Glucagon-like peptide 1 agonists and the development and growth of pancreatic β-cells

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List, James F., and Joel F. Habener. Glucagon-like peptide 1 agonists and the development and growth of pancreatic β-cells. Am J Physiol Endocrinol Metab 286: E875–E881, 2004.—Glucagon-like peptide 1 (GLP-1) is an intestine-derived insulinotropic hormone that stimulates glucose-dependent insulin production and secretion from pancreatic β-cells. Other recognized actions of GLP-1 are to suppress glucagon secretion and hepatic glucose output, delay gastric emptying, reduce food intake, and promote glucose disposal in peripheral tissues. All of these actions are potentially beneficial for the treatment of type 2 diabetes mellitus. Several GLP-1 agonists are in clinical trials for the treatment of diabetes. More recently, GLP-1 agonists have been shown to stimulate the growth and differentiation of pancreatic β-cells, as well as to exert cytoprotective, antiapoptotic effects on β-cells. Recent evidence indicates that GLP-1 agonists act on receptors on pancreas-derived stem/progenitor cells to prompt their differentiation into β-cells. These new findings suggest an approach to create β-cells in vitro by expanding stem/progenitor cells and then to convert them into β-cells by treatment with GLP-1. Thus GLP-1 may be a means by which to create β-cells ex vivo for transplantation into patients with insulinopenic type 1 diabetes and severe forms of type 2 diabetes.

stem cells; diabetes

DIABETES MELLITUS is a major public health problem characterized by an absolute (type 1 diabetes) or relative (type 2 diabetes) deficiency of pancreatic β-cell mass and function. A goal of research in diabetes is to find a way to increase the number of functional insulin-producing β-cells. Toward this end, encouraging results have been obtained in the treatment of patients with type 1 diabetes via the transplantation into the liver of isolated donor islets of Langerhans (57). This approach, however, is severely limited by an inadequate supply of donor islets available for transplantation. More generally applicable methods to enhance functional β-cell mass are needed. Several lines of evidence point to the glucoincretin hormone glucagon-like peptide 1 (GLP-1) as possessing properties that are uniquely suited to encouraging the growth and differentiation of β-cells in tissue culture and in vivo and in suppression of β-cell apoptosis (Fig. 1). For supplemental perspectives of the proproliferative and antiapoptotic actions of GLP-1 on pancreatic β-cells, the reader is referred to several recent review articles (15, 19, 24, 30). Several more general GLP-1 reviews are also available (2, 13, 14, 25, 26, 38, 43, 50, 70). In addition, a useful resource is the website maintained by D. Drucker at the University of Toronto (www.glucagon.com).

PROPERTIES OF GLP-1

GLP-1 is produced from proglucagon in the enteroendocrine L cells of the gut in response to an enteral nutrient load and is also produced in pancreatic islets. GLP-1 acts through a G protein-coupled receptor to exert its functions. This receptor is expressed in many tissues, including pancreatic islets, the central nervous system, lung, kidney, heart, and the gut (6, 66). GLP-1 is coupled to its receptor through stimulatory Gα and adenylyl cyclase to increase intracellular cAMP (66). GLP-1 can induce other intracellular signals as well, including increases in intracellular calcium (41, 73), phosphoinositol 3-kinase (PI3K) activity (10), and mitogen-activated protein kinase activity (45) (Fig. 1). GLP-1 functions to lower postprandial blood glucose via augmentation of glucose-stimulated insulin secretion by β-cells, suppression of appetite, delay of gastric emptying, and suppression of glucagon secretion (36). GLP-1 has extrametabolic effects as well on the cardiovascular, pulmonary, and hypothalamic-pituitary systems (36).

GLP-1 STIMULATES β-CELL PROLIFERATION AND AN INCREASE IN β-CELL MASS

In rodent models of diabetes, the salutary effects of GLP-1 on glucose levels last long beyond the physical half-life of the compound in the circulation (Table 1). Treatment with GLP-1 or exendin-4 (a high-affinity agonist of the GLP-1 receptor originally isolated from Gila monster saliva) for as little as 2–5 days leads to an improvement in glucose metabolism for months. This “memory effect” is likely a reflection of the
ability of GLP-1 to enhance β-cell mass. β-Cell mass exhibits considerable plasticity, increasing over just a few days in response to glucose infusion, pancreatic injury, and extrapancreatic hormones, including placental lactogen, prolactin, growth hormone, and parathyroid hormone-related peptide. GLP-1 and exendin-4 increase β-cell mass in rodent models; treatment for 2 days to 2 wk leads to a 1.4- to 6.2-fold increase in β-cell mass (Table 1). β-Cell hyperplasia is responsible for the increase in mass (72). The efficacy of GLP-1 to enhance β-cell mass varies with the metabolic state of the animal, possibly a reflection of an independent effect of hyperglycemia on β-cell mass (63).

Table 1. Enhancement of β-cell mass by GLP-1 agonist therapy and memory effect

<table>
<thead>
<tr>
<th>Rodent Model</th>
<th>Peptide Dosing</th>
<th>Increase in β-Cell Mass</th>
<th>Durability of Metabolic Improvement (Beyond End of Treatment)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partially pancreatectomized S-D rats</td>
<td>Exendin-4 ip daily × 10 days</td>
<td>1.4-fold</td>
<td>≥15 days</td>
<td>72</td>
</tr>
<tr>
<td>Glucose-intolerant Wistar rats</td>
<td>GLP-1 infusion × 2–5 days</td>
<td>1.5-fold</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>C57B1/6 mice</td>
<td>Exendin-4 ip daily × 2 wk</td>
<td>1.7-fold</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Neonatal STZ-treated rats</td>
<td>GLP-1 or exendin-4 sc daily × 5 days</td>
<td>2.5- to 3.5-fold</td>
<td>≥2 mo</td>
<td>69</td>
</tr>
<tr>
<td>Goto-Kakizaki rats</td>
<td>GLP-1 or exendin-4 sc daily × 5 days</td>
<td>1.8- to 1.9-fold</td>
<td>≥2 mo</td>
<td>68</td>
</tr>
<tr>
<td>db/db Mice</td>
<td>Exendin-4 ip daily × 14 days</td>
<td>1.4-fold</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>ZDF rats</td>
<td>GLP-1 infusion × 2 days</td>
<td></td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>STZ-treated C57BL/6 mice</td>
<td>Exendin-4 ip daily × 10 days</td>
<td>≥9 to ≥16 days</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>db/db Mice</td>
<td>ZP-10A (GLP-1 agonist) × 50 days</td>
<td>≥20 to ≥27 days</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal growth-retarded S-D rats</td>
<td>Exendin-4 sc daily × 6 days</td>
<td>6.2-fold</td>
<td>≥8 mo</td>
<td>59</td>
</tr>
</tbody>
</table>

S-D, Sprague-Dawley; ZDF, Zucker diabetic fatty; STZ, streptozotocin; GLP-1, glucagon-like peptide 1.
GLP-1 INHIBITS APOPTOSIS OF β-CELLS

One mechanism at work in the GLP-1-stimulated expansion of β-cell mass is the inhibition of apoptosis. GLP-1 or exendin-4 decreases apoptosis in the islets of Zucker diabetic fatty (ZDF) rats (22), in the islets of streptozotocin-treated (40) or db/db (71) mice, in isolated rat β-cells exposed to apoptosis-inducing cytokines (40), in isolated human islets (21), in cells of the rat insulinoma cell line RINm5F exposed to palmitate (37), and in cells of the mouse insulinoma line MIN6 exposed to H_{2}O_{2} (29). GLP-1's effect on apoptosis appears to be mediated by the GLP-1 receptor, and expression of the GLP-1 receptor in a nonpancreatic cell line renders these cells sensitive to the inhibition of programmed cell death by GLP-1 (40).

ZDF rats treated with GLP-1 have a decrease in islet staining for the proapoptotic protein caspase 3 (22). db/db Mice treated with exendin-4 have an increase in total pancreatic caspase 3 but a decrease in the amount of active caspase 3 as well as an increase in the survival kinase Akt/PKB (71). Human islets treated with GLP-1 have a downregulation of caspase 3 both at the level of messenger RNA and at the level of active protein and an upregulation of the antiapoptotic protein Bcl-2 (21). RINm5F cells treated with GLP-1 or exendin-4 have a decrease in caspase 3 activity (37).

In MIN6 cells, GLP-1 receptor activation is coupled through cAMP and protein kinase A (PKA) to the phosphorylation and activation of cAMP response element-binding protein (CREB), which, in turn, induces production of insulin receptor substrate (IRS)-2. IRS-2, in the setting of insulin or IGF-I signaling, phosphorylates Akt/PKB, with consequent protection from apoptosis (34). Levels of the antiapoptotic proteins Bcl-2 and Bcl-xl also increase in MIN6 cells in response to GLP-1 in a cAMP-dependent fashion (29). Similarly, although protection from apoptosis in RINm5F cells can come from activation of the cAMP-dependent guanine nucleotide exchange factor (cAMP-GEF) pathway, the cAMP-PKA pathway predominates in the cytoprotective effect mediated by GLP-1 or exendin-4 in these cells (37). Consistent with the physiological relevance of this pathway in β-cell survival, the expression of a dominant negative CREB in the β-cells of transgenic mice leads to a reduction in β-cell mass and a diabetic phenotype (34). PI3K also appears to be important in GLP-1 activation of cell survival pathways. Inhibition of PI3K abolishes much of the antiapoptotic effect of GLP-1 in MIN6 cells (29).

GLP-1 STIMULATES PROLIFERATION OF β-CELLS

A second mechanism responsible for the expansion of β-cell mass is enhanced cell proliferation (Table 1). Mice treated with GLP-1 or exendin-4 show increased cell proliferation by bromodeoxyuridine (BrdU) labeling or by tritiated thymidine incorporation within islets (10, 17, 40). Insulin-expressing cells are stimulated by the administration of GLP-1 in vivo to proliferate. Goto-Kakizaki rats treated with GLP-1 or exendin-4 show an increase in BrdU labeling in cells that are positive for insulin by immunohistochemical staining (68). Similarly, in ZDF rats treated with GLP-1, increased cellular replication as assessed by immuno- staining for Ki-67, a marker of cell proliferation, is seen in insulin-positive cells (22). It remains to be determined whether or not these proliferating insulin-positive cells represent mature β-cells or are immature progenitor β-cells and what the limit is to the replicative capacity of the cells.

Insuloma cell lines have been used to study the cellular proproliferative effect of GLP-1. In rat insuloma cell lines INS-1 and INS(823/13), GLP-1 treatment increases the expression and DNA binding activity of the transcription factor pancreatic duodenal homeobox (PDX)-1, and increases cellular replication as assessed by tritiated thymidine incorporation (8, 10). In these cells, GLP-1 activates immediate early genes involved in cell proliferation such as c-fos, c-jun, junD, and nur77 (64). Betacellulin, a member of the epidermal growth factor (EGF) family, stimulates cell proliferation in INS-1 and INS(823/13) cell lines (8, 33). Betacellulin signaling through the EGF receptor (EGFR) appears to be key to the proproliferative effects of GLP-1 in this system. Disruption of betacellulin signaling abolishes the proproliferative effect (8). The proproliferative effect of betacellulin is also abolished by inhibition of c-src, an activator of endogenous EGFR ligands (8). In this system, GLP-1 also activates PI3K (10) and atypical PKC-ζ (9), supporting a model with a cascade of signaling in which activation of the GLP-1 receptor leads (via c-src) to the autocrine or paracrine release of betacellulin, activation of the EGFR, and activation of PI3K and PKC-ζ (8) (Fig. 1). The stage of β-cell development that corresponds to these autocrine/paracrine effects remains unclear. INS-1 cells, for example, express the stem cell factor receptor c-kit, whereas c-kit is expressed in only a few (55) or none (20) of native islet β-cells. Furthermore, in several rat insuloma cell lines examined (52, 53), including INS-1, insulin is expressed in only a subpopulation of cells; a substantial fraction of the cells are insulin negative (unpublished observation).

GLP-1 induces increased replication of both insulin-positive and insulin-negative pancreatic cells. The insulin-negative cells may represent a pool of β-cell precursors resident in the pancreas that are able to proliferate and differentiate in response to appropriate signals. In the ZDF rat model, GLP-1 stimulates increased replication of insulin-negative cells both within islets and in the exocrine pancreatic parenchyma (22). In aging Wistar rats, GLP-1 stimulates increased cellular replication in the exocrine compartment, as assessed by staining for proliferating cell nuclear antigen (PCNA), a marker of cell proliferation (51). Pancreatic ducts are another site at which GLP-1 stimulates cell proliferation. Exendin-4 increases the incorporation of BrdU in ductal epithelium of sham-pancreatetomized Sprague-Dawley rats (69, 72), and GLP-1 increases numbers of PCNA-positive duct cells in aging Wistar rats (51).

Extraislet cellular proliferation stimulated by GLP-1 agonists is accompanied by the appearance of extraislet cells that express insulin. Sprague-Dawley rats treated with exendin-4 have an increase in the number of single cells located within the exocrine parenchyma that stain positively for insulin (69, 72). In neonatal Wistar rats treated with the β-cell toxin streptozotocin, treatment with GLP-1 or exendin-4 increases single β-cells and β-cell clusters associated with pancreatic ducts (69, 72).

GLP-1 STIMULATES DIFFERENTIATION OF STEM/PROGENITOR CELLS TO AN ENDOCRINE PHENOTYPE

The differentiating effects of GLP-1 on pancreatic cells have been studied in cell culture (Table 2). Several pancreatic cell lines derived from carcinomas expressing ductal and exocrine markers can be coaxed to produce islet hormones by treatment
with GLP-1 agonists. In AR42J, ARIP, and Capan-1 cell lines, treatment with GLP-1 or exendin-4 rapidly induces the expression of insulin, glucagon, and other islet endocrine cell markers (31, 75, 76). Similarly, when GLP-1 is applied to multipotent nestin-positive cells derived from human islets (NIPs) (78), insulin production is induced (1). These cultures also spontaneously produce both GLP-1 and insulin, the former possibly triggering the latter through paracrine signaling (1, 78).

Several lines of evidence implicate the homeodomain protein PDX-1 as a candidate mediator of the actions of GLP-1 to increase β-cell mass. PDX-1 plays an important role in pancreatic development; it is expressed in the early pancreatic bud, and homozygous inactivating mutations of PDX-1 cause pancreatic agenesis (35, 62). PDX-1 is also expressed in β-cells, where it serves as a transcription factor activating the expression of β-cell-specific genes such as insulin, glucokinase, GLUT2, and islet amyloid polypeptide (30). Mutations in the PDX-1 gene are responsible for the phenotype of mature onset diabetes of the young type 4 (60). In rodents, GLP-1 agonists induce increased pancreatic PDX-1 expression. In mice, treatment with GLP-1 or exendin-4 induces an increase in the promoter activity and protein levels of PDX-1 in ductal and exocrine tissues (61). In a rat model of intratherine growth retardation involving bilateral uterine artery ligation in pregnant dams, the administration of daily subcutaneous injections of exendin-4 for 6 days in the neonatal period prevents a decline in the expression of PDX-1 and diminution in β-cell mass. In this model, the GLP-1 agonist exerts a remarkable effect in preventing obesity, insulin resistance, and diabetes that otherwise would occur 15–26 wk later (59).

GLP-1 agonists also stimulate an increase in PDX-1 in pancreas-derived cell lines. In the insulinoma line INS-1, GLP-1 induces an increase in PDX-1 DNA binding activity (10). In AR42J ducal carcinoma-derived cells and in nestin-positive progenitor cells derived from rat and human islets (NIPs), GLP-1-induced differentiation into insulin-producing cells is accompanied by a rise in PDX-1 levels (1, 75, 77). In the Capan-1 cell line, treatment with exendin-4 leads to upregulation of hepatocyte nuclear factor (HNF)-3β binding activity to the PDX-1 promoter as an early event, followed by increases in PDX-1 and differentiation into insulin-producing cells (75, 77). PDX-1 is required for the induction of insulin expression in response to GLP-1 in pancreatic cell lines. AR42J, ARIP, Capan-1, and NIPs express PDX-1. The PANC-1 line, although it expresses the GLP-1 receptor, does not express PDX-1 and fails to produce insulin. When stably transfected with a PDX-1 expression vector, however, PANC-1 cells respond to GLP-1 and differentiate into insulin-producing cells (31). Similarly, aggregates of Blox-5 cells, an insulin-negative line of transformed human β-cells, fail to produce insulin in response to exendin-4. When Blox-5 cells are first infected with a retroviral vector directing PDX-1 expression and are then stimulated with exendin-4, however, they produce insulin (11). Notably, in mouse liver cells that are bathed in GLP-1 from the intestine and pancreas via the portal circulation, the expression of PDX-1 by an adenovirus vector leads to ectopic insulin production (23).

### CELLULAR PROLIFERATION VS. DIFFERENTIATION

In development, there is a general dichotomy between proliferation and differentiation. This circumstance is seen, for example, in the blood-forming cells, in which the replication of precursor cells of the nonlymphocyte lineages precedes terminal differentiation into nonreplicating mature blood cells. In the endocrine system, the replicative capacity of terminally differentiated, mature β-cells has not been established definitively (3–5, 12, 16, 18, 39, 46, 48, 58, 74). In models of β-cell development from embryonic stem cells, adult tissue-derived stem cells, and pancreatic adenocarcinoma cell lines, the differentiation of progenitor cells into insulin-expressing cells is accompanied by a cessation of cellular replication (1, 7, 42, 76, 78). In these experimental systems, inhibition of cell division may be required for triggering the final stages of cell differentiation (27, 54).

Several lines of experimental evidence indicate that GLP-1 stimulates both cellular proliferation and endocrine differentiation in the pancreas. The dichotomy between replication and differentiation is seen in the case of GLP-1’s effects on AR42J cells, in which proliferation and differentiation to a β-cell phenotype in response to GLP-1 appear to be sequentially united. In AR42J cells, GLP-1 causes a burst of cellular replication followed by differentiation to an insulin-expressing phenotype and a cessation of proliferation (76). Peptide mediators other than GLP-1 may also stimulate the growth and differentiation of β-cells, again with a dichotomy between proliferation and differentiation. For example, betacellulin, an analog of EGF, stimulates the replication of INS-1 cells (33). When betacellulin is applied to embryonic pancreas explants (E12.5), however, it increases differentiation of progenitor cell lines.
cells toward a β-cell phenotype without significantly increasing the replication of insulin-expressing cells (32).

CONCLUDING STATEMENTS

Although it appears to be established that GLP-1 stimulates the expansion of β-cell mass both in vivo and in vitro, the direct interaction of GLP-1 with the GLP-1 receptor may not be an absolute requirement for the development of β-cells. In mice carrying a homozygous inactive mutation of the GLP-1 receptor, islets and β-cells do develop (56), albeit somewhat aberrantly, possibly as the result of compensatory signaling from such factors as glucose-dependent insulinotropic polypeptide (49). The exact role of GLP-1 in the normal development of the endocrine pancreas remains to be elucidated.

GLP-1 isopeptides other than GLP-1-(7–36) amide and GLP-1-(7–37) may have important effects in the regulation of the development of the endocrine pancreas as well. Studies of GLP-1 generally have employed the amidated, 30-amino acid residue peptide GLP-1-(7–36) amide, the 31-amino acid glycin extended GLP-1-(7–37), and the GLP-1 agonist exendin-4. The 30-mer and 31-mer GLP-1s are formed from the cleavage of proglucagon by prohormone convertase 1/3 (PC1/3) at the monobasic site Arg109 and the dibasic site Arg109–Arg110. Alternative processing by PC1/3 at Lys90–Arg109 occurs in vivo, liberating a peptide that is six amino acid residues longer at the amino terminus (44, 47). The longer peptide lacks insulinotropic activity, and a specific physiological function has not been assigned to it. However, the administration of GLP-1-(1–37) has been reported to induce insulin expression in murine intestinal epithelial cells, both in tissue culture and in vivo (65).

The ability of GLP-1 agonists to increase β-cell mass in vivo makes these agents attractive as treatments for diabetes. Indeed, GLP-1 agonists are being used in clinical trials for the treatment of both type 1 and type 2 diabetes. Farther on the horizon, GLP-1 agonists may play a central role in strategies to treat type 1 and type 2 diabetes. Farther on the horizon, GLP-1 agonists may play a central role in strategies to treat type 1 and type 2 diabetes. GLP-1 agonists are being used in clinical trials for the treatment of both type 1 and type 2 diabetes. Farther on the horizon, GLP-1 agonists may play a central role in strategies to treat type 1 and type 2 diabetes.

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REFERENCES


