The biology of incretin hormones

Review

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Summary

Gut peptides, exemplified by glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are secreted in a nutrient-dependent manner and stimulate glucose-dependent insulin secretion. Both GIP and GLP-1 also promote β cell proliferation and inhibit apoptosis, leading to expansion of β cell mass. GLP-1, but not GIP, controls glycemia via additional actions on glucose sensors, inhibition of gastric emptying, food intake and glucagon secretion. Furthermore, GLP-1, unlike GIP, potently stimulates insulin secretion and reduces blood glucose in human subjects with type 2 diabetes. This article summarizes current concepts of incretin action and highlights the potential therapeutic utility of GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors for the treatment of type 2 diabetes.

A complex set of physiological responses is activated following meal intake, providing neural and endocrine signals regulating the digestion, absorption, and assimilation of ingested nutrients (Figures 1 and 2). Following the development and utilization of insulin radioimmunoassays for the study of glucose tolerance, a series of studies demonstrated that plasma levels of insulin were significantly greater following oral administration of glucose relative to levels achieved following intravenous glucose challenge in normal human subjects. This gut-associated potentiation of insulin secretion was attributable to one or more humoral or neural factors, termed incretins, that potentiated insulin secretion following enteral nutrient ingestion. Consistent with these observations, administration of intestinal extracts stimulated insulin secretion in dogs. The identity of the putative incretin factor(s) remained elusive until the purification and characterization of the first incretin, glucose-dependent insulinotropic polypeptide (GIP) in the 1970s. Although GIP was shown to be a potent stimulator of glucose-dependent insulin secretion, removal of GIP from gut extracts via immunoabsorption did not eliminate the incretin effect, providing evidence for the existence of additional peptides with incretin-like activity (Ebert et al., 1983). Over a decade later, a second peptide with incretin activity was identified following cloning and characterization of the proglucagon gene. Glucagon-like peptide-1 (GLP-1), a peptide coencoded carboxyterminal to glucagon in the proglucagon gene, was shown to potently stimulate glucose-dependent insulin secretion in both preclinical and human studies (Drucker et al., 1987; Kreymann et al., 1987). This Review summarizes the physiological actions of these hormones (Figure 1) and the therapeutic potential of enhancing incretin action for the treatment of type 2 diabetes.

Incretin synthesis and secretion

GIP is a 42 amino acid peptide produced predominantly in duodenal K cells in the proximal small intestine. GIP has also been localized to the central nervous system, where it may play a role in control of cell survival (Nyberg et al., 2005). The predominant stimulus for GIP secretion is nutrient intake; circulating levels of GIP are low in the fasted state and rise within minutes of food ingestion. As GIP contains an alanine at position 2, it is an excellent substrate for dipeptidyl peptidase-4, an essential enzyme regulating the degradation of both GIP and GLP-1. Full-length GIP(1–42) is rapidly converted to bioinactive GIP(3–42) within minutes of secretion from the gut K cell (Kieffer et al., 1995). Hence, circulating immunoreactive GIP represents a mixture of active GIP(1–42) and inactive GIP(3–42), and experimental analysis of levels of circulating GIP requires discrimination between the intact versus the cleaved peptides. In contrast, GLP-1 is produced in enteroendocrine cells in the distal small bowel and colon. Plasma levels of GLP-1 also rise rapidly within minutes of food intake, hence it seems likely that both neural and/or endocrine factors promote GLP-1 secretion from distal L cells, well before digested nutrients traverse the small bowel to make direct contact with enteroendocrine L cells. Although gastrin-releasing peptide and GIP stimulate GLP-1 secretion in some species, the identity of the endocrine or neural factors promoting rapid release of GLP-1 in humans remains unclear. Similarly, neurotransmitters such as VIP and PACAP likely mediate insulinotropic action following meal ingestion, but function as neurotransmitters, rather than circulating incretins.

Proglucagon is processed to glicentin, oxyntomodulin, GLP-1, and GLP-2 in gut L cells, via processing that requires prohormone convertase-1. Bioactive GLP-1 is generated from GLP-1(1–37) and exists as two equipotent circulating molecular forms, GLP-1(7–37) and GLP-1(7–36)amide. GLP-1(7–36)amide represents the majority of circulating active GLP-1 in human plasma (Orskov et al., 1994). Both forms of GLP-1, like GIP, also contain an alanine at position 2 and are rapidly degraded by DPP-4 to GLP-1(9–36)amide or GLP-1(9–37) following release from gut L cells. Although a separate receptor for GLP-1(9–36)amide has not yet been identified, evidence supports a role for this peptide in glucose clearance or regulation of cardiovascular function. The degradation of GLP-1 is remarkably rapid such that a substantial proportion of immunoreactive GLP-1 in the portal and systemic circulation has already been cleaved by DPP-4. In addition to the importance of DPP-4 for inactivation of GLP-1 and GIP, both peptides are also rapidly cleared from the circulation via the kidney.

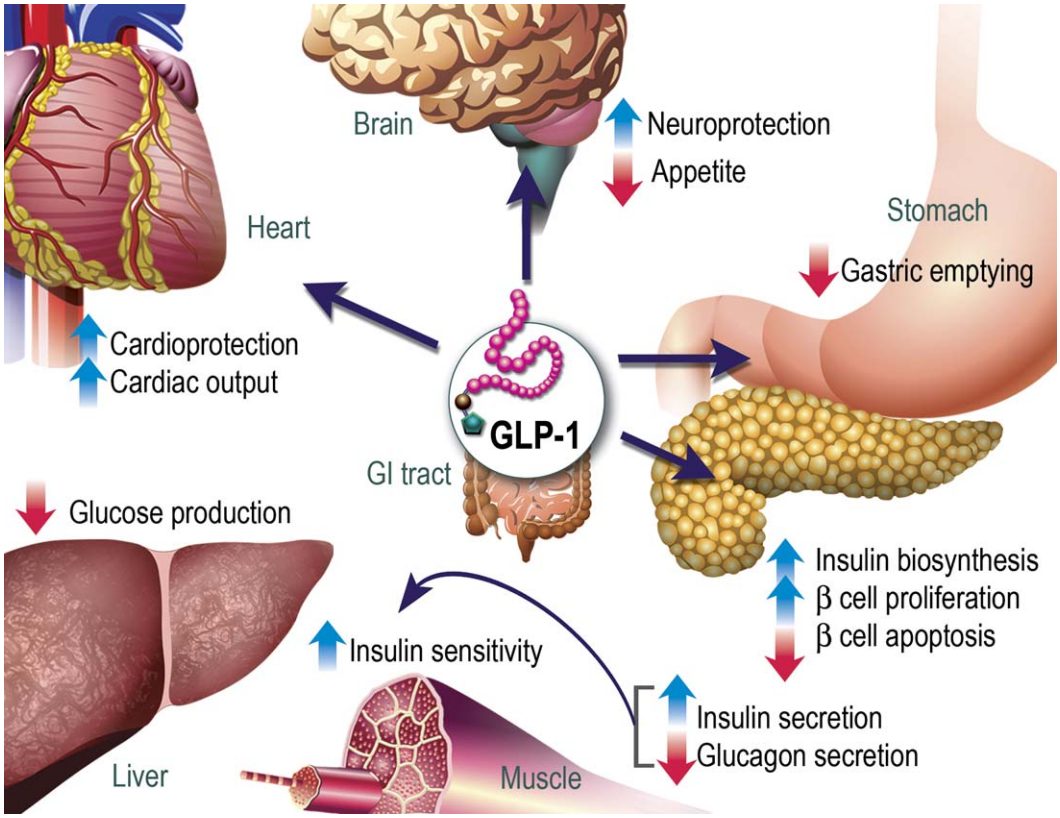

Figure 1. GLP-1 actions in peripheral tissues

GLP-1 acts directly on the endocrine pancreas, heart, stomach, and brain, whereas actions on liver and muscle are indirect.

Molecular mechanisms of incretin action on the endocrine pancreas

The human GLP-1 receptor (GLP-1R) is a 463 amino acid heptahelical G protein-coupled receptor widely expressed in pancreatic islets, kidney, lung, heart, and multiple regions of the peripheral and central nervous system. Within islets, the GLP-1R is predominantly localized to β cells, although GLP-1R expression on islet α and δ cells has also been reported. Although numerous studies allege the functional existence of a second GLP-1 receptor, only a single GLP-1R coupled to glucose homeostasis has yet been identified. Engagement of the GLP-1R stimulates cyclic AMP formation and activation of downstream pathways coupled to protein kinase A and cAMP-regulated guanine nucleotide exchange factors (Holz, 2004). GLP-1R agonists promote cyclic AMP response element binding protein (CREB) phosphorylation and also regulate CREB activity through glucose-dependent stimulation of the cytoplasmic to nuclear translocation of TORC2, a CREB coactivator. GLP-1R activation is also coupled to increased intracellular calcium, inhibition of voltage-dependent K^+ (Kv) currents and activation of immediate early gene expression through effects on Erk1/2, protein kinase C, and phosphatidylinositol 3-kinase (PI3K).

The human GIP receptor exists as two isoforms, 466 and 493 amino acids, expressed in islet β cells, adipose tissue, heart, and brain. GIP receptor activation is also coupled to adenylyl cyclase activation, an increase in intracellular Ca^{2+}, and arachidonic acid efflux. GIP stimulates growth factor-dependent pathways including MAPK (extracellular signal-regulated kinases 1 and 2 [ERK 1/2]), PI3K, and protein kinase B (Akt). Both the GLP-1 and GIP receptors undergo rapid and reversible homologous and heterologous desensitization in vitro, however GIP, but not GLP-1 appears to induce rapid receptor desensitization in vivo. The genes encoding the human GLP-1 or GIP receptors have not been linked to enhanced genetic susceptibility for diabetes.

Both GLP-1 and GIP stimulate glucose-dependent insulin secretion via activation of their specific G protein-coupled receptors expressed directly on islet β cells. The precise mechanisms by which GIP and GLP-1 stimulate insulin secretion only at elevated levels of plasma glucose remain unclear. Both incretins stimulate cyclic AMP formation and protein kinase A activation (Figure 3), although inhibitors of PKA do not completely abrogate the effects of incretins on insulin secretion. The PKA-independent stimulation of insulin secretion by incretins has been attributed to guanine nucleotide exchange factors (GEFs), particularly cyclic AMP-GEFII (Epac2) (Ozaki et al., 2000), and reduction of GEFII expression substantially attenuates the effects of GLP-1 on insulin secretion (Kashima et al., 2001). A role for sulfonylurea receptor (SUR) subunits in modulating GLP-1R-dependent K_{ATP} channel closure has been described. Although GLP-1 and GIP stimulate cyclic AMP formation in $SUR1^{-/-}$ islets, the insulinotropic actions of both incretins are markedly diminished in $SUR1^{-/-}$ mice, likely due to defective coupling of cyclic AMP to pathways regulating insulin exocytosis (Nakazaki et al., 2002; Shiota et al., 2002). These findings are consistent with a modulatory role for SUR1 in the cyclic AMP-dependent regulation of Ca^{2+}-induced exocytosis. In contrast, GLP-1, but not GIP, retains insulinotropic actions in $Kir6.2^{-/-}$ mice (Miki et al., 2005), providing

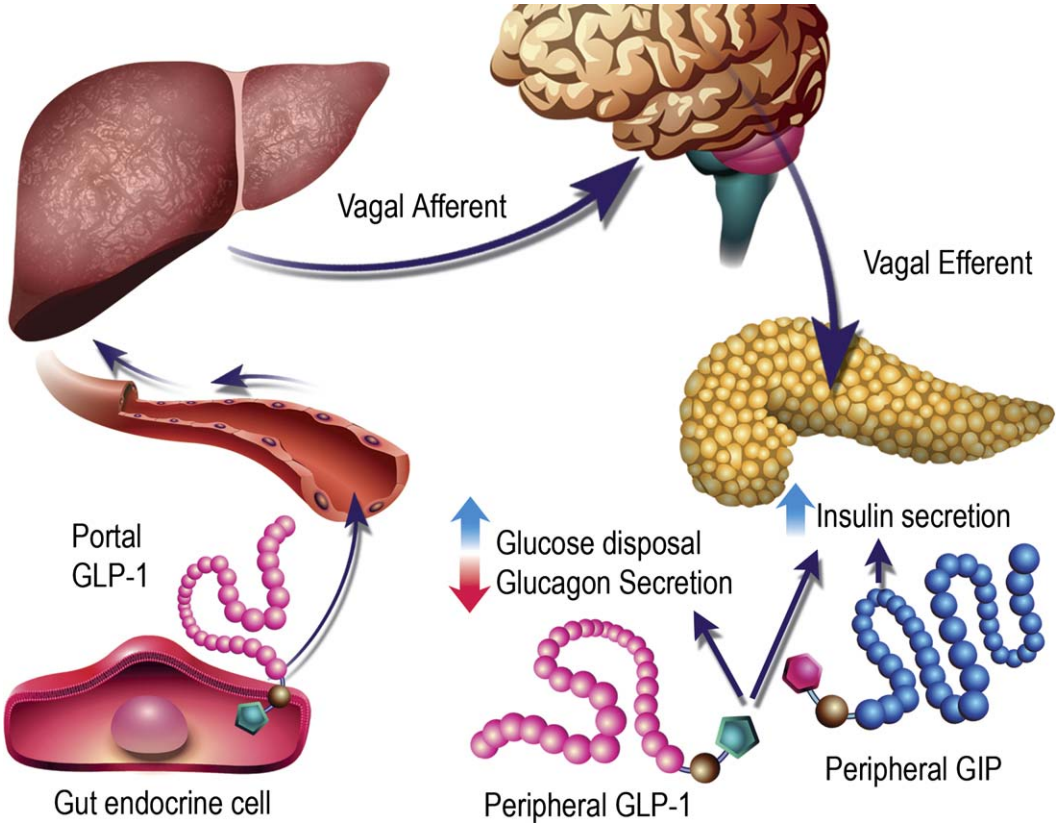

Figure 2. Contrasting roles of GLP-1 and GIP on glucose homeostasis

GLP-1 secreted into the portal vein activates a portal glucose sensor that signals, via vagal afferents, the central nervous system and in turn, vagal efferents enhance insulin secretion. Both GLP-1 and GIP also directly activate insulin secretion via binding to their distinct receptors on islet β cells.

further evidence for divergence of incretin signaling pathways and complexity of K_{ATP} channel subunit action in the β cell.

Unlike other secretagogues acting primarily through the K_{ATP} channel, GLP-1 also replenishes insulin stores via stimulation of proinsulin gene expression (Drucker et al., 1987). These effects are mediated via increases in proinsulin gene transcription and mRNA stability through cyclic AMP-dependent PKA-independent mechanisms. The transcription factor Pdx-1 is an important target for the actions of GLP-1 on insulin gene expression. GLP-1 increases Pdx-1 expression through enhancement of Pdx-1 gene expression, and augmentation of Pdx-1 binding to the insulin gene promoter (Wang et al., 1999). Reduction or elimination of Pdx-1 expression is associated with reduction of GLP-1 receptor expression and loss of GLP-1 action on the β cell, in studies using cell lines or islets in vitro or in mice with a β cell-specific inactivation of the Pdx-1 gene in vivo (Li et al., 2005b; Wang et al., 2005).

GLP-1 also lowers glucose via inhibition of glucagon secretion from islet α cells. The inhibition of glucagon secretion may be direct via GLP-1 receptors expressed on α cells or indirect via stimulation of insulin and somatostatin secretion. Mice with β cell-specific inactivation of the pdx-1 gene exhibit defective suppression of glucagon secretion following exendin-4 administration, illustrating the importance of the β cell in the inhibition of α cell secretory activity (Li et al., 2005b). Of direct clinical relevance, the GLP-1R-dependent suppression of glucagon secretion is regulated in a glucose-dependent manner, thereby reducing the risk of hypoglycemia by relieving inhibition of the α cell once glucose falls to the normal or hypoglycemia range (Degn et al., 2004; Nauck et al., 2002).

Activation of GLP-1R signaling in rodent and human pancreatic exocrine cell lines initiates a program of differentiation toward a more endocrine-like phenotype, in association with increased expression of genes such as Pdx-1, glucokinase, and GLUT-2 (Zhou et al., 2002). GLP-1R agonists may induce differentiation via induction of transcription factors such as Foxa2, leading to increased Pdx-1 gene transcription. Pdx-1 appears critical for transducing the effects of GLP-1R agonists on exocrine cell differentiation, as PANC-1 cells fail to differentiate in the absence of Pdx-1 expression (Hui et al., 2001). GLP-1 has also been shown to promote the differentiation of progenitors derived from human islets into functioning β cells.

GLP-1R agonists also enhance β cell proliferation in studies employing islet cell lines, normal islets, or rodents. Unlike the glucose-dependent actions of GLP-1 on insulin secretion, GLP-1R agonists enhance β cell proliferation and expand β cell mass even in normoglycemic rodents (Edvell and Lindstrom, 1999; Kim et al., 2003; Xu et al., 1999). Remarkably, a transient 5 day neonatal treatment with GLP-1 or the GLP-1R agonist exendin-4 in Wistar rats exposed to a single dose of streptozotocin at birth results in improved β cell mass, findings persistent even in 2-month-old animals (Tourrel et al., 2001). Similarly, transient treatment of rats subjected to a period of intrauterine growth retardation with exendin-4 following birth leads to expansion of β cell mass in rats and prevents the development of diabetes (Stoffers et al., 2003).

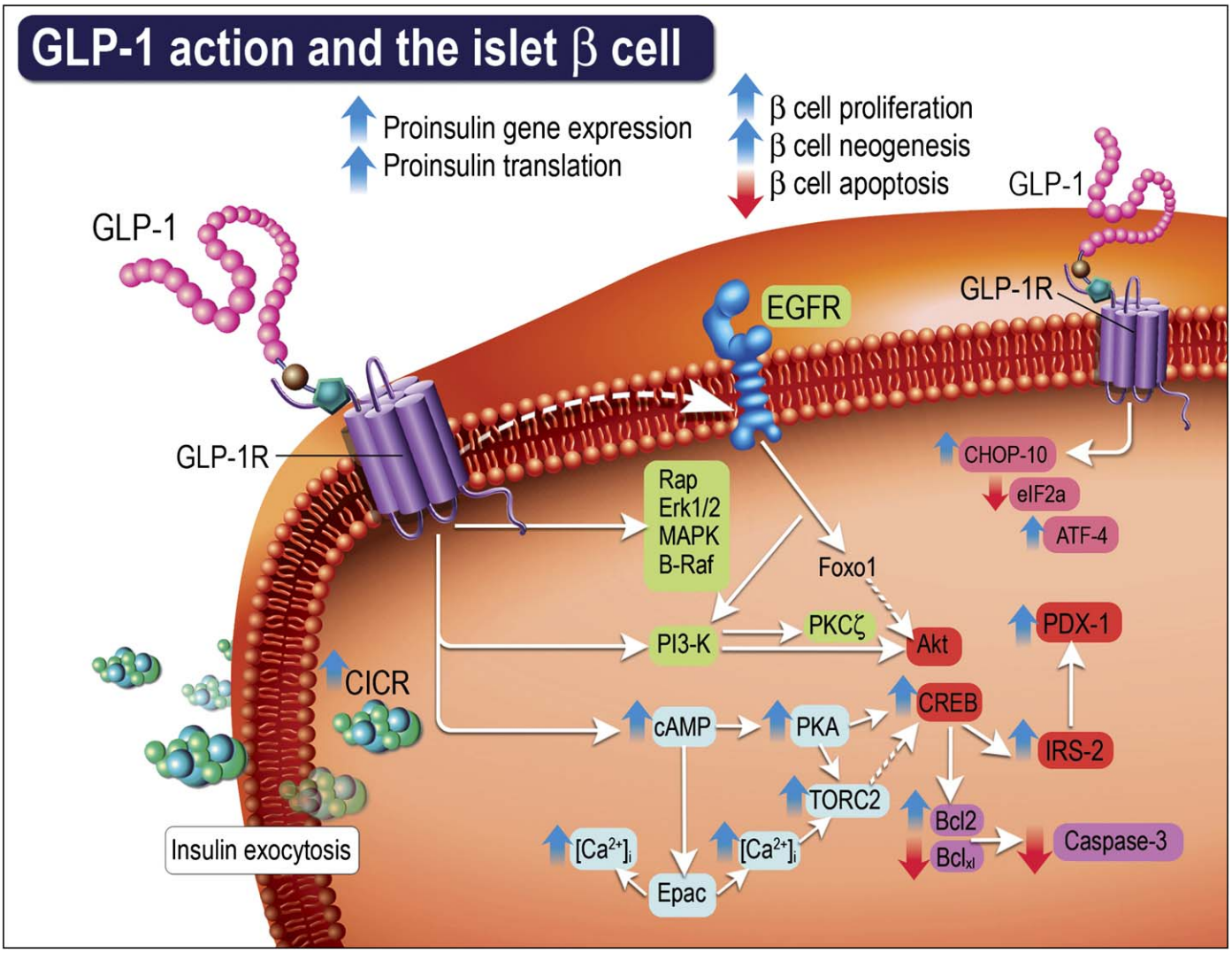

Figure 3. GLP-1 receptor signal transduction pathways in the pancreatic β cell

GLP-1 receptor activation leads to insulin release via stimulation of exocytotic pathways and recruits signaling mechanisms leading to promotion of cell proliferation and survival.

GLP-1R agonists promote expansion of islet mass in association with increased Pdx-1 expression in diabetic mice (Stoffers et al., 2000) and in rats following partial pancreatectomy (Xu et al., 1999). The proliferative and antiapoptotic actions of GLP-1R agonists on the β cell are dependent on the expression of Pdx-1. Impairment of Pdx-1 expression is associated with reduced expression of the GLP-1 receptor and decreased responsivity to exendin-4 in vitro (Wang et al., 2005). Furthermore, mice with β cell-specific inactivation of the Pdx-1 gene exhibit an increased rate of β cell apoptosis, and exendin-4 fails to stimulate β cell proliferation or inhibit apoptosis in Pdx-1$^{-/-}$ islets (Li et al., 2005b).

GLP-1R agonists also promote preservation and expansion of β cell mass through inhibition of apoptotic pathways (Figure 3). GLP-1 reduces caspase-3 expression and nuclear fragmentation in islets of Zucker diabetic rats, and exendin-4 markedly attenuates β cell apoptosis in db/db mice (Wang and Brubaker, 2002) or in wild-type mice following exposure to streptozotocin (Li et al., 2003). Given the potential importance of reactive oxygen species as mediators of β cell cytoxocity, it is noteworthy that GLP-1R activation reduces apoptosis in MIN6 cells exposed to hydrogen peroxide in a cAMP- and PI3K-dependent manner, in association with increased expression of Bcl-2 and Bcl-xL and reduced cleavage of poly-(ADP-ribose)-polymerase (Hui et al., 2003). Similarly, both GLP-1 and exendin-4 reduced palmitate-mediated caspase-3 activation and apoptosis in a PKA-dependent manner in Rinm5F cells (Kwon et al., 2003). GLP-1-mediated stimulation of cyclic AMP leads to enhanced expression of CREB, induction of IRS2, and potentiation of Akt activation (Jhala et al., 2003). Conversely, abrogation of Akt activity using a dominant negative Akt cDNA eliminated the prosurvival actions of exendin-4 in murine islet cells following exposure to cytokines in vitro (Li et al., 2005a). GLP-1R activation also reduces ER stress in murine islets in vivo, reduces eIF2α phosphorylation, promotes induction of ATF4, CHOP, and sXBP-1, and modulates the PERK arm of the endoplasmic reticulum stress pathway in islet β cells.

GLP-1R agonists stimulate proliferation of β cells in part through transactivation of the epidermal growth factor receptor

(EGFR) (Buteau et al., 2003) (Figure 3). GLP-1 also inhibits the transcriptional regulator Foxo1 in islet cells through phosphorylation-dependent nuclear exclusion in an EGFR-dependent manner, and exendin-4 failed to stimulate β cell replication or expansion of islet mass in transgenic mice with constitutive expression of Foxo1 in the nucleus. Similarly, a functional IRS-2 signaling pathway is essential for the exendin-4-dependent stimulation of Akt phosphorylation, Pdx-1 expression and β cell growth, but not for stimulation of insulin secretion in murine islets (Park et al., 2005). The proliferative and antiapoptotic actions of GLP-1 have raised the possibility that GLP-1 may be useful for preservation or expansion of islet mass in the setting of islet transplantation. Although administration of exendin-4 to mice following islet transplantation did not produce improved glucose control, pretreatment of cultured islets with exendin-4 prior to transplantation improved reversal of hyperglycemia following transplantation (King et al., 2005).

The actions of GLP-1 on insulin secretion, β cell proliferation, and survival have also been demonstrated in experiments with human islets. GLP-1 induced membrane depolarization, inhibition of whole-cell K_{ATP} currents, and potentiation of Ca^{2+}-dependent exocytosis in isolated human β cells (Gromada et al., 1998). Both GLP-1 and GIP accelerated Ca^{2+} influx through voltage-dependent (L-type) Ca^{2+} channels and potentiated exocytosis at a site distal to a rise in the cytoplasmic Ca^{2+} concentration (Gromada et al., 1998). Similarly, GLP-1 produced a rapid increase in intracellular calcium that was inhibited by a cAMP antagonist ([Rp]-cAMPS), an L-type Ca^{2+} channel antagonist (nimodipine), an antagonist of the endoplasmic reticulum Ca^{2+} ATPase (thapsigargin), or by ryanodine (Holz et al., 1999). GLP-1 promotes a growth-factor-dependent pathway in human islets in vitro via activation of Rap and B-Raf, in association with increased activity of extracellular signal-regulated kinase (ERK), Akt, and PI3K (Park et al., 2005; Trumper et al., 2005). GLP-1 improved glucose-stimulated insulin secretion, increased Bcl-2 and reduced Bax expression, and enhanced survival of human islet cells in 72 hr cultures (Farilla et al., 2003). GLP-1 also reduced apoptosis in human islets induced by elevated concentrations of glucose or palmitate, alone or in combination (Buteau et al., 2004). Hence, the available evidence suggests that GLP-1R signaling pathways (Figure 3) are highly conserved in rodent versus human islets.

GIP has also been shown to exert proliferative and antiapoptotic actions on islet β cells. GIP improved survival of rat INS-1 cells after serum or glucose deprivation or following exposure to wortmannin or streptozotocin (Ehses et al., 2003). The prosurvival actions of GIP were associated with reduced caspase-3 activation and were dependent on the p38 MAPK pathway. Similarly, GIP promotes cell survival in INS-1(832/13) cells subjected to glucolipotoxicity and in murine islets via downregulation of Bax transcription, due to GIP-mediated reduction in nuclear Foxo1 expression (Kim et al., 2005). Moreover, a 2 week infusion of GIP also downregulated Bax and increased Bcl-2 expression in pancreatic β cells of ZDF rats (Kim et al., 2005). Although the insulinotropic actions of GIP are diminished in hyperglycemic rodents due in part to reduced levels of GIP receptor expression (Lynn et al., 2001), much less is known about the chronic effects of diabetes on preservation of GIP-dependent pathways linked to cell growth and survival.

Extrapancreatic actions of incretins

An important determinant of GLP-1 action on control of postprandial glucose is deceleration of the rate of gastric emptying, which occurs within minutes of pharmacological exogenous GLP-1R agonist administration. The mechanism(s) by which GLP-1 inhibits gastric emptying appear complex and involve communication with the central and peripheral nervous system (Figure 2). Gastric distension increases the expression of c-Fos in brainstem neurons producing GLP-1 (Vrang et al., 2003) and vagal afferent denervation abolishes the effects of GLP-1 on gastric emptying in the rat (Imeryuz et al., 1997). Although small peptides such as GLP-1 and exendin-4 are capable of rapidly crossing the blood-brain barrier and directly access the central nervous system (CNS), higher molecular weight GLP-1R agonists that do not cross the blood-brain barrier are still capable of inhibiting gastric emptying and food intake (Baggio et al., 2004). These findings emphasize the importance of ascending vagal afferents for GLP-1R-dependent control of gastrointestinal motility. Antagonism of the inhibitory effects of GLP-1 on gastric emptying via the prokinetic agent erythromycin results in a much greater GLP-1-stimulated rise in plasma insulin and a loss of the suppression of glucagon secretion following meal ingestion (Meier et al., 2005). GLP-1 receptors are also directly expressed in the stomach on gastric parietal cells, where GLP-1 may directly regulate gastric acid secretion (Schmidtler et al., 1994), however the effects of GLP-1 on gastric acid secretion were abolished in vagotomized human subjects (Wettergren et al., 1997). Hence, considerable evidence supports the importance of vagal innervation for GLP-1R-dependent control of gastric secretion and motility.

Both intracerebroventricular (icv) and peripheral administration of GLP-1R agonists inhibits food intake in rodents (Tang-Christensen et al., 1996; Turton et al., 1996), and GLP-1 receptors have been localized to hypothalamic nuclei important for the regulation of satiety. Repeated icv administration of GLP-1 in rats produces weight loss, whereas icv administration of the GLP-1R antagonist exendin(9–39) for 3 days produced weight gain, and exendin(9–39) administered together with NPY potentiated the increases in food intake and weight gain observed with NPY alone (Meeran et al., 1999). Chronic peripheral administration of GLP-1R agonists is consistently associated with reductions in food intake and weight loss in preclinical studies (Szayna et al., 2000; Young et al., 1999). In contrast, GIP has little effect on gastric emptying or the control of food intake.

GLP-1 also elicits a potent aversive effect in rodents, including development of a conditioned taste aversion (CTA) (Thiele et al., 1997), which may contribute to the anorectic actions of this peptide. Furthermore, central administration of a GLP-1 receptor antagonist attenuates the development of a CTA in response to lithium chloride administration in rats, suggesting that endogenous GLP-1R circuits are important for transduction of aversive signals (Rinaman, 1999; Seeley et al., 2000; Thiele et al., 1998). Treatment of neonatal rats with monosodium glutamate abolishes the anorectic response to GLP-1, implicating a role for the arcuate nucleus and circumventricular organs in the transduction of the GLP-1 anorectic response (Tang-Christensen et al., 1998). Similarly, the sites of GLP-1 action for generation of a CTA versus anorexia have been elucidated via region-specific administration of GLP-1 agonists and antagonists in rats. Administration of GLP-1 into either the lateral or fourth ventricle produced comparable inhibition of food intake whereas only lateral ventricular GLP-1 produced a conditioned taste aversion. Conversely, administration of GLP-1 directly into the central nucleus of the amygdala (CeA) produced a strong CTA without generation of anorexia (Kinzig et al., 2002). These observations

indicate that distinct regions of the CNS mediate the overlapping actions of GLP-1 on aversive and anorectic pathways.

GLP-1 actions and peripheral glucose sensors

GLP-1 may also mediate its effects on glucose control independent of insulin secretion through activation of peripheral sensors linked to enhanced glucose disposal (Figure 2). Intraportal GLP-1, but not GIP, augments the firing of hepatic vagal afferents and pancreatic vagal efferents, ascending nerves communicating signals to the brain and then through neural relays, to the pancreas, respectively (Nakabayashi et al., 1996). Similarly, the ganglionic blocker chlorisondamine inhibited the stimulatory effects of portal GLP-1 on insulin release in rats, evidence implicating a GLP-1R-dependent neural signal emanating from the portal circulation (Balkan and Li, 2000). Although the GLP-1R antagonist exendin(9–39) did not inhibit the GLP-1-dependent activation of vagal afferent firing in rats, exendin(9–39) eliminated the enhanced portal-mediated glucose clearance in mice, and GLP-$1R^{-/-}$ mice do not exhibit enhanced glucose clearance after portal glucose infusion (Burcelin et al., 2001). The importance of portal GLP-1 for enhanced glucose clearance has also been demonstrated in dogs. More recent studies have suggested that GLP-1 action in the brain promotes a reduction in insulin-stimulated glucose uptake in muscle and favors enhanced liver glycogen storage, signals communicated via neural pathways (Knauf et al., 2005). Hence, the coordinate release of digested nutrients and GLP-1 into the portal circulation may augment glucose clearance independent of the peripheral actions of circulating GLP-1.

GLP-1 actions in the heart

GLP-1 receptors are expressed in the rodent and human heart, however the specific cellular localization of GLP-1R expression within the heart has not yet been reported. In mice and rats, GLP-1R agonists rapidly increase heart rate and blood pressure (Barragan et al., 1994). The effect of intravenously administered GLP-1 on arterial blood pressure and heart rate is eliminated by the icv or intravenous administration of the antagonist exendin-(9–39) (Barragan et al., 1999), consistent with a role for central GLP-1R+ neurons in control of the cardiovascular response. Furthermore, exendin-4 and GLP-1 activate c-Fos expression in the adrenal medulla and in neurons in autonomic control sites in the rat brain, and rapidly activate tyrosine hydroxylase transcription in brainstem catecholamine neurons (Yamamoto et al., 2002). GLP-1 increases cyclic AMP in isolated cardiomyocytes without affecting contractility, hence the precise role of the GLP-1R in cardiac cells remains incompletely understood (Vila Petroff et al., 2001). In contrast, although GLP-1R agonists transiently increase levels of cortisol in human subjects, no significant changes in catecholamines, heart rate, or blood pressure have been detected following acute administration of GLP-1R agonists. A role for GLP-1 in the improvement of endothelial function in patients with type 2 diabetes has also been described (Nystrom et al., 2004).

GLP-1 also improves myocardial function and cardiac output (Figure 1) in experimental models of cardiac injury or heart failure. GLP-1 increased cardiac output, and reduced left ventricular end diastolic pressure, in association with improved myocardial insulin sensitivity and myocardial glucose uptake in dogs with rapid pacing-induced congestive heart failure (Nikolaidis et al., 2004a). Consistent with the cytoprotective actions of GLP-1 in the endocrine pancreas, GLP-1 reduced infarct size in the isolated perfused rat heart and in animal models of myocardial ischemia (Bose et al., 2005; Nikolaidis et al., 2005a). The cardioprotective actions of GLP-1 were abolished in the presence of the cyclic AMP inhibitor Rp-cAMP, the PI3kinase inhibitor LY294002, and the p42/44 mitogen-activated protein kinase inhibitor (MAPK) UO126 (Bose et al., 2005). Remarkably, GLP-1 also exerts beneficial effects on cardiac function in human subjects following myocardial infarction and angioplasty. A 72 hr infusion of GLP-1 in patients with acute myocardial infarction and an ejection fraction less than 40% resulted in significantly improved left ventricular ejection fraction and improved regional and global wall motion scores, in association with a trend toward earlier hospital discharge (Nikolaidis et al., 2004b). Whether the beneficial effects of GLP-1 on the injured heart are primarily direct via activation of cardiac GLP-1R signaling or indirect via GLP-1R-dependent improvement in levels of glucose and insulin requires further investigation. Furthermore, the demonstration that GLP-1(9–36)amide improves myocardial glucose uptake and ventricular contractility in dogs with pacing-induced dilated cardiomyopathy suggests that some of the cardiovascular effects of native GLP-1 may be mediated by a distinct GLP-1(9–36) receptor (Nikolaidis et al., 2005b).

Physiological actions of endogenous GLP-1 and GIP

The importance of endogenous incretin action has been examined in studies employing peptide antagonists or in incretin receptor knockout mice. The GLP-1R antagonist exendin(9–39) binds to the GLP-1 receptor and has been used to demonstrate the essential physiological role of endogenous GLP-1 for glucose homeostasis in mice, rats and human subjects. Exendin(9–39) increases both fasting and postprandial glycemia and reduces meal-stimulated levels of circulating insulin in rodents, baboons, and human subjects (Baggio et al., 2000; D'Alessio et al., 1996; Edwards et al., 1999; Kolligs et al., 1995; Schirra et al., 2005). Exendin(9–39) also increases plasma levels of glucagon in human subjects at normal or elevated levels of glucose, consistent with the importance of endogenous GLP-1 as a tonic inhibitor of glucagon secretion (Schirra et al., 1998). Endogenous GLP-1 is also essential for control of gastric emptying, as exendin(9–39) enhanced gastric emptying following oral glucose administration in rats (Imeryuz et al., 1997) and increased antro-pyloro-duodenal motility in human subjects (Schirra et al., 2005). The importance of GIP for glucose homeostasis has been studied using peptide antagonists of GIP action or antisera directed against the GIP receptor in rats and mice. These experiments have demonstrated a predominant role for GIP in the regulation of postprandial glucose clearance. In contrast to studies with GLP-1, endogenous GIP does not appear to be important for control of fasting glucose (Baggio et al., 2000; Lewis et al., 2000; Tseng et al., 1996).

Disruption of the murine GLP-1R results in mild fasting hyperglycemia and impaired glucose tolerance in association with defective insulin secretion following both oral and intraperitoneal glucose challenge (Scrocchi et al., 1996, 1998b). In contrast, despite the importance of exogenous GLP-1 for satiety and weight loss, food intake and body weight are normal in GLP-$1R^{-/-}$ mice on a normal or high-fat diet (Scrocchi et al., 1996; Scrocchi and Drucker, 1998). Similarly, plasma levels of glucagon and

regulation of gastric emptying are not perturbed in the absence of GLP-1R signaling (Baggio et al., 2004; Pederson et al., 1998; Scrocchi et al., 1998b). GLP-1R$^{-/-}$ mice also exhibit phenotypes in the central nervous system and heart. Although the reproductive function of the hypothalamic-pituitary axis is not significantly perturbed following loss of GLP-1R action, GLP-1R$^{-/-}$ mice exhibit increased corticosterone responses to stress (MacLusky et al., 2000). Furthermore, GLP-1R$^{-/-}$ mice exhibit learning deficits and enhanced sensitivity to seizure activity and neuronal injury following kainate administration, and these phenotypes were corrected following restoration of GLP-1R expression using adenoviral gene transfer (During et al., 2003). GLP-1R$^{-/-}$ mice also exhibit defects in cardiac structure and myocardial contractility, implying an essential role for GLP-1R signaling in the control of cardiac development and/or function (Gros et al., 2003).

Targeted disruption of the murine GIPR produces a modest impairment of oral glucose tolerance, with no abnormalities in fasting glucose or intraperitoneal glucose clearance (Miyawaki et al., 1999). Although food intake and body weight are normal in GIPR$^{-/-}$ mice on a regular diet, these mice exhibit resistance to diet-induced obesity and reduced expansion of adipocyte mass following several months of high-fat feeding (Miyawaki et al., 2002, 1999). Furthermore, ob:ob:GIPR$^{-/-}$ mice exhibit reduced weight gain, decreased adiposity, enhanced thermogenesis, and improved glucose homeostasis, implicating a role for GIPR signaling in the control of adipocyte mass and energy expenditure (Miyawaki et al., 2002). Consistent with these findings, although chronic administration of GIP receptor antagonists impairs glucose tolerance in normal mice (Irwin et al., 2004), daily administration of the GIP antagonist (Pro3)-GIP to ob/ob mice markedly lowered levels of glucose and insulin in association with increased insulin sensitivity (Gault et al., 2005). Furthermore, (Pro3)-GIP treatment attenuated the development of islet hypertrophy and β cell hyperplasia. Hence, transient or genetic disruption of GIP action in rodents ameliorates diabetes, likely through modulation of fat accumulation in adipocytes, thereby leading to diminution of insulin resistance (Gault et al., 2005). Although the importance of GIP action in the CNS is not well understood, both GIP and the GIPR are expressed in the brain, and GIPR$^{-/-}$ mice exhibit reduced numbers of cells in the hippocampal dentate gyrus, implicating a role for GIP in neurogenesis (Nyberg et al., 2005). GIP also exerts anabolic and proliferative actions in bone, and GIPR$^{-/-}$ mice exhibit reduced bone mass, abnormal bone architecture, and altered bone turnover (Xie et al., 2005), however GIP does not modulate bone resorption in human subjects.

Surprisingly, genetic disruption of both GLP-1 and GIP receptors in a single DIRKO mouse produces only a modest perturbation in glucose homeostasis (Hansotia et al., 2004; Preitner et al., 2004), raising the possibility that the β cell is capable of adapting to the absence of GIP and GLP-1 action without adverse consequences for glucose homeostasis. Moreover, the phenotype of the Glp-1R$^{-/-}$ and DIRKO mice may also reflect the importance of disrupted GLP-1R signaling in the brain, as emerging evidence implicates an important role for central GLP-1R-dependent pathways in the control of glucose disposal and energy homeostasis (Knauf et al., 2005).

Incretin action and the treatment of type 2 diabetes

Continuous administration of GLP-1 lowers blood glucose to near normal levels in both the fasting and postprandial state in diabetic human subjects via inhibition of gastric emptying and glucagon secretion, and stimulation of insulin secretion (Rachman et al., 1997; Toft-Nielsen et al., 1999). The importance of basal levels of GLP-1R signaling for control of glycemia independent of meals is illustrated by studies demonstrating that continuous GLP-1 infusion from midnight to 8 AM significantly reduced glucose concentrations during the overnight period in subjects with type 2 diabetes (Rachman et al., 1997). The actions of GLP-1 on gastric emptying in human subjects are sufficiently potent so as to markedly attenuate meal-related glucose excursion, thereby potentially reducing insulin secretion from the pancreatic β cell, depending on the level of ambient glucose excursion achieved following GLP-1 administration (Nauck et al., 1997; Todd et al., 1997). Consistent with the importance of glucagon suppression and gastric emptying for GLP-1 action, short-term studies demonstrate that GLP-1 significantly lowers blood glucose in subjects with type 1 diabetes (Dupre et al., 1995; Gutniak et al., 1992).

Proof of concept for the feasibility of using native GLP-1 for therapeutic purposes was obtained in a 6 week study of patients with type 2 diabetes. GLP-1 delivered via continuous subcutaneous infusion significantly lowered both fasting and postprandial glucose, in association with a 1.3% reduction in HbA1c (Zander et al., 2002). GLP-1 therapy was well tolerated, and associated with reduced levels of free fatty acids, improved insulin sensitivity, and a 1.9 kg reduction in body weight (Zander et al., 2002). As the native GLP-1 peptide undergoes rapid enzymatic inactivation by DPP-4, the efficacy of degradation resistant GLP-1R agonists suitable for once or twice daily administration has been examined (Table 1).

Exendin-4 is a naturally occurring 39 amino acid GLP-1 receptor agonist originally isolated from the venom of the *Heloderma suspectum* lizard (Eng et al., 1992). Exendin-4 is encoded by a distinct gene in the lizard, which also contains 2 genes for proglucagon (Chen and Drucker, 1997), however a gene for exendin-4 has not yet been detected in mammalian species. Alignment of native GLP-1 and exendin-4 amino acid sequences demonstrates 53% amino acid identity, and exendin-4 is a highly potent GLP-1R agonist both in vitro and in vivo. Exendin-4 contains a glycine residue at position 2, thereby conferring resistance to cleavage by DPP-4.

Exendin-4 mimics all of the glucose-lowering actions of GLP-1, yet is several orders of magnitude more potent than native GLP-1 following parenteral administration, due to its enhanced pharmacokinetic profile (Young et al., 1999). Twice-daily administration of three different doses of exendin-4 to patients with type 2 diabetes receiving one or two oral antidiabetic agents demonstrated significant lowering of blood glucose and a reduction of HbA1c of 0.7%–1.1% over a 28 day treatment period (Fineman et al., 2003). Phase 3 clinical trials assessed the efficacy of exendin-4, 5 or 10 μg twice daily, added to patients not achieving optimal glucose control with either metformin, a sulfonylurea, or both, for 30 weeks. Exendin-4 (exenatide) was effective in lowering HbA1c in all 3 treatment groups by about 0.9%, with nausea the principal side effect noted in all three studies (Buse et al., 2004; DeFronzo et al., 2005; Kendall et al., 2005). Approximately 34%–46% of patients achieved a HbA1c less than 7% after addition of exenatide (Buse et al., 2004; DeFronzo et al., 2005; Kendall et al., 2005). Patients receiving concomitant sulfonylurea therapy experienced an increased rate of mild to moderate hypoglycemia, however exendin-4 therapy was

Table 1. Properties of GLP-1R agonists versus DPP-4 inhibitors for the treatment of type 2 diabetes

GLP-1R Agonists	DPP-4 Inhibitors
Injectable	Orally available
May produce nausea and vomiting	Well tolerated
Satiety and weight loss	Weight neutral
GLP-1 receptor-dependent MOA	GLP-1 and GIP receptor-dependent MOA
Pharmacological GLP-1R potentiation	Enhancement of endogenous incretin action

MOA = mechanism of action.

associated with modest degrees of weight loss in all treatment groups (Buse et al., 2004; DeFronzo et al., 2005; Kendall et al., 2005). Although anti-exendin-4 antibodies were detected in 41%–49% of treated patients after 30 weeks (Buse et al., 2004; DeFronzo et al., 2005; Kendall et al., 2005), the presence or absence of antibodies did not correlate with the therapeutic response in exendin-4-treated subjects (Buse et al., 2004; DeFronzo et al., 2005; Kendall et al., 2005).

Exenatide has also been compared with insulin glargine as adjunctive therapy for patients with type 2 diabetes not optimally controlled on oral agents with a mean initial starting HbA1c of 8.2%–8.3%. Exenatide and insulin glargine produced comparable reductions in HbA1c (~1.1%) over a 26 week treatment period (Heine et al., 2005). Exenatide produced a greater reduction in postpranial glucose whereas insulin glargine was more effective at lowering fasting glucose. The incidence of gastrointestinal side effects, including nausea, vomiting, and diarrhea, was significantly greater in patients treated with Exenatide, however Exenatide therapy was associated with a mean 2.3 kg weight loss, whereas patients treated with insulin glargine gained ~1.8 kg (Heine et al., 2005).

Complementary approaches for development of GLP-1R agonists include the generation of longer-acting DPP-4-resistant GLP-1 analogs. Liraglutide (NN2211) is a fatty acylated GLP-1 molecule that exhibits a prolonged pharmacokinetic profile after a single injection due to noncovalent association with albumin (Juhl et al., 2002). Liraglutide mimics all of the actions of native GLP-1 and effectively lowers blood glucose in human subjects with type 2 diabetes (Madsbad et al., 2004). The long circulating $t_{1/2}$ of albumin, ~11 days in human subjects, has fostered the development of CJC-1131 and Albugon, albumin-linked GLP-1R agonists with more prolonged durations of action in vivo (Baggio et al., 2004; Kim et al., 2003). Furthermore, a long-acting version of exendin-4 (Exenatide-LAR) developed using a polylactide-glycolide microsphere suspension appears to control glucose for weeks after a single injection in diabetic rats (Gedulin et al., 2005) and is being evaluated in Phase 2 clinical trials in human subjects with type 2 diabetes.

Dipeptidyl Peptidase-4

A complementary approach for enhancing the action of GLP-1 and GIP involves inhibiting the action of CD26, also known as DPP-4, the key enzyme responsible for cleaving and inactivating both of these 2 peptides at the penultimate alanine residue (Mentlein et al., 1993). DPP-4 is a complex, widely expressed enzyme that exists in 2 principal forms; a membrane anchored, largely extracellular protein capable of stimulation of intracellular signal transduction pathways independent of its enzymatic activity, and a circulating soluble enzyme which retains enzymatic activity. The biological importance of DPP-4 has been examined in animals following administration of both selective and nonselective DPP-4 inhibitors, and in rats and mice with inactivating DPP-4 mutations. Fischer 344/CRJ rats with a spontaneous inactivating DPP-4 mutation exhibit reduced numbers of CD4(+) T lymphocytes in response to ovalbumin immunization, in association with significantly reduced ovalbumin-specific IgE-titres (Kruschinski et al., 2005). Similarly, CD26 knockout mice exhibit an altered distribution of splenic T lymphocytes and a reduced level of circulating CD4(+) NKT lymphocytes (Yan et al., 2003). Furthermore, serum levels of total IgG, IgG1, IgG2a, and IgE were lower in sera of $CD26^{-/-}$ mice following immunization with pokeweed mitogen, in association with reduced generation of IL-4 and IL-2 and delayed IFN-γ production (Yan et al., 2003). Moreover, $CD26^{-/-}$ mice exhibit defects in nociception (Guieu et al., 2005) and enhanced severity of experimental arthritis (Busso et al., 2005). Whether highly specific submaximal inhibition of only the catalytic activity of DPP-4 will be associated with similar phenotypes in human subjects chronically treated with DPP-4 inhibitors is not known.

Genetic evidence supports an essential role for DPP-4 in the control of glucose homeostasis. Fischer 344/CRJ rats exhibit increased levels of GLP-1 and reduced glycemic excursion following glucose challenge (Nagakura et al., 2001). Similarly, mice with a targeted inactivation of the DPP-4 gene exhibit improved glucose tolerance, enhanced levels of GLP-1, GIP, and insulin, improved insulin sensitivity, and resistance to diet-induced obesity (Conarello et al., 2003; Marguet et al., 2000). Similarly, chemical inhibitors of DPP-4 prevent the inactivation of both GLP-1 and GIP and lower blood glucose in both preclinical (Deacon, 2004, 1998) and human studies (Ahren et al., 2004a, 2002). Inhibition of DPP-4 activity in 4–52 week studies reduces the levels of HbA1c, in association with prevention of weight gain, potentiation of β cell function, and suppression of plasma glucagon in human subjects with type 2 diabetes (Ahren et al., 2004a, 2004b, 2005).

Numerous peptides and chemokines contain an alanine or proline at position 2 and are susceptible to cleavage by DPP-4, hence the precise identity of the peptide substrates that mediate the glucose-lowering actions of DPP-4 inhibitors remains unclear. DPP-4 inhibitors completely lose their ability to lower blood glucose in mice with genetic disruption of both GLP-1 and GIP receptors (Hansotia et al., 2004). The results of experiments using nonselective versus highly selective DPP-4 inhibitors suggests that DPP-4-selective inhibitors are comparatively safe when administered in high doses to mice, rats, and dogs and do not inhibit human T cell activation in vitro (Lankas et al., 2005). Whether long term selective inhibition of DPP-4 activity in human subjects will significantly perturb the biological activity of peptides such as pituitary adenylate cyclase-activating polypeptide (Zhu et al., 2003), stromal cell-derived factor-1 (SDF-1) (Busso et al., 2005), or substance P (Guieu et al., 2005) remains unknown.

Conclusion

Our understanding of incretin biology has expanded exponentially over the past two decades. Both GLP-1 and GIP exert actions well beyond the β cell, and the roles of these peptides in

peripheral organs such as adipose tissue, the brain, and the heart are receiving increasing attention. Our knowledge of incretin biology remains incomplete, and additional studies on the mechanism of action of both GIP and GLP-1 are warranted. For example, what are the effects of GIPR agonists or DPP-4 inhibitors in adipose tissue, and is it beneficial or deleterious to antagonize GIP action in type 2 diabetes? Why does the human diabetic β cell lose responsivity to GIP but not to GLP-1? What are the consequences of inhibiting DPP-4 on peptide substrates not related to control of glucose homeostasis? Furthermore, the emerging clinical use of strategies based on enhancement of incretin action (GLP-1R agonists and DPP-4 inhibitors) raises additional questions related to mechanisms of action in human subjects. For example, can GLP-1R agonists be used safely without engendering immune responses in a subset of treated individuals? Is there a potential for the proliferative and antiapoptotic actions of GLP-1R agonists to prevent the decline in β cell mass and function characteristic of the natural history of type 2 diabetes? Similarly, will therapy with GLP-1R agonists or DPP-4 inhibitors (Table 1) prove any more durable and control HbA1c better for longer periods of time than currently available agents? Conversely, is there a risk that prolonged GLP-1 receptor activation will lead to uncontrolled cell proliferation and potentially C cell hyperplasia in the thyroid or nesidioblastosis and the development of pancreatic endocrine tumors? Taken together, the potential promise of incretin therapy for the treatment of type 2 diabetes suggests a detailed understanding of incretin biology in multiple systems appears warranted.

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