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Suppression of β Cell Energy Metabolism and Insulin Release by PGC-1α

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Summary

β cell dysfunction is an important component of type 2 diabetes, but the molecular basis for this defect is poorly understood. The transcriptional coactivator PGC-1α mRNA and protein levels are significantly elevated in islets from multiple animal models of diabetes; adenovirus-mediated expression of PGC-1α to levels similar to those present in diabetic rodents produces a marked inhibition of glucose-stimulated insulin secretion from islets in culture and in live mice. This inhibition coincides with changes in metabolic gene expression associated with impaired β cell function, including the induction of glucose-6-phosphatase and suppression of GLUT2, glucokinase, and glycerol-3-phosphate dehydrogenase. These changes result in blunting of the glucose-induced rise in cellular ATP levels and membrane electrical activity responsible for Ca^{2+} influx and insulin exocytosis. These results strongly suggest that PGC-1α plays a key functional role in the β cell and is involved in the pathogenesis of the diabetic phenotype.

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Introduction

The cardinal triad of metabolic disturbances in type 2 diabetes includes diminished insulin-stimulated glucose uptake by skeletal muscle and fat, increased hepatic glucose production, and impaired insulin secretion by pancreatic β cells (reviewed in DeFronzo, 1997). Human diabetic subjects ultimately develop profound insulin secretory defects in islets, including loss of the early response to intravenous glucose, delayed and blunted responses to mixed nutrient meals, and alterations in the normal pulsatile pattern of insulin secretion (reviewed in Evans and Krentz, 2001; Kahn, 2001; Bell and Polonsky, 2001). While the molecular mechanisms underlying the β cell dysfunction in diabetes remain obscure and are rather difficult to study in humans, animal models have revealed a multitude of changes in gene expression in islets that are associated with progression of disease (Johnson et al., 1990; Tokuyama et al., 1995; Laybutt et al., 2002). It has been convincingly shown that impaired insulin secretion in vivo coincides with major alterations in carbohydrate and lipid metabolism in the β cell.

The principal connection between altered β cell metabolism and abnormal insulin secretion derives from the widely accepted model of insulin release, in which glucose metabolism and the subsequent rise in the cellular ATP/ADP ratio constitutes a major signal that triggers the insulin secretory cascade (reviewed in Newgard and McGarry, 1995; Deeney et al., 2000). The closure of the ATP-sensitive K^+ channel in response to rising ATP levels causes membrane depolarization and subsequent activation of voltage-sensitive Ca^{2+} channels. Ultimately, the influx of Ca^{2+} leads to formation of the core complex of exocytosis proteins and activation of protein kinases, which then mediate exocytosis of insulin.

Islets from diabetic animals display significant defects in glucose oxidation associated with a reduction in the expression of GLUT2, the β cell glucose transporter, and certain enzymes of glucose catabolism, including glucokinase and glycerol-3-phosphate dehydrogenase, and an increase in glucose-6-phosphatase (Johnson et al., 1990; Tokuyama et al., 1995; Laybutt et al., 2002; Newgard and Matschinsky, 2001; Khan et al., 1989, 1990; Chan et al., 1996; Ostenson et al., 1993; Fabregat et al., 1996; MacDonald et al., 1996). The resulting increase in nonproductive glucose cycling and decrease in glucose catabolism are associated with reduced cellular ATP/ADP ratios. In addition, there is an elevation of uncoupling protein-2 (UCP-2) in the β cells of obese mice (Zhang et al., 2001). Genetic data indicates that the increase in the uncoupling of mitochondrial oxidative phosphorylation leads to a fall in insulin secretion (Zhang et al., 2001). Thus, multiple abnormalities in several key steps governing glucose and energy metabolism in the β cell may act in concert to suppress the nutrient-derived intracellular signals in the setting of diabetes.

PGC-1α is a highly regulated transcriptional coactivator of nuclear receptors and other transcription factors that has been linked to the control of energy metabolism in multiple cell types (Puigserver et al., 1998; Wu et al.,

1999; Lehmann, et al., 2000; Yoon et al., 2001; Herzig et al., 2001; Michael et al., 2001; Lin et al., 2002). While our original studies showed that PGC-1α could increase mitochondrial biogenesis and uncoupling in brown fat and muscle (Puigserver et al., 1998; Wu et al., 1999), more recent studies have illustrated a role for this coactivator in muscle fiber-type switching and glucose metabolism in the liver (Yoon et al., 2001; Herzig et al., 2001; Lin et al., 2002). Specifically, PGC-1α is a very potent stimulator of hepatic gluconeogenesis in cultured cells and in livers of intact rats, providing a molecular framework for the transcriptional regulation of this process (Yoon et al., 2001; Herzig et al., 2001). PGC-1α is elevated in the liver in fasting and in diabetes, and increases hepatic glucose output by inducing an array of key gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase, and glucose-6-phosphatase. This linkage with glucose metabolism motivated an examination of a possible role for PGC-1α in the β cell.

We show here that the islet PGC-1α levels are markedly elevated in diabetic animals with defective insulin secretion. Expression of this coactivator at levels approximating those found in islets of diabetic animal levels results in suppression of glucose-induced insulin release from islets in culture and in mice following transplantation. This occurs with an altered expression of metabolic genes in a pattern that bears a remarkable resemblance to that seen in type 2 diabetes. These data strongly suggest that PGC-1α plays an important role in the pathogenesis of β cell dysfunction in type 2 diabetes.

Results

PGC-1α Is Expressed in Pancreatic Islets and Elevated in Animal Models of Type 2 Diabetes

We examined the expression of PGC-1α in isolated pancreatic islets and transformed β cells (INS-1) by real-time reverse transcription-polymerase chain reaction (RT-PCR). Both insulinoma cells and pancreatic islets expressed PGC-1α at 30%–40% of the levels found in liver from ad libitum fed mice (Figure 1A). We also assessed PGC-1α expression in animal models of type 2 diabetes associated with impaired β cell function, such as Zucker diabetic fatty (ZDF) rats and ob/ob mice. At 9 weeks and 3 months respectively, the ZDF rats and ob/ob mice are overtly diabetic, but the β cells are still predominantly in a hyperplastic phase. As shown in previous studies (Tokuyama et al., 1995), islets from the ZDF rat have elevated gene expression for the catalytic subunit of glucose-6-phosphatase (shared by liver and β cells) and reduced glucokinase and GLUT2 (Figure 1B). Interestingly, the islet PGC-1α levels were found to be significantly elevated in ZDF rats and ob/ob mice, both at the mRNA and protein levels (Figures 1B and 1C). The PGC-1α mRNA levels were increased 3- to 4-fold in the obese, diabetic models compared to the control animals, while the protein levels were elevated to an even greater extent, 5-fold in ZDF and 11-fold in ob/ob mice. Another model of β cell decompensation is partial pancreatectomy (approximately 90%) which, following a 7–10 day period of limited recovery, stably reproduces the chronic hyperglycemia and relative β cell insufficiency associated with type 2 and early type 1 diabetes (Laybutt et al., 2002). The β cell dysfunction in this model likely originates from environmental triggers different from those of obese congenital models such as ZDF rats or ob/ob mice, but they share common functional abnormalities in insulin secretion. PGC-1α mRNA levels were elevated 3-fold in islets from 90% pancreatectomized rats compared to the controls at 2 and 4 weeks following surgery (Figure 1D). This elevation also correlated temporally with increases in glucose-6-phosphatase gene expression (Laybutt et al., 2002). Thus, islet PGC-1α expression is consistently induced in several very distinct models of diabetes, both genetic and nongenetic, that are associated with prominent β cell secretory defects.

PGC-1α Negatively Regulates Insulin Secretion in Islets and β Cell Lines

To investigate the effects of PGC-1α on glucose-stimulated insulin secretion in the β cell, this protein was expressed in isolated rat islets using an adenoviral vector. A multiplicity of infection (moi) of 30 was sufficient to achieve a high-efficiency transduction (60%–70%) of intact pancreatic islets, with good penetration into the core, as determined by confocal microscopic detection of the GFP marker gene expression (data not shown). Perifusion experiments with islets infected with the control adenovirus showed that, as expected, insulin release was stimulated 4- to 5-fold by a step increase in the glucose concentration in the perifusion buffer from 3 to 16.7 mM (Figure 2). In a series of three separate experiments, PGC-1α expression caused a decrease in glucose-stimulated insulin secretion ranging from a 40% reduction to a complete abolishment of response, with an average of approximately 60%. Two representative experiments are shown in Figure 2. PGC-1α mRNA levels were elevated approximately 7-fold in these experiments (Figure 3A). The basal secretion in 3 mM glucose was often slightly higher in PGC-1α-expressing islets, but both the early and the delayed phases of insulin secretion were negatively affected following glucose stimulation (Figure 2), reminiscent of the secretory patterns that characterize type 2 diabetes. PGC-1α also caused a dampening of the oscillatory patterns in insulin secretion that normally occurs with a period of roughly 5–10 min, both in isolated islets and in vivo (Tornheim, 1997; O'Rahilly et al., 1998). Importantly, no significant difference was seen in the magnitude of the secretory response when depolarizing concentrations of KCl (40 mM) was used to induce basic exocytosis of insulin-containing secretory granules (Figure 2, Experiment A), indicating that the exocytosis machinery remained intact in PGC-1α-expressing islets. The insulin contents, extracted at the end of the secretion experiment, were only modestly decreased (roughly 15%) in PGC-1α-expressing islets compared to the control (7.2 ± 0.6 versus 6.2 ± 0.5 ng/islet.ml). These results show that PGC-1α can, at levels observed in diabetes, suppress glucose-induced insulin release without affecting the basic secretory apparatus.

Similar results were obtained with the INS-1-derived cell line 832/13, which displays a particularly robust response to glucose stimulation (Hohmeier et al., 2000).

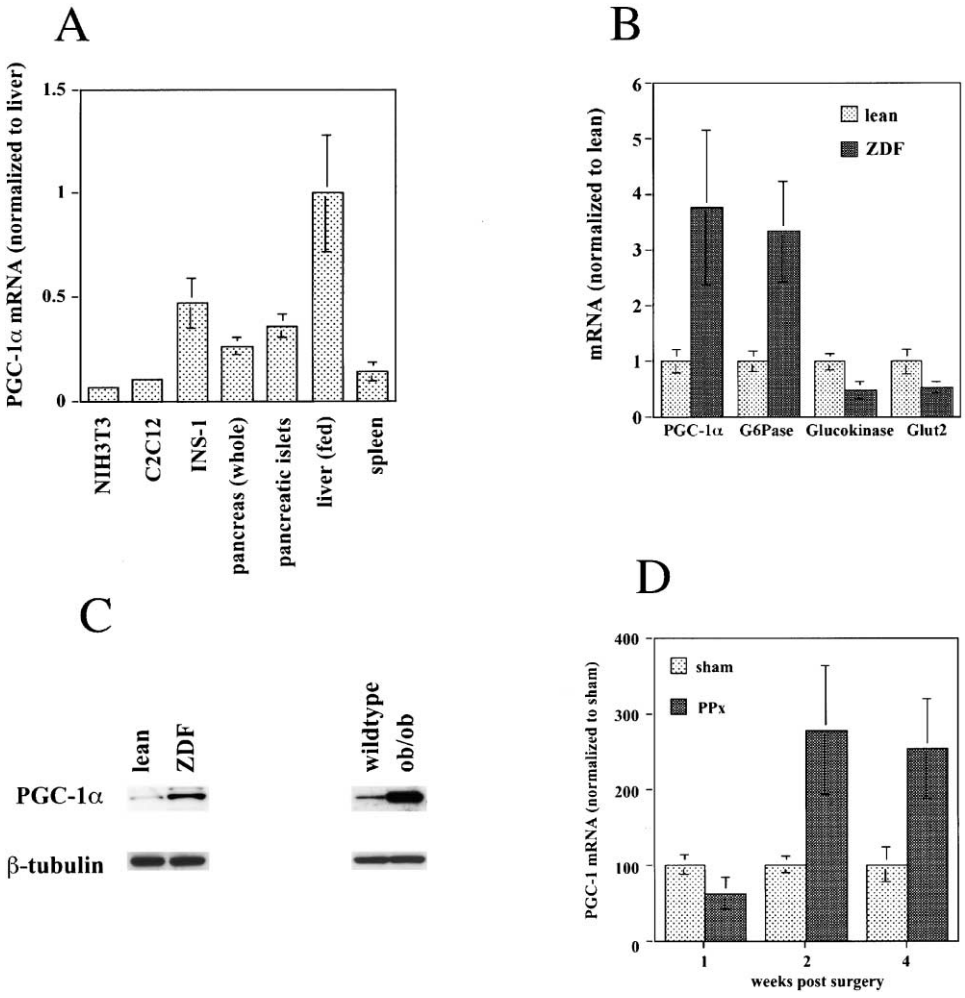

Figure 1. Expression of PGC-1α in Pancreatic Islets

(A) Relative abundance of PGC-1α transcript in selected tissues and cell lines. Total RNA was extracted from pooled male mouse tissues (n = 3–5) or cell lines, and 1 μg per tissue was used for reverse transcription, followed by real-time PCR.

(B) Gene expression changes in islets from diabetic ZDF rats. Total RNA was extracted from pooled islets and subjected to real-time RT-PCR as in (A).

(C) Elevation of PGC-1α in islets from diabetic animals. Protein extracts were made from pooled islets isolated from 9-week-old male rats (ZDF *fa/fa* rats or +/+ lean littermates) or 3-month-old mice (+/+ and ob/ob mice) and analyzed by immunoblotting for PGC-1α. Animals were fed a diet with fat constituting 4.5%–6% of total calories. Both the ZDF rats (serum glucose 300–500 mg/dl) and the ob/ob mice (200–350 mg/dl) were overtly diabetic at the time of sacrifice.

(D) Elevation of PGC-1α mRNA in islets from pancreatectomized rats. Male rats were subjected to 90% partial pancreatectomy (PPx) or sham surgery. Islets were isolated at 1, 2, and 4 weeks after surgery and the mRNA levels determined by RT-PCR as previously described (Laybutt et al., 2002).

PGC-1α expression via adenoviral vectors also caused a clear inhibition of insulin secretion in these cells (data not shown).

To investigate the molecular basis of impaired insulin secretion caused by PGC-1α, we used intact islets to examine the levels of mRNAs encoding several β cell genes involved in nutrient metabolism essential for insulin secretion. PGC-1α expression led to a large increase (7-fold) in the mRNA encoding the catalytic subunit of glucose-6-phosphatase, while reducing glucokinase by approximately 75% and GLUT2 by more than 50% (Figure 3A). In contrast, hexokinase, which has a lower K_M for glucose than glucokinase, was expressed at a slightly higher level. PGC-1α also decreased glycerol-3-phosphate dehydrogenase mRNA by 40%. It is striking that these changes in GLUT2, glucokinase, glycerol-3-phosphate dehydrogenase, and glucose-6-phosphatase transcript levels produced by PGC-1α expression closely mirror those described in the context of β cell dysfunction in the islets of obese, diabetic ZDF rats (Johnson et al., 1990; Tokuyama et al., 1995). UCP-2 mRNA, which has been shown to be elevated in obese, diabetic mice (Zhang et al., 2001), was unchanged by PGC-1α expression.

Expression of PGC-1α in islets also reduced the levels of several islet-enriched transcription factors important for β cell development and function, including HNF-4α, HNF-1α, and PDX-1 (Figure 3B). Mutations in these transcription factors are also associated with the MODY1, 3, and 4 syndromes, respectively, whereas the MODY2

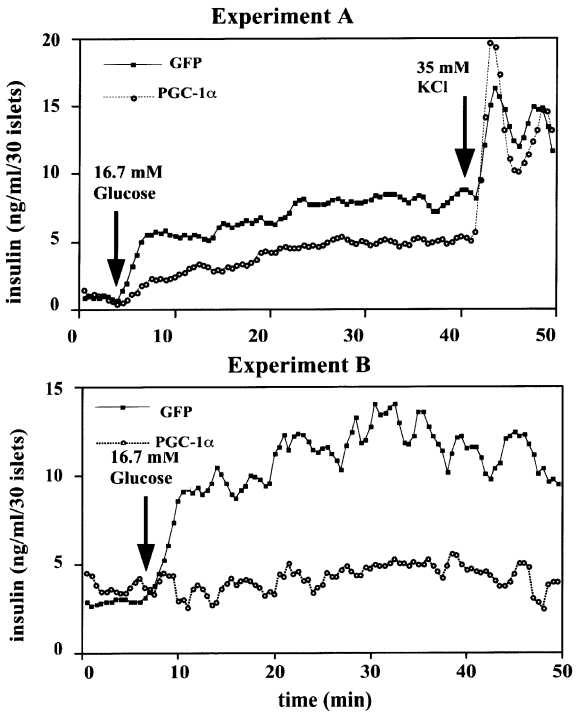

Figure 2. Suppression of Insulin Secretion by PGC-1α Expression in Islets

Isolated rat islets were infected with either GFP- or PGC-1α-expressing adenovirus. Perifusion studies were performed 48–72 hr after infection with a step increase in the glucose concentration in the perifusion buffer from 3 to 16.7 mM at the times indicated by the arrows.

gene encodes the enzyme glucokinase (Shih and Stoffel, 2001). Because these transcription factors themselves may target GLUT2 and a subset of the glycolytic genes, it is conceivable that PGC-1α may act through suppression of these transcription factors. In contrast, the transcription factor Sp1, which is present in all cell types, was not affected by PGC-1α expression.

The altered expression of metabolic genes in PGC-1α-expressing islets may ultimately influence glucose-induced fluctuations in cellular ATP levels, which in turn are coupled to stimulation of insulin release. As expected, ATP levels were significantly increased in control islets when the glucose concentration was stepped up from 3 to 16.7 mM. While islets expressing ectopic PGC-1α had slightly higher basal ATP levels compared to the controls, little further increase was induced by exposure to high glucose concentrations (Figure 3C). These results suggest that the blunting of glucose-stimulated insulin secretion in PGC-1α-expressing islets may be a consequence of diminished ability to drive ATP production in response to glucose stimulation.

PGC-1α Suppresses Membrane Depolarization in the β Cell

Changes in the rate of cellular ATP production can impact upon insulin secretion by controlling β cell membrane depolarization and influx of Ca^{2+}, the ultimate mediator of insulin exocytosis (reviewed in Newgard and McGarry, 1995; Deeney et al., 2000). To critically evaluate the possibility that PGC-1α can modulate this key step in glucose-induced insulin release, we performed patch-clamp recordings of glucose-induced electrical activity in isolated mouse β cells subjected to infection with an adenovirus expressing the control GFP protein (Figure 4A, upper trace) or PGC-1α (lower trace). Resting membrane potential did not differ between cells expressing GFP or PGC-1α during perifusion with 3 mM glucose (Figure 4B). However, when challenged with 16.7 mM glucose, PGC-1α-expressing β cells exhibited significantly less frequent spikes of action potentials than control cells (Figures 4A and 4C). These data strongly suggest that PGC-1α controls β cell insulin secretion, at least in part, by modulating the key metabolic and ionic events leading up to membrane depolarization and insulin exocytosis.

Ectopic Expression of Glucose-6-Phosphatase Mimics the PGC-1α-Mediated Inhibition of Insulin Secretion while Restoration of Glucokinase Partially Ameliorates the PGC-1α Effect

Elevated islet glucose-6-phosphatase activity has been implicated in defective insulin secretion in diabetic and obese hyperglycemic animals (Tokuyama et al., 1995; Khan et al., 1989, 1990; Chan et al., 1996; Ostenson et al., 1993). Because PGC-1α strongly induced glucose-6-phosphatase gene expression, we investigated the possibility that the suppression of insulin secretion observed here may be influenced by activation of this gene and the consequent increase in futile glucose cycling. Isolated rat islets were infected with either control adenovirus or those expressing PGC-1α or glucose-6-phosphatase (Trinh et al., 1997). As expected, expression of PGC-1α at an moi of 30 in islets led to a substantial attenuation of insulin secretion at stimulatory concentrations of glucose, on average reducing the total insulin output by about 60%. Expression of hepatic glucose-6-phosphatase at an moi of 60 resulted in levels of this mRNA comparable to those produced by PGC-1α expression (Figure 5A). Interestingly, this degree of glucose-6-phosphatase expression in islets mimicked the effect of PGC-1α to a significant extent, producing a significant decrease in glucose-stimulated insulin release (Figure 5B). This result is also consistent with previous data obtained by overexpression of glucose-6-phosphatase in insulinoma cell lines (Trinh et al., 1997; Fulceri et al., 2000; Iizuka et al., 2000).

Similar experiments were performed with an adenoviral vector expressing glucokinase, which allowed us to attenuate the drop in the glucokinase mRNA caused by PGC-1α. Glucokinase controls the rate-limiting step of glycolysis, serving as the β cell glucose sensor, and experimental attenuation of glucokinase impairs insulin secretion in isolated islets and in vivo (Efrat et al., 1994; Piston et al., 1999). Ectopic expression of glucokinase with the control GFP protein did not appear to have a major impact on the secretion profile, as reported previously (Becker et al., 1996). However, coexpression of glucokinase with PGC-1α essentially eliminated the

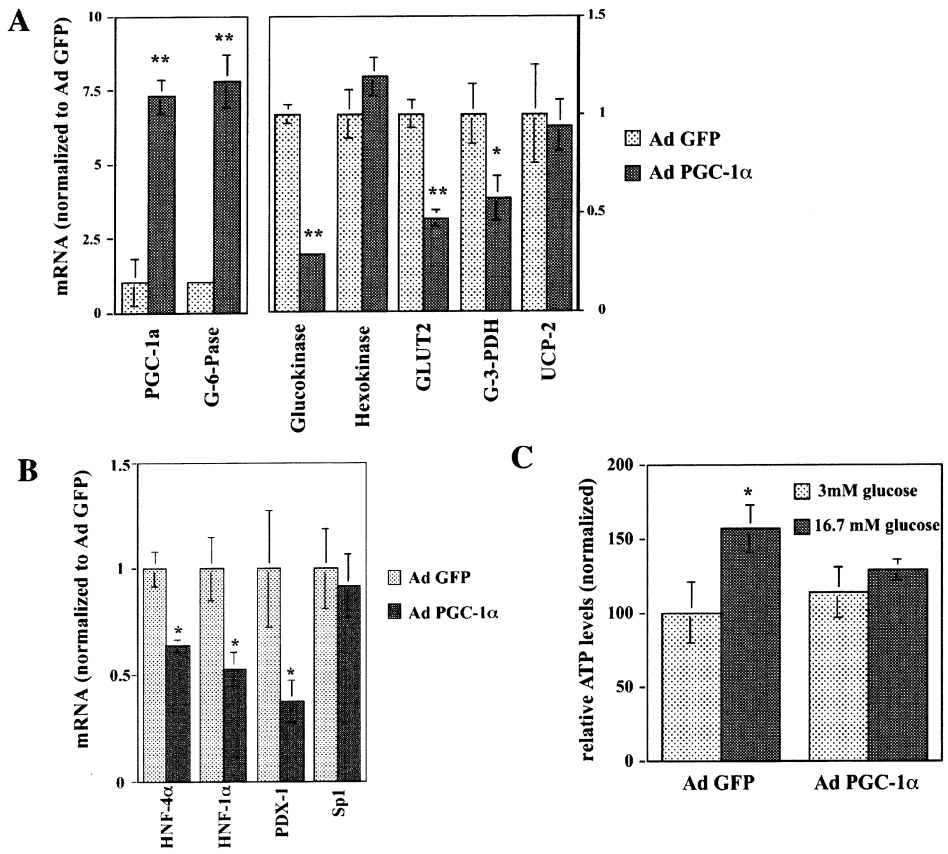

Figure 3. Effect of PGC-1α on the β Cell Metabolic Gene Expression and ATP Production

(A) PGC-1α regulates enzymes of glucose sensing and cycling. Total RNA was prepared from isolated rat islets infected with GFP or PGC-1 α adenovirus and was subjected to real-time RT-PCR. *$p < 0.05$; **$p < 0.01$ versus GFP for each gene.

(B) Effects of PGC-1α expression on islet transcription factors. Total RNA was extracted from islets and was subjected to real-time RT-PCR as in (A). *$p < 0.05$ versus GFP for each gene.

(C) PGC-1α blunts glucose-induced increase in ATP production. Adenovirally infected rat islets were cultured overnight and incubated in 3 or 16.7 mM glucose for 1 hr. Islets were then extracted and the ATP concentrations measured by a luminometric assay. *$p < 0.05$ versus 3 mM glucose.

drop in glucokinase expression that accompanies PGC-1α expression (Figure 5C). This led to a significant improvement in insulin secretion at stimulatory glucose concentrations (Figure 5D), showing that suppression of glucose phosphorylation constitutes an additional mechanism by which PGC-1α regulates the metabolic response in the β cell. These results, taken together, strongly suggest that major perturbations in β cell glucose sensing and metabolism underlie the observed effect of PGC-1α on insulin secretion.

PGC-1α Modulates the Antidiabetic Effects of Islet Transplantion In Vivo

We investigated the role of PGC-1α in β cell function in vivo through transplantation studies. Transplantation of 500–1000 islets under kidney capsules of mice rendered diabetic through injection of the β cell toxin streptozotocin (STZ) has been shown to be sufficient for normalization of glucose levels in these animals (Kaneto et al., 2002). Adenoviral infection of islets prior to transplantation has been shown to result in robust expression of the transgene in grafts for over 4 weeks (Kaneto et al., 2002). Isolated rat islets were infected with viruses containing the cDNA for PGC-1α or GFP, and transplanted under renal capsules of STZ-induced diabetic mice. The islet grafts remained viable and similar in size and appearance at 2 and 4 weeks, but with altered patterns of gene expression (data not shown and below). Weekly measurements of nonfasting blood glucose levels revealed that animals that received transplants of GFP-expressing islets were able to normalize glucose levels within 1 week, while those receiving the PGC-1α-expressing islets remained diabetic (Figure 6A). These changes correlated with greatly reduced insulin levels in mice receiving the PGC-1α-expressing islets (Figure 6B). Intraperitoneal glucose tolerance test administered at the end of 4 weeks demonstrated a persistently impaired response to a glucose load in the PGC-1α group and a normal response in the GFP group (Figures 6C and 6D). There was no significant difference in weight gain between the two groups over the 4 week period (data not shown).

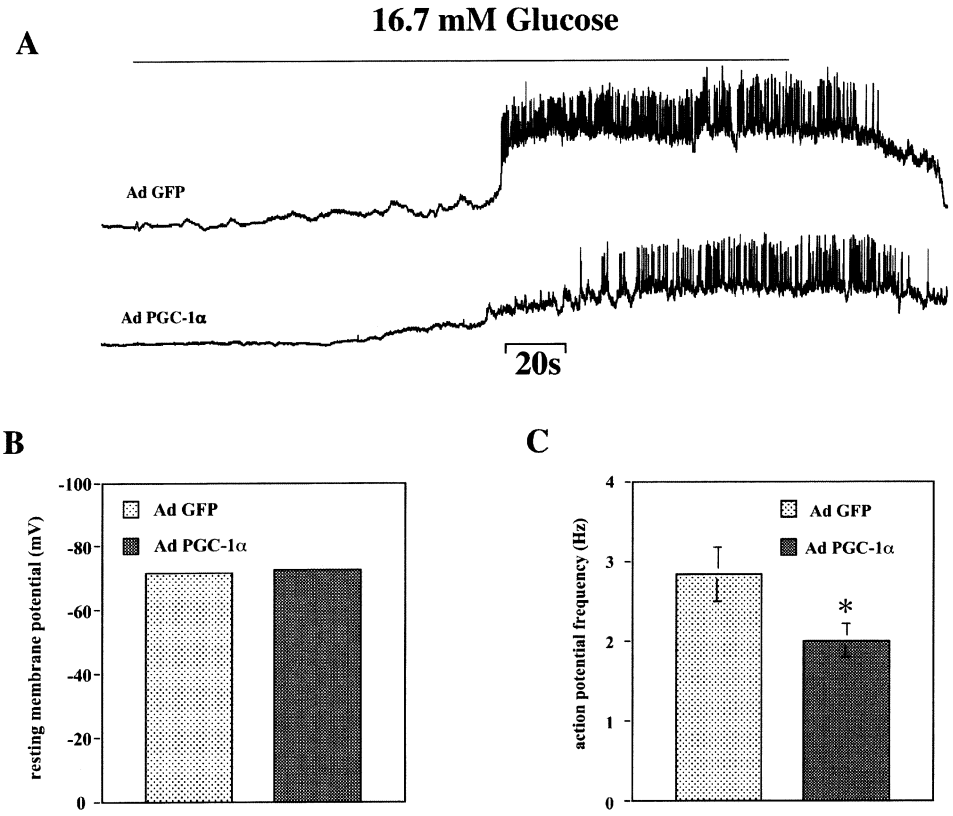

Figure 4. PGC-1α Expression Decreases β Cell Action Potential Frequency

(A) Examples of membrane potential traces recorded from a control cell (Ad GFP infection, upper) and a cell infected with Ad PGC-1α (lower). Action potential in the cell infected with Ad PGC-1α fired at a markedly slower rate than that in the cell subjected to Ad GFP infection.

(B) No significant difference in the resting membrane potential was detected between control cells ($n = 14$) and cells infected with Ad PGC-1α ($n = 14$).

(C) Summary graph shows that cells infected with Ad PGC-1α ($n = 14$) displayed a significantly slower action potential firing rate than control cells ($n = 14$). *$p < 0.05$ versus Ad GFP.

We also analyzed the correlative changes in gene expression at the end of the 4 week experiment by excising the islet grafts from the animals. The grafts taken from the PGC-1α group showed a persistent elevation of PGC-1α and glucose-6-phosphatase mRNA levels, averaging a 3.2- and 3.9-fold elevation at 4 weeks compared to the GFP group (Figure 6E). UCP-2 mRNA, which was not elevated immediately following PGC-1α expression in culture, was increased 2.2-fold at 4 weeks following transplantation. Certain other islet genes, such as hexokinase, did not differ appreciably between the two groups, mirroring the results from cultured islets. These data demonstrate that elevation of PGC-1α levels in the range observed in diabetes can alter islet gene expression and markedly impair insulin secretion in vivo, likely contributing to glucose intolerance and overt diabetes mellitus.

Discussion

Glucose-stimulated insulin release by β cells is a multistep process that begins with glucose entry and metabolism, causing a rise in the cellular ATP/ADP ratio. The closure of the ATP-dependent K^+ channel and subsequent membrane depolarization then leads to Ca^{2+} influx, triggering exocytosis of insulin (reviewed in Newgard and McGarry, 1995; Deeney et al., 2000). Because the secretion of insulin is tightly coupled to the rate of glucose metabolism, defects in the regulation of glucose oxidation in the β cell, such as reduced activity of glycolytic enzymes and increased glucose cycling, have been viewed as key contributory factors in impairment of insulin secretion (Johnson et al., 1990; Tokuyama et al., 1995; Khan et al., 1989, 1990; Chan et al., 1996; Ostenson et al., 1993; Trinh et al., 1997). Genetic evidence for this comes from patients carrying mutations in glucokinase, who exhibit defective insulin secretion in one of the MODY forms of diabetes (Froguel et al., 1992). The notions have been further supported by a mathematical model of β cell glucose metabolism and insulin release (Sweet and Matschinsky, 1995), but a unifying mechanistic basis for the multiple enzymatic changes observed in diabetic islets has not been elucidated.

It is shown here that the transcriptional coactivator PGC-1α, found at elevated levels in the islets of multiple models of diabetes, alters expression of several key genes of β cell nutrient metabolism in a pattern that is remarkably similar to that seen in diabetic animals. PGC-1α profoundly impairs glucose-stimulated insulin

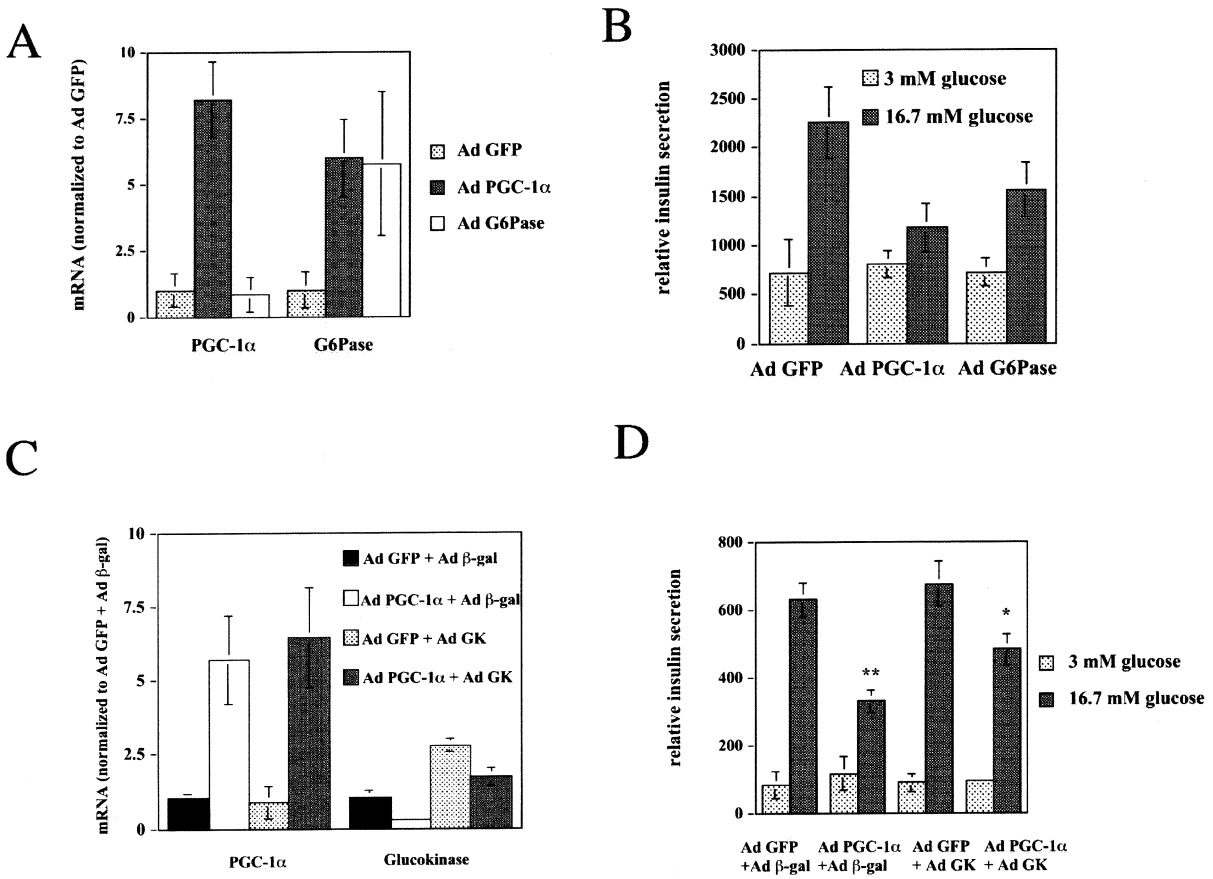

Figure 5. Glucose-6-Phosphatase Expression Mimicks PGC-1α Activity, and Restoration of Glucokinase Partially Ameliorates the Insulin Secretion Defect in PGC-1α-Expressing Islets

(A) Expression of glucose-6-phosphatse gene in islets. Isolated islets were infected with either control, PGC-1α, or glucose-6-phosphatase adenovirus, and total RNA was prepared for analysis of gene expression by real-time RT-PCR.

(B) Measurement of insulin release from glucose-6-phosphatase-expressing islets. Glucose-stimulated insulin release was measured from batches of islets by incubating in basal Krebs buffer (3 mM glucose) and then adding basal or stimulation buffer (16.7 mM). Insulin content of the culture media was measured by ELISA.

(C) Restoration of glucokinase mRNA by adenoviral infection. Isolated rat islets were infected with either GFP or PGC-1α adenovirus, followed by a second infection with either β-gal or GK adenovirus. Total RNA was prepared from islets and analyzed by real-time RT-PCR as in (A).

(D) Measurement of insulin release. Batches of infected islets were incubated in basal Krebs buffer (3 mM glucose) followed by basal or stimulation buffer (16.7 mM), and secreted insulin was measured as in (B). *$p < 0.05$; **$p < 0.01$ versus Ad GFP + Ad β-gal.

secretion, at least in part, as a result of these changes in metabolic enzymes. It is important to note that the effects observed through PGC-1α expression occur at levels that approximate the levels actually observed in diabetic islets. While the calculations of relative PGC-1α levels achieved through use of the adenoviral vectors would be very misleading if only a small number of β cells per islet were infected, the use of a GFP tag in the adenoviral vectors gives confidence that the majority of β cells in target islets were, in fact, successfully transduced. The fact that the expression of certain genes (e.g., glucokinase and PDX-1) was markedly reduced by PGC-1α expression is also not consistent with a severe overexpression occurring in a relatively small number of β cells per islet.

That abnormal regulation of PGC-1α is a shared feature of several distinct models of diabetes suggests its involvement in the common pathophysiological pathway leading to β cell failure. While the primary stimulus that triggers the induction of PGC-1α in diabetes is presently unknown, it could be related to the relative deficiency in insulin signaling that characterizes all of the animal models of diabetes used in these studies. We have previously shown that PGC-1α is negatively regulated (albeit indirectly) by insulin in the liver (Yoon et al., 2001; Puigserver et al., 2003). Altered insulin signaling in β cells leading to impaired glucose-stimulated insulin secretion has been demonstrated with animals lacking the insulin receptor on the surface of β cells through genetic ablation (Kulkarni et al., 1999). In addition to relative insulin deficiency, another possible cause for the elevation of PGC-1α in diabetes is the chronic exposure to elevated levels of circulating free fatty acids that accompanies obesity and diabetes. Alternatively, chronic, mild hyperglycemia or increased levels glucagon may contribute to the elevation of PGC-1α.

Since PGC-1α is a transcriptional coactivator, it is likely to affect β cell function through docking on a

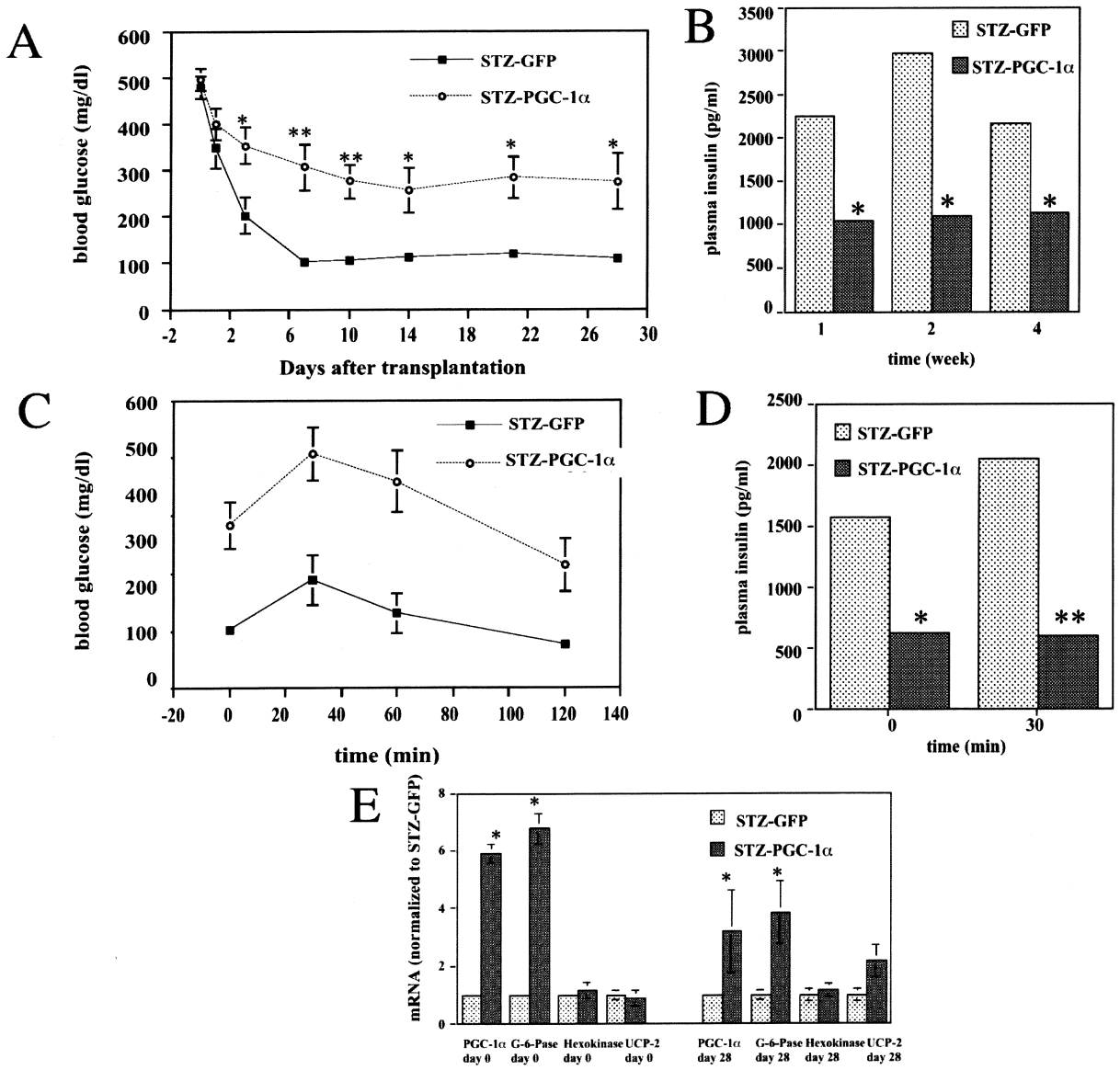

Figure 6. PGC-1α Alters Insulin Secretion from Transplanted Islets

(A) Rat islets were infected with GFP or PGC-1α adenovirus, and 600 islets were transplanted into STZ-induced diabetic mice (n = 7 and 9 respectively). Nonfasting blood glucose levels and body weights were measured weekly. *p < 0.05; **p < 0.01 versus STZ-GFP.

(B) Plasma insulin levels were measured at 1, 2, and 4 weeks posttransplantation by radioimmunoassay. *p < 0.05 versus STZ-GFP.

(C) Glucose tolerance test was performed at 4 weeks after transplantation by intraperitoneal injection of glucose and serial measurement of blood glucose levels over a 2 hr period.

(D) Plasma insulin levels were measured during the glucose tolerance test at 4 weeks. *p < 0.05; **p < 0.01 versus STZ-GFP.

(E) Islet grafts were excised, and gene expression was analyzed by real-time RT-PCR. *p < 0.05 versus STZ-GFP.

set of specific transcription factors bound to relevant promoters. Prominent among these is the forkhead family member FOXO1. This factor has been shown to bind to the glucose-6-phosphatase promoter in liver, and produces an impairment of insulin secretion when elevated in β cells. Most recently, we have shown that PGC-1α binds directly to FOXO1 and increases its activity on the glucose-6-phosphatase promoter in liver cells (P.P. and B.M.S.; Puigserver et al., 2003). Coactivation of FOXO1 in β cells may also lead to suppression of key islet transcription factors such as PDX-1 (Nakae et al., 2002), accounting for some of the observed changes in metabolic gene expression. HNF-4α is also a potentially interesting target for PGC-1α, but here the data is more confusing. HNF-4α has been shown to be a positive modulator of β cell insulin secretion, and mutations in HNF-4α are a cause of β cell dysfunction in one form of MODY diabetes (Yamagata et al., 1996). PGC-1α expression leads to a modest decrease in HNF-4α levels. However, PGC-1α also binds avidly to HNF-4α and greatly increases its transcriptional activity (Yoon et al., 2001); how this could impact on β cell signaling is less

clear. Ultimately, the relative roles of various transcription factors as potential targets for PGC-1α coactivation must be tested in islets, using both gain- and loss-of-function approaches. The physiological role of PGC-1β, a close homolog of PGC-1α, should also be examined.

It is of interest that PGC-1α did not increase expression of mRNA for UCP-2 in cultured islets, especially since this coactivator has been shown to induce UCP-2 mRNA in cultured muscle cells (Wu et al., 1999), and UCP-2 has been shown to be a negative regulator of glucose-stimulated insulin secretion (Zhang et al., 2001). Despite the lack of a direct effect at the gene expression level in cultured islets, it is still possible that PGC-1α may regulate UCP-2 activity, perhaps by controlling the rate of mitochondrial generation of reactive oxygen species, an effector recently shown to control UCP-2 activity (Echtay et al., 2002). Furthermore, the data from islet transplantation studies indicate that UCP-2 mRNA is moderately induced in vivo following chronic elevation of PGC-1α in islets. The role of PGC-1α as a regulator of β cell UCP-2 function requires more study.

The present findings raise the possibility that PGC-1α may serve as a target in the β cell for treatment of diabetes. To this end, identification of the mechanism by which PGC-1α is induced in β cells of diabetic animals will be of particular interest. Also of importance will be determination of the specific transcription factors through which PGC-1α achieves the suppression of insulin secretion. Inhibition of those key docking events may reverse certain aspects of defective β cell function in diabetes.

Experimental Procedures

Real-Time RT-PCR

Total RNA was prepared from cells or from tissues with Trizol (Life Technologies) following the manufacturer's instructions. 1 μg of total RNA was transferred to 100 μl cDNA synthesis reactions containing 1× TaqMan RT buffer (Perkin Elmer Biosystems), 5.5 mM $MgCl_2$, 500 μM dNTPs, 2.5 μM random hexamers, 0.4 U/μl RNase inhibitor, and 1.25 U/μl MultiScribe Reverse Transcriptase (Perkin Elmer Biosystems). The reactions were incubated for 10 min at 25°C, for 30 min at 48°C, followed by 5 min at 95°C. PCR reactions were then carried out in a volume of 50 μl containing 5 μl of cDNA template, 300 nM of each primer, and 1× SYBR Green PCR Master Mix (Perkin Elmer Biosystems). Incubation steps were at 50°C for 2 min and at 95°C for 10 min, followed by 40 cycles of switching between 95°C for 15 s and 60°C for 1 min. The products were detected with the ABI Prism 7700 Sequence Detector (Perkin Elmer Biosystems) and the threshold cycle values (C_T) were determined as a measure of the cycle number at which a statistically significant increase in fluorescence intensity is first detected. The RNA samples were run in duplicates or triplicates for the cDNA synthesis and in the following PCR amplifications. The cDNA synthesis step included a control reaction without the reverse transcriptase, and the PCR step included a control reaction without the template to rule out contamination and/or genomic amplification. In addition, the TATA box binding protein (TBP) gene was used as an endogenous control to account for possible variations due to differences in initial RNA quality and the efficiency of the cDNA synthesis reaction. The abundance of the amplified DNA was then determined from the C_T values and was normalized to the value for the control gene TBP to yield the relative abundance. When comparing samples from two or more experimental groups, the relative abundance values were normalized to the average relative abundance for the control group and the resulting ratios were presented in figures. The oligonucleotide primers were as follows: PGC-1α (forward) 5′-GGCCCGGTACAGTGAGTGTT-3′, (reverse) 5′-GCACTGAGGACTTGCTGAGTT-3′; G6Pase (forward) 5′-GTGTATTCTCCTGCGGTCCG-3′, (reverse) 5′-CGAAGATAGCGAGAGTAGA-3′; glucokinase (forward) 5′-ATGTGCGTCAACACGGAGTG-3′, (reverse) 5′-GGGTTCGCTGAGCTTTCATC-3′; hexokinase (forward) 5′-AATCCTGGTGAAGATGGCCA-3′, (reverse) 5′-TGGCGGACACGTCACTAGTG-3′; GLUT2 (forward) 5′-GCTTTGCAGTAGGCGGAATG-3′, (reverse) 5′-CAACGAGAGGCTGTTTGCAG-3′; G3PDH (forward) 5′-AGGCCGACTGACTCCGTTCT-3′, (reverse) 5′-TTTGAAACGCCATTGCTCA-3′; UCP2 (forward) 5′-GTGTATTCTCCTGCGGTCCG-3′, (reverse) 5′-TGGCTATCATGGCCTGATCC-3′; and TBP (forward) 5′-ACCCTTCACCAATGACTCCTATG-3′, (reverse) 5′-TGACTGCAGCAAATCGCTTGG-3′.

Islet Isolation and Adenoviral Infections

Islets were isolated as previously described (Laybutt et al., 2002) using an intraductal collagenase technique. Islets of similar size (100–150 μm) were picked while viewed under a microscope, divided into two groups, and were infected with adenovirus expressing either GFP or PGC-1. Virus-treated islets were maintained in 10% fetal bovine serum (FBS) (Hyclone)-RPMI (Cellgro) at 37°C with 5% CO_2%/95% room air. Glucose-stimulated insulin secretion assays were performed 48–72 hr after infection, at which time the efficiency of adenoviral transduction was also determined by fluorescence confocal microscopy using the Zeiss LSM microscope (Carl Zeiss). The size of the islets and the number of cells per islet were comparable between the two groups, without evidence of increased apoptosis in one particular group.

Islet Perifusion Studies

Groups of 30 islets were sandwiched between two layers of Cytodex-3 microcarrier beads (Sigma, St. Louis, MO) in a column of 1 cm diameter and 1.3 cm height. They were perifused (Cunningham et al., 1996) with a HEPES-buffered Krebs salt solution containing 119 mM NaCl, 4.6 mM KCl, 1 mM $MgSO_4$, 0.15 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 20 mM HEPES, pH 7.4, 0.05% (w/v) BSA (fraction V; Sigma), and the designated concentrations of glucose. Islets were perifused at a gravity flow rate of 0.3 ml/min for 30 min with a basal concentration of 3 mM glucose prior to collection. Eluted fractions were then collected every 30 s. At a predetermined time point, glucose concentration was increased from 3 to 16.7 mM. Temperature was maintained at 37°C throughout. Samples were assayed for insulin by radioimmunoassay (Linco Research).

Batch Measurement of Glucose-Stimulated Insulin Secretion from Islets

Islets were washed in HEPES-buffered Krebs salt solution with 2% BSA and were pelleted in 15 ml centrifuge tubes (40 islets per tube). After a 15 min incubation in basal Krebs buffer (2% BSA, 3 mM glucose) at 37°C, basal or stimulation buffer was added to each tube, and the islets were incubated for 1 hr at 37°C. Supernatants were removed and assayed for insulin content by ELISA (Crystal Chem) using a rat insulin standard. The islets were extracted in acid ethanol at 4°C and assayed for insulin content by ELISA.

Western Blot Analysis

Islets were lysed in a buffer containing 100 mM Tris (pH 8.5), 250 mM NaCl, 1% NP-40, 1 mM EDTA, protease inhibitors (Boehringer Mannheim), and 0.1% phenylmethylsulfonyl fluoride, and were centrifuged at 14,000 rpm for 10 min to remove particulate matter. Proteins were separated by SDS-PAGE, transferred to immobilin P membrane (Millipore), and were probed with polyclonal antisera against PGC-1α or monoclonal anti-β-tubulin antibody (Boehringer Mannheim).

Islet Extraction and ATP Measurements

Islets were pelleted, extracted in 30–50 μl of 0.1 M NaOH/0.5 mM EDTA, incubated at 60°C for 20 min, and stored at −80°C. The ATP concentrations were determined using a luciferin-based luminometric assay (Ronner et al., 1999).

Electrophysiology

Mouse pancreatic β cells were infected with either Ad PGC-1α or Ad GFP and cultured for 48–72 hr on coverslips. Cells expressing

the GFP marker gene were selected for membrane potential recordings by using the perforated-patch variant of the whole-cell patch-clamp recording technique. Pipettes were pulled from borosilicate glass capillaries (Hilgenberg, Malsfeld, Germany) on a horizontal programmable puller (DMZ Universal Puller; Zeitz-Instrumente, Augsburg, Germany). Typical electrode resistance was 2–4 MΩ. Electrodes were filled with 76 mM K_2SO_4, 1 mM $MgCl_2$, 10 mM KCl, 10 mM NaCl, and 10 mM HEPES (pH 7.35), as well as amphotericin B (0.24 mg/ml) to permeabilize the cell membrane and allow low-resistance electrical access without breaking the patch. The cells were bathed in a solution containing 138 mM NaCl, 5.6 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5 mM HEPES, and 3 mM glucose (pH 7.4) for 1 hr before membrane potential registration. Membrane potential was recorded during perifusion with 3 and 16.7 mM glucose in current-clamp mode with an Axopatch 200 amplifier (Axon Instruments, Foster City, CA). All recordings were made at 34°C.

Islet Transplantation

Male nude Swiss mice were made diabetic by intraperitoneal injection of streptozotocin (STZ) (250 mg/kg) as previously described (Kaneto et al., 2002). Freshly isolated rat islets were infected with adenovirus expressing either GFP or PGC-1α, maintained in culture for 2 days, and 600 islets were transplanted under kidney capsules of the diabetic mice as described (Kaneto et al., 2002). Following transplantation, nonfasting blood glucose levels and body weights were measured weekly. Four weeks after transplantation, glucose tolerance test was performed. Blood samples were collected into capillary tubes and plasma insulin determined by radioimmunoassay (Linco Research). Also 4 weeks after transplantation, islet grafts were excised under methoxyflurane anesthesia and lysed in Trizol (Life Technologies) for real-time RT-PCR or semiquantitative RT-PCR as previously described (Kaneto et al., 2002).

Statistical Analysis

Data represent mean ± SE, and differences between groups were analyzed by Student's t test or one-way analysis of variance. $p < 0.05$ was considered to be statistically significant.

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