Barg S, Gopel S. The cell physiology of biphasic insulin secretion. *News Physiol Sci*. 2000;15:72–77.
3. 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.
4. Elrick H, Stimmler L, Hlad CJ, Arai Y. Plasma insulin responses to oral and intravenous glucose administration. *J Clin Endocrinol Metab*. 1964;24:1076–1082.
5. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab*. 1986;63:492–498.
6. 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.

7. 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.
8. Orskov C, Wettergren A, Holst JJ. Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day. *Scand J Gastroenterol*. 1996;31:665–670.
9. Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B, et al. International union of pharmacology. XXXV. The glucagon receptor family. *Pharmacol Rev*. 2003;55:167–194.
10. 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.
11. Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ. Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J Clin Endocrinol Metab*. 2000;85:3575–3581.
12. 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.
13. Siegel EG, Creutzfeldt W. Stimulation of insulin release in isolated rat islets by GIP in physiological concentrations and its relation to islet cyclic AMP content. *Diabetologia*. 1985;28:857–861.
14. 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.
15. Andersen DK, Elahi D, Brown JC, Tobin JD, Andres R. Oral glucose augmentation of insulin secretion. Interactions of gastric inhibitory polypeptide with ambient glucose and insulin levels. *J Clin Invest*. 1978;62:152–161.
16. Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W. Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1-(7-36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. *J Clin Endocrinol Metab*. 1993;76:912–917.
17. Nauck M, Schmidt WE, Ebert R, Strietzel J, Cantor P, Hoffmann G, et al. 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.
18. Vilsboll 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*. 2003;114:115–121.
19. Ebert R, Creutzfeldt W. Influence of gastric inhibitory polypeptide antiserum on glucose-induced insulin secretion in rats. *Endocrinology*. 1982;111:1601–1606.
20. 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.
21. Tseng CC, Kieffer TJ, Jarboe LA, Usdin TB, Wolfe MM. Postprandial stimulation of insulin release by glucose-dependent insulinotropic polypeptide (GIP) – effect of a specific glucose-dependent insulinotropic polypeptide receptor antagonist in the rat. *J Clin Invest*. 1996;98:2440–2445.
22. Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, 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 USA*. 1999;96:14843–14847.
23. Winter KD, Ehses JA, Eeson G, Kim S-J, Nian C, Warnock G, et al. Effects of glucose-dependent insulinotropic polypeptide on the phosphorylation of protein kinase B (PKB/AKT) and its contribution to pancreatic beta-cell survival. *J Invest Med*. 2007;55:S124.
24. Trumper A, Trumper K, Trusheim H, Arnold R, Goke B, Horsch D. Glucose-dependent insulinotropic polypeptide is a growth factor for beta (INS-1) cells by pleiotropic signaling. *Mol Endocrinol*. 2001;15:1559–1570.
25. Trumper A, Trumper K, Horsch D. Mechanisms of mitogenic and anti-apoptotic signaling by glucose-dependent insulinotropic polypeptide in beta(INS-1)-cells. *J Endocrinol*. 2002;174:233–246.
26. Ehses JA, Casilla VR, Doty T, Pospisilik JA, Winter KD, Demuth HU, et al. 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.
27. 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.
28. Eckel RH, Fujimoto WY, Brunzell JD. Gastric inhibitory polypeptide enhanced lipoprotein lipase activity in cultured preadipocytes. *Diabetes*. 1979;28:1141–1142.
29. Oben J, Morgan L, Fletcher J, Marks V. Effect of the entero-pancreatic hormones, gastric inhibitory polypeptide and glucagon-like polypeptide-1(7-36) amide, on fatty acid synthesis in explants of rat adipose tissue. *J Endocrinol*. 1991;130:267–272.
30. Beck B, Max JP. Gastric inhibitory polypeptide enhancement of the insulin effect on fatty acid incorporation into adipose tissue in the rat. *Regul Pept*. 1983;7:3–8.
31. Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou H, et al. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med*. 2002;8:738–742.
32. Bollag RJ, Zhong Q, Phillips P, Min L, Zhong L, Cameron R, et al. Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors. *Endocrinology*. 2000;141:1228–1235.
33. Xie D, Cheng H, Hamrick M, Zhong Q, Ding KH, Correa D, et al. Glucose-dependent insulinotropic polypeptide receptor knockout mice have altered bone turnover. *Bone*. 2005;37:759–769.

34. Ebert R, Unger H, Creutzfeldt W. Preservation of incretin activity after removal of gastric inhibitory polypeptide (GIP) from rat gut extracts by immunoadsorption. *Diabetologia*. 1983;24:449–454.
35. Holst JJ. Enteroglucagon. *Annu Rev Physiol*. 1997;59:257–271.
36. Orskov C, Holst JJ, Poulsen SS, Kirkegaard P. Pancreatic and intestinal processing of proglucagon in man. *Diabetologia*. 1987;30:874–881.
37. Holst JJ, Orskov C, Nielsen OV, Schwartz TW. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett*. 1987;211:169–174.
38. Kreymann B, Yiangou Y, Kanse S, Williams G, Ghatei MA, Bloom SR. Isolation and characterisation of GLP-1 7-36 amide from rat intestine. Elevated levels in diabetic rats. *FEBS Lett*. 1988;242:167–170.
39. Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem*. 1993;214:829–835.
40. Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. *Diabetes*. 1995;44:1126–1131.
41. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest*. 1993;91:301–307.
42. Fehmann HC, Göke R, Göke B. Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocrine Rev*. 1995;16:390–410.
43. Thorens B. Expression cloning of the pancreatic cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci USA*. 1992;89:8641–8645.
44. Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet*. 1987;2:1300–1304.
45. Gutniak M, Orskow 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.
46. 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.
47. Mojsov S, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest*. 1987;79:616–619.
48. Goke R, Wagner B, Fehmann HC, Göke B. Glucose-dependency of the insulin stimulatory effect of glucagon-like peptide-1 (7-36) amide on the rat pancreas. *Res Exp Med (Berl)*. 1993;193:97–103.
49. Qualmann C, Nauck MA, Holst JJ, Orskov C, Creutzfeldt W. Insulinotropic actions of intravenous glucagon-like peptide-1 (GLP-1) [7-36 amide] in the fasting state in healthy subjects. *Acta Diabetol*. 1995;32:13–16.
50. 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.
51. D'Alessio DA, Vogel R, Prigeon R, Laschansky E, Koerker D, Eng J, et al. 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.
52. 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.
53. Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, et al. 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.
54. Edwards CMB, Todd JF, Mahmoudi M, Wang ZL, Wang RM, Ghatei MA, et al. 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.
55. 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.
56. Orskov C, Holst JJ, Nielsen OV. Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology*. 1988;123:2009–2013.
57. Kawai K, Suzuki S, Ohashi S, Mukai H, Ohmori H, Murayama Y, et al. Comparison of the effects of glucagon-like peptide-1-(1-37) and -(7-37) and glucagon on islet hormone release from isolated perfused canine and rat pancreases. *Endocrinology*. 1989;124:1768–1773.
58. 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.
59. Willms B, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Nauck MA. Gastric emptying glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in type 2 (noninsulin-dependent) diabetic patients. *J Clin Endocrinol Metab*. 1996;81:327–332.
60. Young AA, Gedulin BR, Rink TJ. Dose-responses for the slowing of gastric emptying in a rodent model by glucagon-like peptide (7-36)NH2, amylin, cholecystokinin, and other possible regulators of nutrient uptake. *Metabolism*. 1996;45:1–3.
61. Imeryuz N, Yegen BC, Bozkurt A, Coskun T, Villanueva-Penacarrillo ML, Ulusoy NB. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am J Physiol*. 1997;273:G920–G927.

62. Schjoldager BT, Mortensen PE, Christiansen J, Orskov C, Holst JJ. GLP-1 (glucagon-like peptide 1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in humans. *Dig Dis Sci*. 1989;34:703–708.
63. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, et al. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature*. 1996;379:69–72.
64. Shughrue PJ, Lane MV, Merchenthaler I. Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. *Endocrinology*. 1996;137:5159–5162.
65. Donahey JCK, van Dijk G, Woods SC, Seeley RJ. Intraventricular GLP-1 reduces short- but not long-term food intake or body weight in lean and obese rats. *Brain Res*. 1998;779:75–83.
66. Conlon JM, Samson WK, Dobbs RE, Orci L, Unger RH. Glucagon-like polypeptides in canine brain. *Diabetes*. 1979;28: 700–702.
67. Davis HR, Mullins DE, Pines JM, Hoos LM, France CF, Compton DS, et al. Effect of chronic central administration of glucagon-like peptide-1 (7-36) amide on food consumption and body weight in normal and obese rats. *Obes Res*. 1998;6: 147–156.
68. Meeran K, O'shea D, Edwards CMB, Turton MD, Heath MM, Gunn I, 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.
69. 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.
70. Gutzwiller JP, Drewe J, Göke B, Schmidt H, Rohrer B, Lareida J, et al. Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol*. 1999;45:R1541–R1544.
71. 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.
72. 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.
73. Edvell A, Lindstrom P. Initiation of increased pancreatic islet growth in young normoglycemic mice (Umea +/?). *Endocrinology*. 1999;140:778–783.
74. 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.
75. Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes*. 1999;48:2270–2276.
76. Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, et al. 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.
77. Farilla L, Hui H, Bertolotto C, Kang E, Bulotta A, Di Mario U, et al. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in zucker diabetic rats. *Endocrinology*. 2002;143:4397–4408.
78. Abraham EJ, Leech CA, Lin JC, Zulewski H, Habener JF. Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. *Endocrinology*. 2002;143:3152–3161.
79. Hardikar AA, Wang XY, Williams LJ, Kwok J, Wong R, Yao M, et al. Functional maturation of fetal porcine beta-cells by glucagon-like peptide 1 and cholecystokinin. *Endocrinology*. 2002;143:3505–3514.
80. 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*. 1999;48:2358–2366.
81. Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, et al. GLP-1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology*. 2003;144:5149–5158.
82. 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.
83. Henquin JC. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes*. 2000;49:1751–1760.
84. Steiner DF, James DE. Cellular and molecular biology of the beta-cell. *Diabetologia*. 1992;35:S41–S48.
85. Ashcroft FM, Proks P, Smith PA, Ammala C, Bokvist K, Rorsman P. Stimulus-secretion coupling in pancreatic beta cells. *J Cell Biochem*. 1994;55:54–65.
86. Holz GG, Kuhtreiber 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.
87. 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.
88. Suga S, Kanno T, Ogawa Y, Takeo T, Kamimura N, Wakui M. cAMP-independent decrease of ATP-sensitive K+ channel activity by GLP-1 in rat pancreatic beta-cells. *Pflugers Arch*. 2000;440:566–572.
89. Gromada J, Ding WG, Barg S, Renstrom E, Rorsman P. Multisite regulation of insulin secretion by cAMP-increasing agonists: evidence that glucagon-like peptide 1 and glucagon act via distinct receptors. *Pflugers Arch*. 1997;434:515–524.
90. Gromada J, Bokvist K, Ding WG, Holst JJ, Nielsen JH, Rorsman P. Glucagon-like peptide 1(7-36) amide stimulates exocytosis in human pancreatic beta-cells by both proximal and distal regulatory steps in stimulus-secretion coupling. *Diabetes*. 1998;47:57–65.

91. 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.
92. Nakazaki M, Crane A, Hu M, Seghers V, Ullrich S, Aguilar-Bryan L, et al. cAMP-activated protein kinase-independent potentiation of insulin secretion by cAMP is impaired in SUR1 null islets. *Diabetes*. 2002;51:3440–3449.
93. Shiota C, Larsson O, Shelton KD, Shiota M, Efanov AM, Hoy M, 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;277:37176–37182.
94. de Heer J, Holst JJ. Sulfonylurea compounds uncouple the glucose dependence of the insulinotropic effect of glucagon-like peptide 1. *Diabetes*. 2007;56:438–443.
95. Gutniak MK, Juntti-Berggren L, Hellstrom PM, Guenifi A, Holst JJ, Efendic S. Glucagon-like peptide I enhances the insulinotropic effect of glibenclamide in NIDDM patients and in the perfused rat pancreas. *Diabetes Care*. 1996;19: 857–863.
96. Ammala C, Ashcroft FM, Rorsman P. Calcium-independent potentiation of insulin release by cyclic AMP in single beta-cells. *Nature*. 1993;363:356–358.
97. MacDonald PE, Salapatek AM, Wheeler MB. Glucagon-like peptide-1 receptor activation antagonizes voltage-dependent repolarizing K(+) currents in beta-cells: a possible glucose-dependent insulinotropic mechanism. *Diabetes*. 2002;51(Suppl 3):S443–S447.
98. MacDonald PE, Wang X, Xia F, El-Kholy W, Targonsky E, Tsushima RG, et al. Antagonism of rat beta-cell voltage-dependent K+ currents by exendin-4 requires dual activation of the cAMP/PKA and PI3 kinase signalling pathways. *J Biol Chem*. 2003;278:52446–52453.
99. Kim SJ, Choi WS, Han JS, Warnock G, Fedida D, McIntosh CH. A novel mechanism for the suppression of a voltage-gated potassium channel by glucose-dependent insulinotropic polypeptide: protein kinase A-dependent endocytosis. *J Biol Chem*. 2005;280:28692–28700.
100. Yada T, Itoh K, Kakei M, Tanaka H. Glucose metabolism by rat pancreatic beta-cells produces dual change in cytosolic Ca2+. *Jpn J Physiol*. 1993;43:S115–S118.
101. Kang G, Chepurny OG, Holz GG. cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca2+-induced Ca2+ release in INS-1 pancreatic beta-cells. *J Physiol*. 2001;536:375–385.
102. Gromada J, Dissing S, Bokvist K, Renstrom E, Frokjaer-Jensen J, Wulff BS, et al. Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting beta TC3-cells by enhancement of intracellular calcium mobilization. *Diabetes*. 1995;44:767–774.
103. Kang G, Joseph JW, Chepurny OG, Monaco M, Wheeler MB, Bos JL, et al. Epac-selective cAMP analog 8-pCPT-2′-O-Me-cAMP as a stimulus for Ca2+-induced Ca2+ release and exocytosis in pancreatic beta-cells. *J Biol Chem*. 2003;278:8279–8285.
104. Islam MS, Leibiger I, Leibiger B, Rossi D, Sorrentino V, Ekstrom TJ, et al. In situ activation of the type 2 ryanodine receptor in pancreatic beta cells requires cAMP-dependent phosphorylation. *Proc Natl Acad Sci USA*. 1998;95: 6145–6150.
105. Liu YJ, Grapengiesser E, Gylfe E, Hellman B. Crosstalk between the cAMP and inositol trisphosphate-signalling pathways in pancreatic beta-cells. *Arch Biochem Biophys*. 1996;334:295–302.
106. Kang G, Holz GG. Amplification of exocytosis by Ca(2+)-induced Ca(2+) release in INS-1 pancreatic beta cells. *J Physiol*. 2003;546:175–189.
107. Holz G. New insights concerning the glucose-dependent insulin secretagogue action of glucagon-like peptide-1 in pancreatic beta-cells. *Horm Metab Res*. 2004;36:787–794.
108. Tsuboi T, da Silva Xavier G, Holz GG, Jouaville LS, Thomas AP, Rutter GA. Glucagon-like peptide-1 mobilizes intracellular Ca2+ and stimulates mitochondrial ATP synthesis in pancreatic MIN6 beta-cells. *Biochem J*. 2003;369:287–299.
109. Eliasson L, Renstrom 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(Pt 2):399–412.
110. Eliasson L, Ma X, Renstrom E, Barg S, Berggren PO, Galvanovskis J, et al. SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic B-cells. *J Gen Physiol*. 2003;121:181–197.
111. Renstrom E, Eliasson L, Rorsman P. Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells. *J Physiol*. 1997;502(Pt 1):105–118.
112. Hisatomi M, Hidaka H, Niki I. Ca2+/calmodulin and cyclic 3,5′ adenosine monophosphate control movement of secretory granules through protein phosphorylation/dephosphorylation in the pancreatic beta-cell. *Endocrinology*. 1996;137: 4644–4649.
113. Gromada J, Holst JJ, Rorsman P. Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. *Pflugers Arch Eur J Physiol*. 1998;435:583–594.
114. Fehmann HC, Habener JF. Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. *Endocrinology*. 1992;130:159–166.
115. Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci USA*. 1987;84:3434–3438.
116. 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.

117. 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.
118. U.K. Prospective Diabetes Study Group. U.K. Prospective Diabetes Study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. *Diabetes*. 1995;44:1249–1258.
119. Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia*. 1986;29:46–52.
120. Creutzfeldt W, Ebert R, Nauck M, Stockmann F. Disturbances of the entero-insular axis. *Scand J Gastroenterol Suppl*. 1983;82:111–119.
121. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, et al. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab*. 2001;86:3717–3723.
122. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes*. 2001;50:609–613.
123. Amland PF, Jorde R, Aanderud S, Burhol PG, Giercksky KE. Effects of intravenously infused porcine GIP on serum insulin, plasma C-peptide, and pancreatic polypeptide in non-insulin-dependent diabetes in the fasting state. *Scand J Gastroenterol*. 1985;20:315–320.
124. Elahi D, McAloon-Dyke M, Fukagawa NK, Meneilly GS, Sclater AL, Minaker KL, et al. 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.
125. Krarup T, Saurbrey N, Moody AJ, Kuhl C, Madsbad S. Effect of porcine gastric inhibitory polypeptide on beta-cell function in type I and type II diabetes mellitus. *Metabolism*. 1987;36:677–682.
126. Jones IR, Owens DR, Moody AJ, Luzio SD, Morris T, Hayes TM. The effects of glucose-dependent insulinotropic polypeptide infused at physiological concentrations in normal subjects and type 2 (non-insulin-dependent) diabetic patients on glucose tolerance and B-cell secretion. *Diabetologia*. 1987;30:707–712.
127. Meier JJ, Hucking 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.
128. 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.
129. Lynn FC, Thompson SA, Pospisilik JA, Ehses JA, Hinke SA, Pamir N, et al. A novel pathway for regulation of glucose-dependent insulinotropic polypeptide (GIP) receptor expression in beta cells. *FASEB J*. 2003;17:91–93.
130. Toft-Nielsen M-B, Damholt MB, Hilsted L, Hughes TE, Krarup T, Madsbad S, et al. GLP-1 secretion is decreased in NIDDM patients compared to matched control subjects with normal glucose tolerance. *Diabetologia*. 1999;42:A40.
131. Vaag AA, Holst JJ, Volund A, BeckNielsen H. 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.
132. 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*. 1993;36:741–744.
133. 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.
134. Larsen J, Hylleberg B, Ng K, Damsbo P. Glucagon-like peptide-1 infusion must be maintained for 24 h/day to obtain acceptable glycemia in type 2 diabetic patients who are poorly controlled on sulphonylurea treatment. *Diabetes Care*. 2001;24:1416–1421.
135. Todd JF, Edwards CM, Ghatei MA, Mather HM, Bloom SR. Subcutaneous glucagon-like peptide-1 improves postprandial glycaemic control over a 3-week period in patients with early type 2 diabetes. *Clin Sci (Colch)*. 1998;95:325–329.
136. Nauck MA, Wollschlager D, Werner J, Holst JJ, Orskov C, Creutzfeldt W, et al. Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7-36 amide]) in patients with NIDDM. *Diabetologia*. 1996;39:1546–1553.
137. 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.
138. Goke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, et al. 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.
139. Thorens B, Porret A, Buhler 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.
140. Kolterman OG, Kim DD, Shen L, Ruggles JA, Nielsen LL, Fineman MS, et al. Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus. *Am J Health Syst Pharm*. 2005;62:173–181.
141. Parkes DG, Pittner R, Jodka C, Smith P, Young A. Insulinotropic actions of exendin-4 and glucagon-like peptide-1 in vivo and in vitro. *Metabolism*. 2001;50:583–589.
142. 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.

143. Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, et al. Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab*. 2003;88:3082–3089.
144. Gedulin B, Jodka C, Hoyt J. Exendin-4 (AC2993) decreases glucagon secretion during hyperglycemic clamps in diabetic fatty Zucker rats. *Diabetes*. 1999;48(Suppl 1):A199 (Abstract 0864).
145. Nielsen LL, Young AA, Parkes DG. Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. *Regul Pept*. 2004;117:77–88.
146. Degn KB, Brock B, Juhl CB, Djurhuus CB, Grubert J, Kim D, et al. Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. *Diabetes*. 2004;53:2397–2403.
147. Fehse F, Trautmann M, Holst JJ, Halseth AE, Nanayakkara N, Nielsen LL, et al. 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.
148. Szayna M, Doyle ME, Betkey JA, Holloway HW, Spencer RG, Greig NH, et al. Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. *Endocrinology*. 2000;141:1936–1941.
149. Young AA, Gedulin BR, Bhavsar S, Bodkin N, Jodka C, Hansen B, et al. Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (*ob/ob, db/db*) mice, diabetic fatty Zucker rats and diabetic rhesus monkeys (*Macaca mulatta*). *Diabetes*. 1999;48:1026–1034.
150. Bhavsar S, Watkins J, Young A. Comparison of central and peripheral effects of exendin-4 and GLP-1 on food intake in rats. Program and Abstracts: 80th Annual Meeting of the Endocrine Society; 1998:433 (Abstract P3–223).
151. Tourrel C, Bailbe 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.
152. Tourrel C, Bailbe D, Lacorne M, Meile MJ, Kergoat M, Portha B. Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the beta-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. *Diabetes*. 2002;51:1443–1452.
153. Keating GM. Exenatide. *Drugs*. 2005;65:1681–1692.
154. 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.
155. 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.
156. Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, et al. 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.
157. Klonoff DC, Buse JB, Nielsen LL, Guan X, Bowlus CL, Holcombe JH, et al. 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*. 2007;24:275–286.
158. Blonde L, Klein EJ, Han J, Zhang B, Mac SM, Poon TH, et al. Interim analysis of the effects of exenatide treatment on A1C, weight and cardiovascular risk factors over 82 weeks in 314 overweight patients with type 2 diabetes. *Diabetes Obes Metab*. 2006;8:436–447.
159. Amylin Pharmaceuticals Inc. *Byetta ® Exenatide Injection [Prescribing Information]*. San Diego, CA: Amylin Pharmaceuticals, Inc.; 2007.
160. Trautmann ME, Burger J, Johns D, Brodows R, Okerson T, Roberts A, et al. Less hypoglycemia with exenatide versus insulin glargine, despite similar HbA1C improvement, in patients with T2DM adjunctively treated with metformin. *Diabetes*. 2007;56(Suppl 1):A45 (Abstract 172-OR).
161. Kim D, MacConell L, Zhuang D, Kothare PA, Trautmann M, Fineman M, et al. 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*. 2007;30:1487–1493.
162. Agerso H, Jensen LB, Elbrond 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.
163. Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, et al. One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) Markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. *Diabetes*. 2004;53:1187–1194.
164. Chang AM, Jakobsen G, Sturis J, Smith MJ, Bloem CJ, An B, et al. The GLP-1 derivative NN2211 restores beta-cell sensitivity to glucose in type 2 diabetic patients after a single dose. *Diabetes*. 2003;52:1786–1791.
165. Harder H, Nielsen L, Thi TD, Astrup A. The effect of liraglutide, a long-acting glucagon-like peptide 1 derivative, on glycemic control, body composition, and 24-h energy expenditure in patients with type 2 diabetes. *Diabetes Care*. 2004;27:1915–1921.
166. Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agerso H, Veldhuis J, et al. Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. *Diabetes*. 2002;51:424–429.
167. Madsbad S, Schmitz O, Ranstam 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.

168. 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.
169. Vilsboll T, Zdravkovic M, Le-Thi T, Krarup T, Schmitz O, Courreges JP, et al. Liraglutide, a long-acting human GLP-1 analog, given as monotherapy significantly improves glycemic control and lowers body weight without risk of hypoglycemia in patients with type 2 diabetes mellitus. *Diabetes Care*. 2007;30:1608–1610.
170. 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. *Diabetes Med*. 2005;22:1016–1023.
171. Holst JJ. Therapy of type 2 diabetes mellitus based on the actions of glucagon-like peptide-1. *Diabetes Metab Res Rev*. 2002;18:430–441.
172. Ahren B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. *J Clin Endocrinol Metab*. 2004;89:2078–2084.
173. Ristic S, Byiers S, Foley J, Holmes D. Improved glycaemic control with dipeptidyl peptidase-4 inhibition in patients with type 2 diabetes: vildagliptin (LAF237) dose response. *Diabetes Obes Metab*. 2005;7:692–698.
174. McIntosh CH, Demuth HU, Kim SJ, Pospisilik JA, Pederson RA. Applications of dipeptidyl peptidase IV inhibitors in diabetes mellitus. *Int J Biochem Cell Biol*. 2006;38:860–872.
175. Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, 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.
176. Herman GA, Zhao P-L, Dietrich G, Color G, Schrodter A, Keymeulen B, et al. The DPP-IV inhibitor MK-0341 enhances active GLP-1 and reduces glucose following an OGTT in type 2 diabetics. *Diabetes*. 2004;53(Suppl 2):A82 (Abstract 353-OR).
177. Aschner P, Kipnes MS, Lunceford JK, Sancez M, Mickel C, Williams-Herman D, et al. 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.
178. Charbonnel B, Karasik A, Liu J, Wu M, Meininger G. Sitagliptin Study 020 Group. 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.
179. 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.
180. Goldstein B, Feinglos M, Lunceford J, Johnson J, Williams-Herman DE, for the Sitagliptin 036 Study Group*. 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.
181. 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.
182. Nauck MA, Meininger G, Sheng D, Terranella L, Stein PP, Sitagliptin Study 024 Group. 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*. 2007;9:194–205.
183. Ahren B, Pacini G, Foley JE, Schweizer A. Improved meal-related beta-cell function and insulin sensitivity by the dipeptidyl peptidase-IV inhibitor vildagliptin in metformin-treated patients with type 2 diabetes over 1 year. *Diabetes Care*. 2005;28:1936–1940.
184. Bosi E, Camisasca RP, Collober C, Rochotte E, Garber AJ. Effects of vildagliptin on glucose control over 24 weeks in patients with type 2 diabetes inadequately controlled with metformin. *Diabetes Care*. 2007;30:890–895.
185. Pi-Sunyer FX, Schweizer A, Mills D, Dejager S. Efficacy and tolerability of vildagliptin monotherapy in drug-naive patients with type 2 diabetes. *Diabetes Res Clin Pract*. 2007;76:132–138.