Insulinotropic Glucagon-Like Peptide 1 Agonists Stimulate Expression of Homeodomain Protein IDX-1 and Increase Islet Size in Mouse Pancreas

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Diabetes is caused by a failure of the pancreas to produce insulin in amounts sufficient to meet the body's needs. A hallmark of diabetes is an absolute (type 1) or relative (type 2) reduction in the mass of pancreatic β-cells that produce insulin. Mature β-cells have a lifespan of ~48–56 days (rat) and are replaced by the replication of preexisting β-cells and by the differentiation and proliferation of new β-cells (neogenesis) derived from the pancreatic ducts. Here, we show that the insulinotropic hormone glucagon-like peptide (GLP)-1, which is produced by the intestine, enhances the pancreatic expression of the homeodomain transcription factor IDX-1 that is critical for pancreas development and the transcriptional regulation of the insulin gene. Concomitantly, GLP-1 administered to diabetic mice stimulates insulin secretion and effectively lowers their blood sugar levels. GLP-1 also enhances β-cell neogenesis and islet size. Thus, in addition to stimulating insulin secretion, GLP-1 stimulates the expression of the transcription factor IDX-1 while stimulating β-cell neogenesis and may thereby be an effective treatment for diabetes. *Diabetes* 49:741–748, 2000

Transcription factors are critical in early pancreatic development, cell lineage specification, and the expression of differentiation-specific genes (1). At least 5 distinct gene loci encoding pancreatic transcription factors have been identified that, when mutated, lead to both early- and late-onset forms of type 2 diabetes (2,3). One of these loci encodes the homeodomain transcription factor IDX-1 (also known as PDX-1, IPF-1, and STF-1). IDX-1 is required for early pancreas development, and it regulates glucose-responsive insulin gene transcription and the transcription of the β-cell genes GLUT2, glucokinase, and islet amyloid polypeptide (3–5). The homozygous *idx-1* null mouse (6) and a child homozygous for an inactivating mutation in the *idx-1* gene (7) fail to develop a pancreas (pancreatic agenesis). The heterozygous *idx-1* (+/–) mouse develops a pancreas but becomes glucose intolerant during adulthood as a result of smaller islets and decreased numbers of β-cells (8). Furthermore, *idx-1 (ipf-1)* haploinsufficient family members of a child born with pancreatic agenesis who carry one inactive *idx-1* allele manifest early-onset diabetes, maturity-onset diabetes of the young type 4 (MODY4) (9; A.R. Clocquet, J.M.E., D.A.S., D.C. Muller, L. Wideman, G.A. Chin, W.L. Clarke, J.B. Hanks, J.F.H., D. Elahi, unpublished data). IDX-1 expression is impaired in rodent models of diabetes (11,12) and in cell culture models of glucose toxicity in which β-cell dysfunction is due to hyperglycemia (13–15). Elevated levels of fatty acids, which are found in diabetes, also decrease the expression of IDX-1 (16). Thus, it is important to consider that a deficiency of IDX-1 expression is associated with diabetes and that a means to increase the expression of IDX-1 may improve the function of β-cells in diabetes.

In the studies reported in this article, the regulation of IDX-1 by the insulinotropic hormone glucagon-like peptide (GLP)-1 and by its long-acting analog exendin-4 was examined in the pancreas of mice in vivo. GLP-1 is a proglucagon-derived peptide hormone that is synthesized and secreted by the intestinal L-cells in response to the ingestion of nutrients and circulates to the pancreas where it stimulates the synthesis and secretion of insulin in a glucose-dependent manner (17–19). As such, GLP-1 and its more stable and long-acting analogs, such as exendin-4, are under investigation as potential therapeutic agents for the treatment of type 2 diabetes. In this study, we describe the induction of IDX-1 protein in mouse pancreas during a 2-week course of treatment

with GLP-1 or exendin-4. The administration of exendin-4 to diabetic (*db/db*) mice for 2 weeks lowers blood glucose levels, stimulates insulin secretion, and lowers HbA_{1c} levels in these mice. In addition, the administration of exendin-4 to mice results in an increase in islet size and an enhanced activation of a *LacZ* reporter gene driven by the IDX-1 promoter in the pancreatic ducts of transgenic mice.

RESEARCH DESIGN AND METHODS

Peptides and assays. GLP-1(7-36) amide was purchased from Bachem (King of Prussia, PA). Exendin-4 and exendin 9-39 were purchased from Bachem or Peninsula Laboratories (San Carlos, CA). Glucose levels were determined in plasma obtained from freely feeding mice by the glucose oxidase method (20). Insulin and HbA_{1c} levels were determined as described previously (21).

Animals. Mice of the following strains were commercially obtained: *db/db* (Jackson Laboratory, Bar Harbor, ME), C57Bl6, and CD1 (Charles River Laboratories, Charles River, MA; Jackson Laboratory). Transgenic mice harboring a *LacZ* transgene under the transcriptional regulation of 4.6 kb of IDX-1 5′ flanking were characterized previously (22). GLP-1 receptor null mice were described previously (23); these mice are bred in a CD1 background strain.

Treatment protocol. GLP-1 and exendin-4 were administered for 2 weeks. GLP-1 was infused continuously at a rate of 1.5 pmol · kg^{-1} · min^{-1} via an Alzet micro-osmotic pump (Alza, Palo Alto, CA), as described previously (20). Exendin-4 was given once a day via an intraperitoneal injection at a dose of 100 pmol/kg body wt or 1 nmol/kg body wt. In experiments in which the GLP-1 antagonist exendin 9-39 was coadministered with exendin-4, a separate intraperitoneal injection of 10 nmol/kg of exendin 9-39 was given with the exendin-4 injection. Animals were fasted overnight and euthanized by CO_2 asphyxiation, and the pancreases were dissected.

Protein expression. To evaluate IDX-1 protein expression, freshly dissected pancreases were sonicated in SDS lysis buffer (7). Insoluble material was pelleted at 14,000*g*, and the cleared lysate was assayed for protein using the MicroBCA kit (Pierce, Rockford, IL). Equal amounts of protein were resolved on 12% SDS-PAGE, transferred to Immobilon-P membrane (Millipore, Bedford, MA), and visualized with a COOH-terminal peptide-specific polyclonal IDX-1 antiserum (24) using enhanced chemiluminescence (Amersham, Arlington Heights, IL), as described previously. Quantitation of autoradiogram bands was performed using ImageQuant software on a laser densitometer (Molecular Dynamics, Sunnyvale, CA). At least 2 autoradiograms of varying exposure times were evaluated.

Promoter activity. The generation of transgenic mice carrying a *LacZ* transgene under transcriptional control of 4.6 kb of IDX-1 promoter 5′ flanking region has been previously described (22). The *LacZ* gene encodes the enzyme β-galactosidase. Mice from 1 line (BG15) highly expressing *LacZ* were treated with saline or exendin-4 (1 nmol/kg body wt) for 2 weeks and were fasted overnight before being killed. The pancreases were removed, fixed in 4% paraformaldehyde for 45–60 min at 4°C, rinsed in phosphate-buffered saline (PBS), and immersed in 30% sucrose in PBS at 4°C overnight before embedding in OCT compound (Tissue-Tek; Miles Laboratories, Elkhart, IN). Cryostat sections (7 μm) were incubated in X-gal (HistoMark Kit; Kirkegaard and Perry Laboratories, Gaithersburg, MD) overnight at room temperature, postfixed in 4% paraformaldehyde for 10 min, and mounted in aqueous mounting medium (Glycergel; Dako, Carpinteria, CA).

Quantitation of ductal *LacZ* expression, as determined by the conversion of X-gal to the blue-colored product by β-galactosidase, was performed by dividing ducts into 2 categories. The common pancreatic, main, and large interlobular ducts were counted together in the large-duct category. Small interlobular and intralobular ducts were counted together in the small-duct category. The criteria used to determine the identity of each class of duct have been described (25). In the large ducts, the number of lightly versus intensely labeled *LacZ*⁺ cells per millimeter of ductal epithelium was quantitated. To control for quantitative heterogeneity of *LacZ* expression among littermates, cells were considered intensely labeled if the intensity of the X-gal product was equivalent to that of islets on the same section. At least 2 sections, separated by 300 μm, from each mouse were scanned at 200× final magnification. Nonoverlapping adjacent fields were evaluated for the presence of large and small ducts. For each large duct, the length of epithelium was determined on captured images at 400× final magnification by use of an Optronics TEC-470 charged-coupled device (CCD) camera (Optronics Engineering, Goleta, CA) interfaced with IP-Lab Spectrum imaging software (Scanalytus, Vienna, VA); the number of *LacZ*⁺ cells was determined visually from the same image. Between 25 and 35 large-duct profiles were evaluated for each animal, and the total length of

ductal epithelium evaluated was similar in each group. Whereas all large ducts expressed some amount of *LacZ* activity, only a subset of small ducts was *LacZ*⁺. The total number of small ducts expressing *LacZ* was counted and normalized for the area of the section by dividing by the number of adjacent nonoverlapping 20× microscope fields evaluated (final magnification 200×). The normalized number of small ducts for each pancreas was averaged.

Immunofluorescence. Excised pancreases were embedded in OCT compound. Cryosections of 7 μm were fixed in 4% paraformaldehyde for 5 min at room temperature and then double-stained for IDX-1 {rabbit polyclonal anti–rat IDX-1 [COOH-terminal peptide-specific] at 1:750 dilution [24] and insulin [guinea pig anti-human insulin at 1:1,000 dilution (Linco Research, St. Charles, MO)]}. Fluorescent secondary antisera coupled to Cy-3 and Cy-2 (indocarbocyanine) were obtained from Jackson Immunoresearch Laboratories (West Grove, PA). To minimize variability, tissues were processed simultaneously with identical batches of all solutions from the point of harvesting of the tissue through the end of the staining protocol. Assessment of fluorescence intensity was performed for a given experiment in a single session to avoid variations in signal intensity over time.

Slides were viewed on a Nikon epifluorescence microscope equipped with an Optronics TEC-470 CCD camera (Optronics Engineering, Goleta, CA) interfaced with IP-Lab Spectrum imaging software (Signal Analytics, Vienna, VA) using constant image-grabbing parameters as previously described (11). Islets used for quantitation were defined as the region of interest (ROI) by marking the islet border with the computer-interfaced freehand tool. The border of the islet was defined by the outer margin of insulin immunofluorescence. The area of the islet in arbitrary units was recorded. For evaluation of exocrine IDX-1 expression, the entire captured field lacking any islet tissue was selected as the ROI. The average fluorescence intensity (AFI) was determined by analysis of pixel intensity and is independent of the area of the measured ROI. AFI values were normalized to background fluorescence obtained with sections in which primary antiserum was omitted. Between 20 and 30 islets and exocrine fields from each of 4 animals in each treatment group were evaluated. Values are expressed as means ± SE.

RESULTS

Amelioration of diabetes in *db/db* mice by exendin-4. To examine the effects of the administration of the GLP-1 analog exendin-4 on the progression of diabetes in *db/db* mice, exendin-4 was given intraperitoneally at 1 nmol/kg once a day for 2 weeks. Exendin-4 lowered plasma glucose and raised plasma insulin levels (Fig. 1*A* and *B*), indicating that the therapeutic effect of exendin-4 does not desensitize during long-term administration. Notably, this therapeutic effect was apparent in both diabetic and nondiabetic control mice. The magnitude of the glucose-lowering effect was sufficient to cause a measurable borderline-significant decline in levels of HbA_{1c} at the end of 2 weeks (Fig. 1*C*). These findings are in agreement with recent information describing the beneficial effects of the long-term (12–13 weeks) administration of exendin-4 on the glucose homeostasis of *db/db* diabetic mice (21).

Stimulation of IDX-1 protein expression by GLP-1 and exendin-4. To determine whether IDX-1 protein expression is regulated by GLP-1 or exendin-4 in vivo, C57Bl6 control mice were divided into 3 treatment groups (saline, GLP-1, and exendin-4; *n* = 5 in each group). Saline or exendin-4 was administered once a day by intraperitoneal injection, and GLP-1 was infused via a micro-osmotic pump at a constant rate of 1.5 pmol · kg^{-1} · min^{-1}. Western blot analysis of total pancreatic protein revealed a significant induction of IDX-1 protein levels in both treatment groups compared with that in the saline-treated control group (Fig. 2*A*). Quantitation of IDX-1 bands by laser densitometry indicated a 3- to 4-fold increase in both groups, compared with the control group (GLP-1 4.6-fold, exendin-4 3.4-fold) (Fig. 2*B*).

It has been reported that exendin-4 and GLP-1 exert metabolic effects in tissues that do not express the cloned GLP-1 receptor (26–28), thus raising the possibility that additional
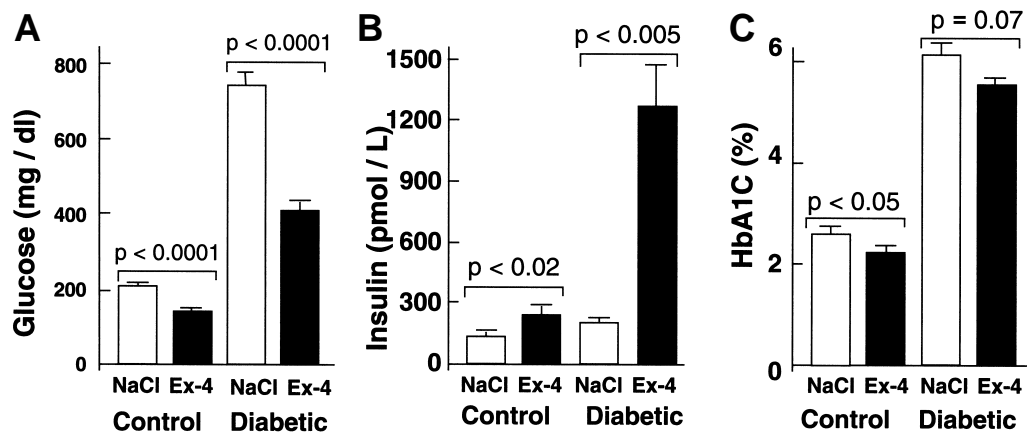
A

B

C

FIG. 1. Amelioration of diabetes by exendin-4 (Ex-4). A 2-week course of once-a-day intraperitoneal administration of exendin-4 (1 nmol/kg) to *db/db* (diabetic) and C57B16 (control) mice resulted in a lowering of plasma glucose levels (*A*), a stimulation of plasma insulin levels (*B*), and a lowering of HbA$_{1c}$ levels (*C*). Values are nonfasting measurements from 6 animals per treatment group and are expressed as means ± SE.

GLP-1 receptors exist. It is also possible that exendin-4 does not exert its metabolic effects entirely through the known GLP-1 receptor. Therefore, to determine whether exendin-4 acts through the identified and characterized GLP-1 receptor, we examined the effectiveness of the GLP-1 antagonist exendin 9-39 (Fig. 2*C*) to inhibit the stimulation of IDX-1 levels in response to the coadministration of exendin-4 agonist.

The exendin 9-39 antagonist completely prevented the induction of IDX-1 in the pancreas (Fig. 2*C*). To further confirm the specificity of exendin-4 acting on GLP-1 receptors, we tested the ability of exendin-4 to stimulate IDX-1 protein levels in GLP-1 receptor –/– mice (23). As observed in the C57Bl6 strain, exendin-4 also stimulated IDX-1 protein levels in the CD1 background mouse strain of the null mice (Fig. 2*C*). In
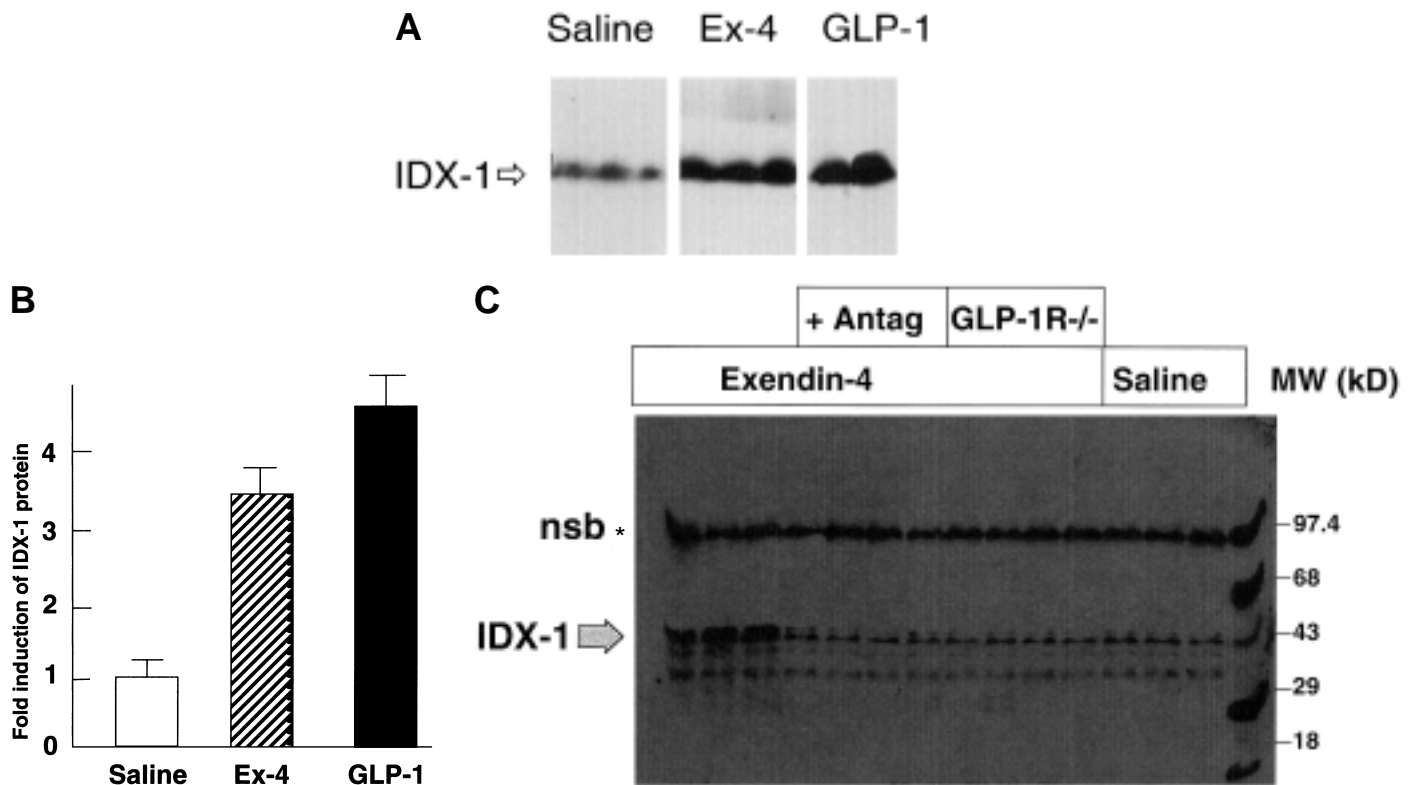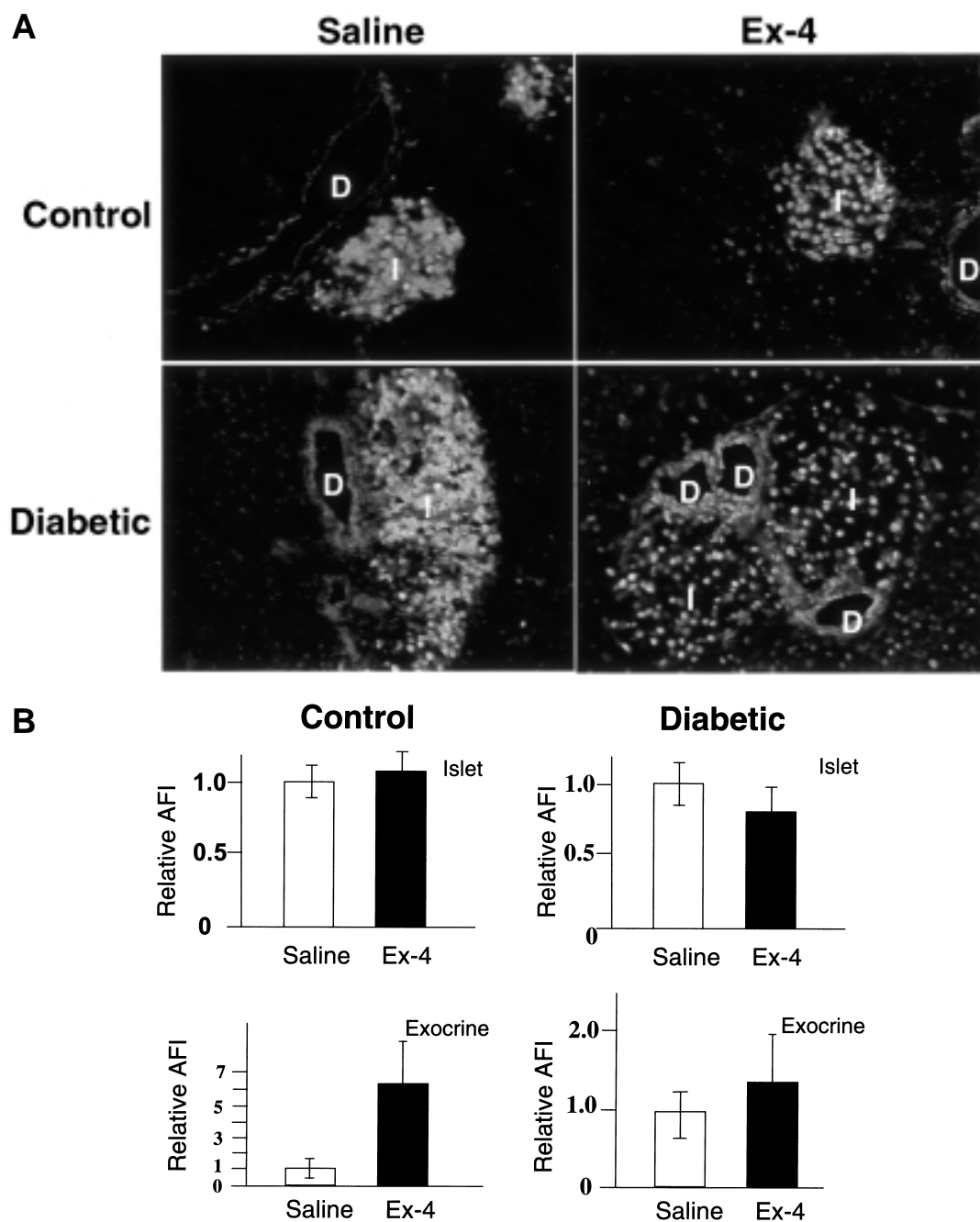
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FIG. 2. GLP-1 and exendin-4 (Ex-4) increase IDX-1 protein in the pancreas. *A*: Western immunoblots of whole-cell extracts of pancreases from C57B16 mice treated with exendin-4 100 pmol/kg intraperitoneally once a day for 2 weeks or a GLP-1 constant infusion at a rate of 1.5 pmol · min^{-1} · kg^{-1} via an osmotic pump. Equal amounts of protein (40 µg) were loaded in each lane. Animals were fasted overnight before being killed. *B*: Densitometry of autoradiogram of *A* reveals a 3- to 4-fold induction of IDX-1 protein in both GLP-1–treated (4.6-fold) and exendin-4–treated (3.4-fold) animals compared with saline-treated controls (*n* = 4 in each group). *C*: Exendin-4 stimulation of IDX-1 protein levels requires the GLP-1 receptor because stimulation of IDX-1 levels is prevented by coadministration of the GLP-1 antagonist exendin 9-39 and does not occur in *glp-1r* null mice. Molecular weight (MW) markers are shown on the right. –/–, *glp-1r* null mice; Antag, exendin 9-39 antagonist; nsb, nonspecific band.

A

B

FIG. 3. Exendin-4 stimulates IDX-1 expression in pancreatic ductal and exocrine tissue. *A*: Immunofluorescence staining for IDX-1 in pancreatic sections from control C56B16 nondiabetic mice (top) and diabetic *db/db* mice (bottom) treated with saline (left) or exendin-4 (right) (100 pmol/kg) for 2 weeks. Islets and ducts are indicated. *B*: IDX-1 levels are increased by exendin-4 treatment in exocrine but not endocrine tissues of nondiabetic C57B16 control and diabetic *db/db* mice. Values are AFI expressed in arbitrary units ± SE. To facilitate comparison, the value for saline-treated control animals was set to 1.0 in each graph, and the exendin treatment group results were normalized accordingly. D, ducts; Ex-4, exendin-4; I, islets.

contrast, no stimulation of IDX-1 protein was detected in the GLP-1 receptor null mice (Fig. 2*C*), indicating that exendin-4 appears to stimulate IDX-1 expression solely through the known GLP-1 receptor.

The stimulation of IDX-1 by exendin-4 was further confirmed by immunofluorescence immunocytochemistry. An enhanced expression of IDX-1 was observed in the ducts and the exocrine tissue of both control nondiabetic and diabetic *db/db* mice treated with exendin-4 for 2 weeks (Fig. 3*A*). In addition, enhanced nuclear localization of IDX-1 in

response to exendin-4 is observed in the ductal and islet cells in control nondiabetic and diabetic *db/db* mice (Fig. 3*A*). Glucose has been reported to promote the nuclear translocation of IDX-1 in primary islets and in MIN6 cells (29,30), and cytoplasmic IDX-1 in pancreatic ductal epithelium has been noted previously (31,32). Thus, the subcellular distribution of IDX-1 may be a mechanism of its regulation by extracellular signals generated by exendin-4.

Semiquantitative analyses by fluorescence immunocytochemistry of pancreases of control nondiabetic mice showed

an ~7-fold induction of IDX-1 expression in exocrine tissue in control nondiabetic mice (1.00 vs. 6.99 in saline- vs. exendin-4–treated C57Bl6 mice, $P \leq 0.01$) and showed no significant effect on intraislet IDX-1 immunofluorescence (1.00 vs. 1.04 in saline- vs. exendin-4–treated animals) (Fig. 3*B*). These data are consistent with the induction of IDX-1 protein observed in whole-pancreas lysates on Western blot, since 98–99% of adult pancreatic tissue consists of exocrine acinar cells (Fig. 2) (1). In the pancreases of *db/db* diabetic mice, exendin-4 also stimulated the expression of IDX-1 in the exocrine pancreas, but this effect was not statistically significant (Fig. 3*B*). The lack of a stimulatory effect of GLP-1 agonists on total-islet IDX-1 measured by fluorescence immunocytochemistry indicates that the effect of GLP-1 on insulin gene transcription is more likely to be mediated via a distinct temporally rapid mechanism, such as phosphorylation of IDX-1. It remains to be proven that the acute effect of GLP-1 on insulin gene transcription involves IDX-1.

Stimulation of IDX-1 promoter activity by exendin-4. To further delineate cell types in which GLP-1 regulates the *idx-1* gene, the effect of exendin-4 was examined on *idx-1* promoter activity in vivo. At the transcriptional level, expression of the *idx-1* gene in β-cells is determined by enhancer sequences located within 6.5 kb of the rat *idx-1* gene promoter (33) and 4.6 kb of the mouse *ipf-1* promoter (22,33). To better understand the spatial and temporal patterns of expression of IDX-1 during development, transgenic mice were created that express the *Escherichia coli LacZ* gene under the control of the 5′-proximal 4.6 kb of the IDX-1 promoter (22). The determinants of both developmental and tissue-type specificity of expression contained within this region of the promoter closely approximate the expression of the endogenous *idx-1* gene (22). When –4.6 kb *idx-1/LacZ* mice were treated with exendin-4 for 2 weeks, there was a 5.9-fold increase in the number of epithelial cells within large-duct cells expressing high levels of an *idx-1* promoter *LacZ* reporter transgene (0.67 ± 0.53 vs. 3.93 ± 1.14 cells per millimeter ductal epithelium [$\times10^{-7}$] in saline- vs. exendin-4–treated animals, respectively) (Fig. 4*A* and *C*). The number of large-duct epithelial cells expressing low levels of *LacZ* was not affected by exendin-4 treatment (56.7 ± 1.2 vs. 44.9 ± 0.6 cells per millimeter ductal epithelium [$\times10^{-7}$] in saline- vs. exendin-4–treated animals). These findings led us to hypothesize that the intensely staining *LacZ*⁺ cells in the large ducts may represent precursor cells that are committed to develop into endocrine cells by virtue of exposure to exendin-4. This notion is consistent with the model proposed by Guz et al. (34), in which IDX-1 expression marks a population of hormone-expressing ductal stem-cell precursors that will go on to differentiate into islet cells. An increased expression of the *idx-1* promoter *LacZ* transgene was also seen in response to exendin-4 in the smaller ducts of the pancreas (Fig. 4*B* and *C*).

Expansion of islet size by exendin-4. To determine whether long-term exposure (2 weeks) to exendin-4 leads to an expansion of β-cell mass by stimulating new islet tissue formation (neogenesis), islet size was evaluated. A 1.7-fold increase was found in islet size in the exendin-4 treatment group compared with that in the saline-treated control group (Fig. 5).

DISCUSSION

The insulinotropic hormone GLP-1 was initially shown to augment glucose-stimulated insulin release, to suppress glucagon
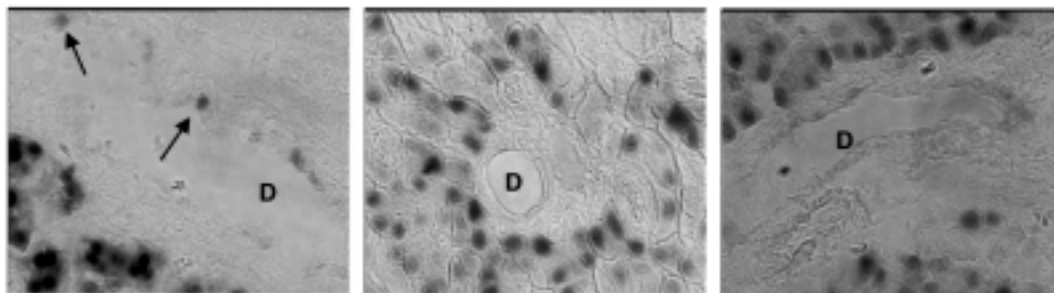
secretion, to delay gastric emptying, and to possibly stimulate glucose uptake by peripheral tissues (17–19). Subsequently, GLP-1 was shown to reduce food intake when administered to rats (35) or human volunteers (36) and to improve glucose homeostasis in glucose-intolerant aged rats (20) and obese diabetic *db/db* mice (21). In addition, GLP-1 receptor null mice develop severe glucose intolerance (23) and have dysmorphic development of the pancreatic islets (D.J.D., unpublished data). Recently, GLP-1 was shown to increase islet cell proliferation and islet mass when administered to Umea +/? normoglycemic mice (37). It was also shown to stimulate the proliferation of INS-1 cells in vitro (38) and to increase β-cell replication, neogenesis, and β-cell mass in rats (39).

In our studies, we show that GLP-1 and the longer-acting GLP-1 agonist exendin-4 stimulate the expression of the IDX-1 homeodomain protein in the pancreas when administered to mice. Expression of both immunoreactive IDX-1 and β-galactosidase expressed from a *LacZ* reporter transgene driven by the IDX-1 promoter occurs in the pancreatic ducts and the exocrine pancreas. This is a potentially important observation for at least 2 reasons: *1*) IDX-1 is required for the growth of the pancreas, and *2*) the epithelium of the pancreatic ducts and the centrolobular terminal ducts in the acinar tissue are the source of the differentiation of new β-cells (β-cell neogenesis) (S.B.-W., unpublished data).
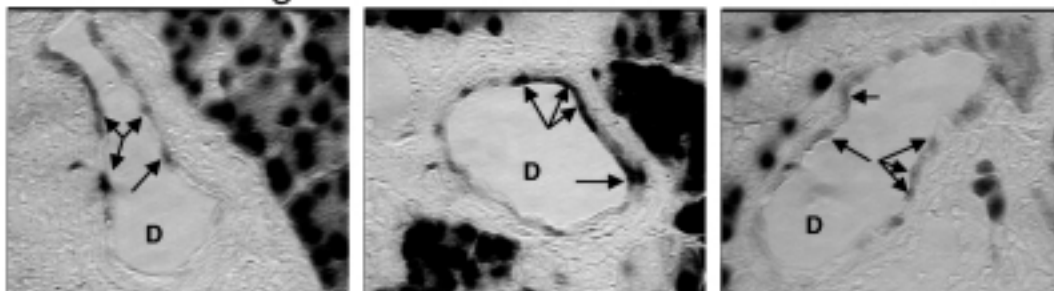
IDX-1 is important for pancreas development because *idx-1* nullizygosity results in a failure of the pancreas to develop both in mice (6,40) and in a child (7; A.R. Clocquet, J.M.E., D.A.S., D.C. Muller, L. Wideman, G.A. Chin, W.L. Clarke, J.B. Hanks, J.F.H., D. Elahi, unpublished data). Furthermore, *idx-1/ipf-1* haploinsufficiency leads to glucose intolerance and small islets in mice (8) and MODY4 in humans. Moreover, there is now evidence for an association of several missense mutations in *idx-1/ipf-1* with late-onset type 2 diabetes (41,42). In addition, persistent hyperglycemia, such as that which occurs in diabetes, downregulates IDX-1 expression in animal models (11,12,43) and in cultured insulinoma cells (13,14), resulting in an inhibition of insulin gene transcription and the consequent production and secretion of insulin. Although it cannot be directly tested, one might assume that IDX-1 expression and β-cell mass could be diminished in individuals with type 2 diabetes. Therefore, we anticipate that pharmacological means to stimulate β-cell neogenesis and/or proliferation (e.g., by use of GLP-1 agonists) would be beneficial for the treatment of diabetes.

It is worth noting that our studies indicate that the effects of GLP-1 and exendin-4 on the induction of IDX-1 in the pancreas appear to be direct rather than indirect, because no such induction was observed either in GLP-1 receptor null mice or in mice that received the GLP-1 antagonist exendin 9-39 with the administration of the exendin-4 agonist. These studies support the idea that exendin-4 exerts its physiological actions, at least in part, via the GLP-1 receptor. The GLP-1 receptor is a 7-membrane–spanning G-protein–coupled receptor that is coupled to the formation of cAMP via stimulation of adenylate cyclase. Thus, cAMP signaling in pancreatic ducts and exocrine pancreas cells could be responsible for the increased expression of IDX-1 and the activation of the IDX-1 promoter, as reported by *LacZ* expression in transgenic mice treated with exendin-4. However, recent studies indicate that GLP-1 also activates mitogen-activated protein (MAP) kinase pathways in cultured
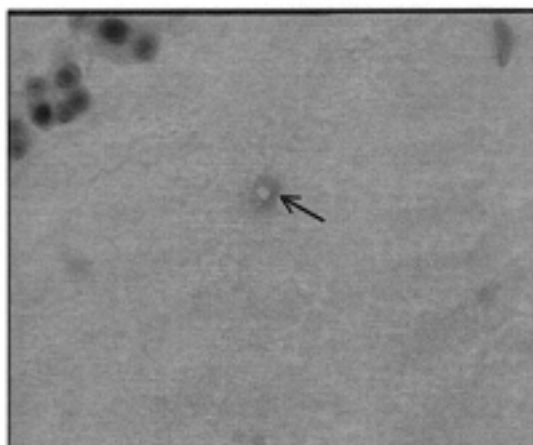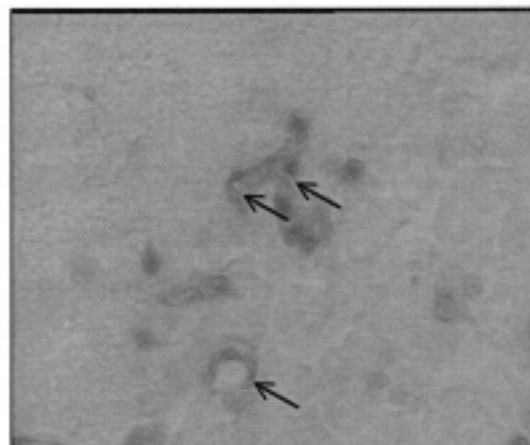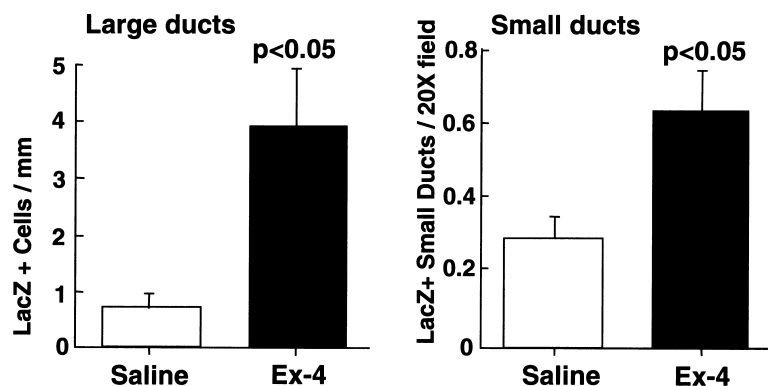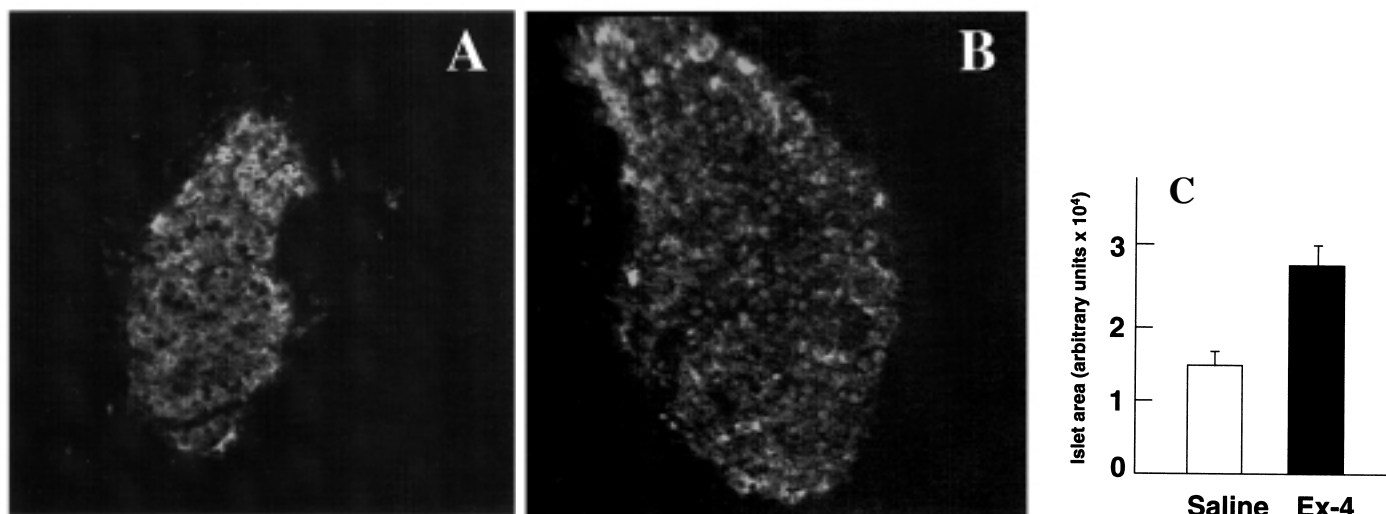
FIG. 4. Exendin-4 stimulates *idx-1* promoter *LacZ* transgene expression in large and small pancreatic ducts. *LacZ* activity in the large (*A*) and small (*B*) pancreatic ducts of –4.6kb *LacZ* transgenic mice treated with saline (upper panel in *A*, left panel in *B*) or exendin-4 (Ex-4) (1 nmol/kg body wt) (lower panel in *A*, right panel in *B*) once a day intraperitoneally for 2 weeks. Arrows in *A* point to *LacZ*⁺ epithelial cells in the large ducts. Arrows in *B* point to *LacZ*⁺ small ducts. *C*: Quantitation of *LacZ* expression in large and small ducts shows a stimulation of the number of intensely labeled cells in the epithelium of the large ducts (left) and a stimulation of the total number of the labeled small ducts (right). In the left panel, the units are expressed as numbers of epithelial cells per millimeter of ductal epithelium.

FIG. 5. Long-term exendin-4 (Ex-4) treatment leads to an increase in islet size. Representative islets from a control (*A*) and an exendin-4–treated (*B*) C57Bl/6 mouse, as shown by insulin immunofluorescence staining. *C*: Islet size is increased by 1.76-fold after 2 weeks of daily exendin-4 treatment (100 pmol/kg).

INS-1 cells (38), in RIN1046-38 cells, and in CHO cells (44). Such an activation of MAP kinase pathways would best explain the apparent growth-promoting actions of GLP-1 on pancreatic islets as shown here, in mice (Umea +/?) (37), and in cultured insulinoma cells (38). One potential mechanism by which GLP-1–stimulated cAMP signaling may activate growth-promoting MAP kinase–signaling pathways is via the activation of one or more guanine nucleotide exchange factors (GEFs), which are known in turn to activate components of the ras-MAP kinase pathway (45). The GEFs are activated by the binding of cAMP, just as the binding of cAMP activates the regulatory subunit of protein kinase A. It is also possible that the effect of GLP-1 on islet neogenesis is partially mediated by the increase in circulating insulin levels that is caused by GLP-1–mediated stimulation of insulin secretion. However, the treatment of AR42J cells derived from a ductal carcinoma with GLP-1 differentiates them into insulin-producing β-cells, indicating that insulin is not required for GLP-1–induced endocrine differentiation in this in vitro cultured cell model (46).

These and the other reported observations of the islet growth-promoting effects of GLP-1 agonists, in addition to its insulin secretogogic actions, provide encouragement for the eventual efficacy of the use of GLP-1 agonists in the treatment of diabetes.

ACKNOWLEDGMENTS

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