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Glucagon-Like Peptide-1 and the Islet β-Cell: Augmentation of Cell Proliferation and Inhibition of Apoptosis

Type 2 diabetes is a heterogeneous disorder that develops as a result of relatively inappropriate insulin secretion often in the setting of defective insulin action (insulin resistance). The factors responsible for insufficient insulin production in patients with type 2 diabetes are complex and are thought to involve a combination of genetic and metabolic defects, including chronic hyperglycemia, increased levels of free fatty acids, and dyslipidemia, which contribute to an inability to sustain adequate insulin secretion, defective β-cell proliferation, and/or increased β-cell apoptosis (1).

Studies of the natural history of type 2 diabetes inform us that a progressive decline in β-cell function is common in diabetic patients independent of the method of treatment (2). Hence, new approaches are clearly needed that not only control metabolic abnormalities such as dyslipidemia and hyperglycemia, but that also preserve β-cell function and mass, perhaps by increasing the number of newly formed β-cells and/or decreasing β-cell death. Emerging experimental evidence has confirmed that elevated levels of glucose and specific monounsaturated fatty acids may be deleterious to β-cell survival in short-term studies of human islets (3). Although detailed information about the relative balance of β-cell proliferation and apoptosis in pancreases from subjects with type 2 diabetes is not available, recent autopsy data suggest that a combination of inappropriately decreased β-cell mass and increased β-cell apoptosis in the diabetic pancreas may be more common than previously appreciated (4, 5). Hence, absolute or relative deficiency of β-cells occurs paradoxically at a time when enhanced functional β-cell mass and increased insulin production is needed to overcome insulin resistance. Not surprisingly, the progressive deterioration in β-cell function that accompanies type 2 diabetes has sparked interest in identifying factors that either promote formation of new β-cells or inhibit the death of vulnerable β-cells (1).

Experimental studies carried out predominantly in rodents have demonstrated that IGFs, hepatocyte growth factor, pituitary adenylate cyclase-activating polypeptide, PTHrP, epidermal growth factor receptor agonists in combination with gastrin, and incompletely characterized proteins such as islet neogenesis-associated protein, are capable of enhancing β-cell mass in the setting of experimental diabetes. Hence, prolonged administration of these agents may expand β-cell mass, ultimately leading to increased insulin secretion and improved glycemic control. Similarly, growth factors may also expand the number of human islet cells in experimental culture systems (6, 7). Nevertheless, none of these proteins has been demonstrated to rapidly lower blood glucose and improve metabolic control in preclinical models of diabetes independent of their chronic effects on islet proliferation or β-cell survival. Hence, pharmaceutical development of molecules exhibiting β-cell growth factor-like activity that do not concomitantly acutely lower blood glucose remains highly challenging as it may take weeks or more likely months to determine their potential efficacy in diabetic human subjects.

New data presented in the article by Farilla *et al.* (8) in this issue of *Endocrinology* now demonstrate that glucagon-like peptide-1 (GLP-1), a gut hormone derived from enteroendocrine L cells, inhibits cell death in freshly isolated human islets cultured *in vitro* for 5 d. The antiapoptotic effects of GLP-1 have previously been demonstrated in diabetic rodents, islet cell lines, purified rat β-cells, and heterologous cells expressing the GLP-1 receptor (9–12). Intriguingly, GLP-1 receptor activation also enhances neuronal survival in diverse cellular and animal models of neuronal toxicity, and elimination of GLP-1 receptor function in mice is associated with increased neurotoxicity after peripheral administration of kainic acid (13, 14). Furthermore, the antiapoptotic actions of GLP-1 are essential for β-cell survival in response to cellular injury, as mice with a disruption of the GLP-1 receptor gene exhibit enhanced β-cell death and more severe hyperglycemia after exposure to streptozotocin (10).

Farilla *et al*. (8) added GLP-1 (10 nM) to human islet cells isolated from three independent donors and observed preserved islet morphology in the GLP-1-treated islets, whereas control islets exhibited a greater degree of degradation and loss of three-dimensional structure. GLP-1 treatment was also associated with reduced morphological features of β-cell apoptosis (nuclear condensation and fragmentation) and decreased expression of active caspase-3, an enzyme that plays an essential role in the final pathway leading to programmed cell death. The authors found that GLP-1-treated islets exhibited a progressive increase in the levels of the prosurvival protein bcl-2, which likely contributed to the antiapoptotic actions of GLP-1 in the islet cultures. GLP-1 treatment also increased the number of insulin-immunopositive cells at the end of the 5-d experiment, and GLP-1-treated islets exhibited significantly increased insulin content and improved glucose-dependent insulin secretion (8). Given the intense interest in optimizing islet transplantation for the treatment of type 1 diabetes (15), and the shortage of available human islets relative to the demand for such therapy, agents that potentially preserve or ideally expand islet number while maintaining β-cell function in freshly isolated islets are of great interest to the diabetes community.

Abbreviations: CREB, cAMP response element binding protein; DPP-IV, dipeptidyl peptidase IV; GLP-1, glucagon-like peptide-1; IRS, insulin receptor substrate.

In addition to direct effects on inhibition of β-cell apoptosis, GLP-1 exerts simultaneous effects on control of glucose homeostasis and islet cell growth (16, 17). GLP-1 reduces food intake, inhibits gastric emptying and glucagon secretion, and enhances glucose-dependent insulin secretion, actions that promote lowering of glycemia and restoration of a normal metabolic milieu (Fig. 1) (18). Furthermore, activation of GLP-1 receptor signaling expands islet mass via stimulation of islet neogenesis and induction of β-cell proliferation in both young and old, normal and diabetic animals in multiple different experimental paradigms (9, 19–22). Conversely, basal levels of GLP-1 receptor signaling appear critical for normal islet development (23) and optimal regeneration of β-cell mass after experimental murine pancreatectomy (24). As GLP-1 treatment of human diabetic subjects improves metabolic control resulting in reduction of hyperglycemia and a decrease in circulating fatty acids (25), GLP-1 may also indirectly attenuate islet glucotoxicity and lipotoxicity, thereby resulting in healthier β-cells and reduced β-cell death (Fig. 1).

How Does GLP-1 Increase Islet Neogenesis, Enhance β-Cell Proliferation, and Promote β-Cell Survival?

What are the mechanisms activated by the GLP-1 receptor leading to enhanced β-cell proliferation, increased β-cell survival, and expansion of islet mass? Analysis of the signal transduction pathways activated by GLP-1 that lead to enhanced islet neogenesis is difficult due to the absence of validated cellular models for recapitulating neogenesis *in vitro*. Studies using islet cell lines have identified phosphatidylinositol-3 kinase, which in turn activates protein kinase Cζ (26) as an important downstream component of the GLP-1-stimulated pathway coupled to cell growth. More recent experiments have identified the epidermal growth factor receptor and c-src as direct transactivated downstream targets of GLP-1, with epidermal growth factor receptor and src antagonists blocking GLP-1-stimulated [^{3}H]thymidine incorporation in β-cell lines and rat islets (27). Even more limited is our understanding of how GLP-1 receptor activation promotes cell survival. Experiments with Min6 islet cells demonstrate the importance of cAMP and phosphatidylinositol-3 kinase activation for enhancement of GLP-1-dependent cell survival, in part via increasing the cytoplasmic levels of antiapoptotic Bcl-2 and Bax-xL, after exposure of cells to hydrogen peroxide (11). Although GLP-1 receptor activation enhances the levels of the prosurvival kinase Akt in heterologous cell lines and in the pancreas of diabetic mice (10, 12), the pathway(s) coupling GLP-1R activation to increased Akt phosphorylation remain incompletely characterized.

Recent experiments using a combination of cell lines and transgenic mice have suggested that GLP-1 increases cell survival via cAMP-dependent stimulation of cAMP response element binding protein (CREB) activity, and subsequent enhancement of the insulin receptor substrate (IRS)-2 growth factor-regulated pathway (28). GLP-1 promotes cAMP-stimulated CREB phosphorylation and mice expressing a dominant negative CREB protein in islet β-cells exhibit a marked reduction in β-cell mass and increased β-cell apoptosis, consistent with the importance of CREB for β-cell survival. Gene expression profiling of Min6 islet cells after overexpression of a dominant negative CREB protein revealed that IRS-2, a key transducer of insulin and IGF signaling, is a downstream target of CREB activity in β-cells. Both exendin-4 and cAMP increased the levels of IRS-2 in MIN6 cells, and a combination of promoter transfection, mobility shift, and chromatin immunoprecipitation assays demonstrated that CREB increases levels of IRS-2 in part via transcriptional activation (28). Intriguingly, the ability of IGF-I to activate Akt was significantly enhanced after prior exposure of MIN6 cells to forskolin, providing an additional link between GLP-1-stimulated accumulation of intracellular cAMP, and insulin or IGF-dependent activation of IRS-2 and Akt (Fig. 1).

GLP-1, Cell Proliferation, Differentiation, and Survival in Human Islets

Although the majority of studies assessing the mechanisms whereby GLP-1 increases islet cell differentiation and cell proliferation have employed rodent cell lines or rat and murine islet cell cultures, more recent experiments have examined the effect of GLP-1 on human islet cells. GLP-1R agonists enhance Ca^{2+} influx and stimulate glucose-dependent insulin secretion from human islet cells (29, 30) and activate cellular pathways leading to formation of differentiated functional β-cells from exocrine or islet precursors

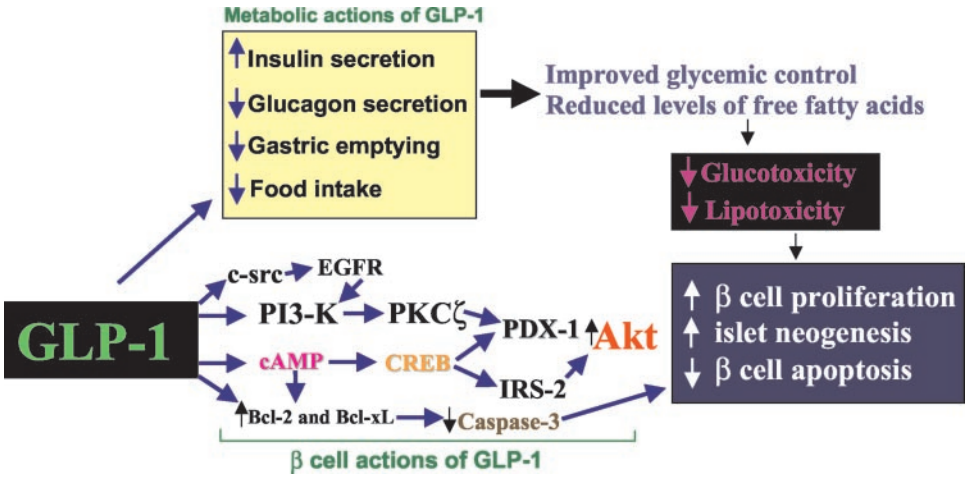

FIG. 1. Actions of GLP-1 that promote expansion of islet β-cell mass. The metabolic actions converge on lowering of blood glucose and lipids, whereas the direct actions on islet precursors and β-cells stimulate β-cell proliferation and inhibit apoptosis.

(31–34). Exendin-4, a potent GLP-1R agonist, induces pancreatic and duodenal homeobox gene-1 expression in human fetal islet cell cultures and promotes functional maturation and proliferation of human islet cell cultures transplanted under the rat kidney capsule (35). Moreover, a subset of nestin-positive human islet-derived progenitor cells express functional GLP-1 receptors and GLP-1 stimulates the differentiation of nestin-positive human islet-derived progenitor cells into insulin-producing cells *in vitro* (33). The current data in the paper by Farilla *et al.* (8) extend the antiapoptotic actions of GLP-1 from rodent models to human islets and raises the possibility that administration of GLP-1R agonists may prove useful for preservation of human β-cells either cultured *in vitro*, after transplantation, or after sustained treatment of diabetic subjects *in vivo*.

What Are the Clinical Implications of the Antiapoptotic Actions of GLP-1?

Although continuous infusion of native GLP-1 markedly improves metabolic parameters in human subjects with type 2 diabetes (25), the degradation of native GLP-1 by dipeptidyl peptidase IV (DPP-IV) has fostered the development of DPP-IV-resistant GLP-1R agonists for human studies (16). A GLP-1R agonist, exendin-4, is currently completing phase 3 clinical trials for the treatment of type 2 diabetes (36). Furthermore, human GLP-1R agonists, such as NN2211 (37) and CJC-1131 (22), are also being evaluated in clinical trials for the treatment of type 2 diabetes. Similarly, drugs that prevent the degradation of GLP-1 by inhibiting the activity of the enzyme DPP-IV have also been shown to exhibit proliferative and antiapoptotic effects on rodent β-cells (38) and are also being evaluated in clinical trials for the treatment of type 2 diabetes (39). Although the major treatment end points in these clinical studies are reduction in glycemia and hemoglobin A1c, it is tempting to speculate that administration of GLP-1 R-agonists to diabetic patients may also be associated with preservation of β-cell function, proliferation of new β-cells, and potentially reduction of β-cell apoptosis. Clearly more work needs to be done, initially with human islet β-cells cultured *in vitro*, and subsequently with human islet cells transplanted into patients with type 1 diabetes, to determine whether GLP-1R agonists have a potential clinical role in the preservation or enhancement of β-cell function in these clinical settings. Despite considerable recent progress using cell and animal models, whether prolonged GLP-1R agonist administration will be associated with reduced rates of β-cell failure and preservation of β-cell function over time in human diabetic subjects is currently unknown. Moreover, the long-term stimulation of cell proliferation, coupled to inhibition of apoptosis, raises theoretical questions about an increased risk of inappropriate cell proliferation and neoplastic transformation in GLP-1R target tissues. These important questions will be technically challenging to assess given the chronic nature of the studies and our current inability to accurately determine functional human β-cell mass in a non-invasive manner.

In view of the positive beneficial effects of GLP-1 on insulin secretion, β-cell proliferation, and inhibition of β-cell death, a clinical trial evaluating the effects of exendin-4 in patients with stable, C-peptide positive type 1 diabetes has been initiated at the National Institutes of Health. The goal of these studies, entitled "The effect of AC2993 (Synthetic Exendin-4) administered alone or in combination with rapamycin and FK506-on islet function in patients with type 1 diabetes" is to ascertain whether exendin-4 treatment might lead to an increase in functional β-cell mass, increased C-peptide production and better glucose control in patients with type 1 diabetes. Although it is not possible to predict whether such studies will produce clinically meaningful improvements in subjects with type 1 diabetes, the favorable metabolic actions of GLP-1 in subjects with type 2 diabetes, taken together with its stimulation of β-cell proliferation, and enhancement of β-cell survival suggests that we may soon be one step closer to the elusive goal of preserving β-cell function and preventing β-cell failure in diabetic patients.

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