Original Article

Peroxisome Proliferator–Activated Receptor-γ Regulates Expression of PDX-1 and NKX6.1 in INS-1 Cells

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In the 60% pancreatotomy (Px) rat model of β-cell adaptation, normoglycemia is maintained by an initial week of β-cell hyperplasia that ceases and is followed by enhanced β-cell function. It is unknown how this complex series of events is regulated. We studied isolated islets and pancreas sections from 14-day post-Px versus sham-operated rats and observed a doubling of β-cell nuclear peroxisome proliferator–activated receptor (PPAR)-γ protein, along with a 2-fold increase in nuclear pancreatic duodenal homeobox (Pdx)-1 protein and a 1.4-fold increase in β-cell nuclear NKx6.1 immunostaining. As PPAR-γ activation is known to both lower proliferation and have prodifferentiation effects in many tissues, we studied PPAR-γ actions in INS-1 cells. A 3-day incubation with the PPAR-γ agonist troglitazone reduced proliferation and increased Pdx-1 and NKx6.1 immunostaining, along with glucokinase and GLUT2. Also, a 75% knockdown of PPAR-γ using RNA interference lowered the mRNA levels of Pdx-1, glucokinase, GLUT2, and proinsulin II by more than half. Our results show a dual effect of PPAR-γ in INS-1 cells: to curtail proliferation and promote maturation, the latter via enhanced expression of Pdx-1 and NKx6.1. Additional studies are needed to determine whether there is a regulatory role for PPAR-γ signaling in the β-cell adaptation following a 60% Px in rats. Diabetes 56:88–95, 2007

The islet β-cell regulates the circulating levels and metabolism of cellular fuels through secretion of insulin. This system is precisely regulated. A reduction in tissue insulin sensitivity (i.e., insulin resistance) is normally counterbalanced by upregulation of insulin secretion and β-cell mass, thus normoglycemia is maintained (1,2). An experimental reduction of β-cell mass also elicits β-cell adaptive responses as shown in rats following a partial pancreaticectomy (Px). Our laboratory has studied nonobese Sprague-Dawley rats following a 60% Px. They maintain normoglycemia through a combination of partial β-cell regeneration and enhanced function of the remaining β-cells; thus, whole-pancreas insulin secretion is similar to sham-operated control rats (3). Investigation of the temporal pattern of the β-cell regeneration demonstrated that during the 1st week post-Px, both duct-derived β-cell neogenesis and islet β-cell hyperplasia occur (4,5). The regeneration wanes in the 2nd week; β-cell mass by 7 weeks post-Px is ~55% of shams versus 40% at the time of surgery (6). By the 4th week post-Px, β-cell function is augmented by a doubling of glucokinase activity (7). The twice-normal glucokinase activity and 55% of normal β-cell mass result in overall β-cell glucose metabolism and glucose-induced insulin secretion, which are identical to shams over the physiologic range of glycemia (3). It is unknown how this complex series of events is controlled and, in particular, how the transition from β-cell regeneration to enhanced function occurs.

Peroxisome proliferator–activated receptor (PPAR)-γ is a member of a subset of nuclear receptors that plays a key role in lipid and glucose homeostasis. Best known is its regulation of adipocyte differentiation and adipocyte-mediated effects on skeletal muscle insulin sensitivity (8–11). In addition, PPAR-γ has antiproliferation properties in many tissues (12–15), including β-cells. The latter was discovered in mice, with a β-cell–specific ablation of PPAR-γ, that were found to have β-cell hyperplasia and increased β-cell mass (16) and also corroborated in vitro with PPAR-γ agonists (16,17). Another reported effect in β-cells is protection against apoptosis (18). It is not known whether there is a physiologic role for PPAR-γ in β-cell mass regulation and the downstream signaling pathways that are activated.

A network of transcription factors regulates pancreatic ontogeny and fetal β-cell development (19,20). Expression for many of these factors is maintained in mature β-cells. Most studied is the pancreatic duodenal homeobox (Pdx-1) that transcriptionally regulates proinsulin, glucokinase, islet amyloid polyypeptide, and GLUT2 and augments β-cell function and survival (21–25). Also of interest is Nkx6.1, a β-cell–specific transcription factor that is important in the terminal differentiation of β-cells (26) and is known to affect glucose-induced insulin secretion (27). Pdx-1 haploinsufficiency impairs β-cell compensation to insulin resistance (28,29). Whether these factors are involved in the β-cell adaptation following a loss of β-cell mass is unknown.

In this study, we examined 60% Px rats at the transition between the mass augmentation and enhanced function phases of the β-cell adaptation, finding increased β-cell expression of PPAR-γ, Pdx-1, and Nkx6.1. This spurred us...
to study PPAR-γ regulatory effects in INS-1 cells. We observed the decrease in proliferation described by others (16,17) but also discovered that PPAR-γ transcriptionally regulates pdx-1 and Nkx6.1. These results show a dual action of PPAR-γ in INS-1 cells to suppress proliferation and promote differentiation. Additional studies are needed to determine whether there is a regulatory role for PPAR-γ signaling in the β-cell adaptation to a 60% Px in rats.

RESEARCH DESIGN AND METHODS

60% Px rats. Male Sprague-Dawley rats (90–120 g) underwent a 60% Px using our previously described protocol (7). Briefly, the portion of the pancreas bordered by the spleen and stomach to the pylorus was removed by abrasion using cotton applicators. Control rats (shams) underwent laparotomy and mobilization of the pancreas. All rats were given a rat diet and tap water ad libitum and were studied 2, 5, or 14 days after surgery. Islets were isolated by pancreas duct infiltration with collagenase, Histopaque gradient separation, and handpicking. Protocols were in accordance with the principles of laboratory animal care and were approved by the Institutional Animal Use and Care Committee of the University of Vermont.

INS-1 cells. INS-1 (382/13) cells (a gift from Christopher Newgard, Duke University) were cultured in RPMI-1640 containing 10% FCS, 10 mmol/l HEPES, 2 mmol/l L-glutamine, 1 mmol/l sodium pyruvate, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 mmol/l HEPES, 150 mmol/l NaCl, 1% Triton X-100, 5 mmol/l EDTA, 5 mmol/l protein assay kit (Pierce). For some experiments, cells were trypsinized and grown in the presence of 10 µmol/l troglitazone (Sigma) or DMSO, with daily media replenishment.

PPAR-γ RNA interference in INS-1 cells. Four pooled SMART selection-designed small interfering RNA (siRNA) duplexes with “UU 3”-overhang and cosp. For some experiments, cells were trypsinized and grown in the presence of 10 µmol/l troglitazone (Sigma) or DMSO, with daily media replenishment.

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RNA extraction and RT-PCR. Total RNA was extracted from islets or INS-1 cells using the RNeasy Mini kit plus single-step on-column DNase digestion (QIAGEN). cDNAs were synthesized using 50 ng extracted RNA with ImProm-II reverse transcriptase (Promega), dNTPs, and random hexamer primers. PCR analyses were carried out in a PTC-200 Peltier Thermal Cycler (MJ Research) using the cDNAs, together with TaqDNA Polymerase (Roche) and the appropriate primers (primers are available from the authors on request). The thermal cycle program used an initial denaturing step at 94°C for 3 min followed by 35 cycles (at 94°C for 15 s, 55°C for 30 s, and 72°C for 1 min) and a final extension step of 7 min at 72°C for the genes examined, except pyruvate carboxylase (20 cycles at 55°C annealing temperature followed by 15 cycles at 55°C) and sterol regulatory element–binding protein-1c and PPAR-α (58°C annealing temperature). α-Tubulin was used as an internal control for all genes, except for PPAR-α, PPAR-γ, and pyruvate carboxylase, where cyclophilin B was used. Gel images were captured using Gel Doc EQ gel documentation system (Bio-Rad) and analyzed using Quantity One software (Bio-Rad), with the amount of each product expressed relative to the control gene.

Insulin secretion. Freshly isolated islets were rested 2 h in RPMI medium containing 5.5 mmol/l glucose. INS-1 cells (3 × 105 cells/well) seeded in 24-well plates were cultured in RPMI until 70% confluence. Triplicate batches of 10 islets or wells of INS-1 cells underwent 1 h preincubation in prewarmed, oxygenated Krebs-Ringer bicarbonate HEPES buffer containing 2.8 mmol/l glucose and 0.5% BSA (wt/vol), followed by 1 h in Krebs-Ringer bicarbonate HEPES buffer containing 2.8, 8.3, or 16.7 mmol/l glucose. The incubation buffer was removed for insulin measurement by rat insulin radioimmunoassay kit (Linco). Insulin protein content of each sample was measured using BCA protein assay kit (Pierce).

Immunoblotting. Freshly isolated islets were extracted in lysis buffer (50 mmol/l HEPES, 150 mmol/l NaCl, 1% Triton X-100, 5 mmol/l EDTA, 5 mmol/l EGTA, 20 mmol/l sodium pyrophosphate, 20 mmol/l sodium fluoride, 1 mmol/l activated sodium orthovanadate, 1 mmol/l phenylmethylsulfonyl fluoride, and 1 µg/ml each leupeptin, aprotinin, and pepstatin A). Some islets were separated by immunoprecipitation and Tandem Mass Tag reagents (Novagen). Inslet proteins (60 µg whole-islet extracts or 50 µg islet fractions) were separated by 10% SDS-PAGE and electrophoretically transferred onto polyvinylidene fluoride membranes (Bio-Rad). Membranes were incubated overnight at 4°C with rabbit anti-PPAR-γ (Affinity BioReagents) or rabbit anti-Pdx-1 (a gift from Chris Wright, Vanderbilt University), washed, and incubated with goat anti-rabbit horseradish peroxidase–conjugated antibody (Jackson ImmunoResearch) for 1 h at room temperature. Detection was by chemiluminescence (Amersham) and autoradiography using HyperFilm-ECL (Amersham). The protocol for slight mobilization of the pancreas and a final extension step of 7 min at 72°C for the genes examined, except pyruvate carboxylase, where cyclophilin B was used. Gel images were captured using Gel Doc EQ gel documentation system (Bio-Rad) and analyzed using Quantity One software (Bio-Rad), with the amount of each product expressed relative to the control gene.

Data presentation. Studies of isolated islets from Px and sham rats used islets from single rats for each data point. All data are expressed as means ± SEM.
RESULTS

60% Px rats. Sprague-Dawley rats were studied 5 and 14 days after 60% Px or sham Px. A few experiments included rats that were 2 days postsurgery. Consistent with our prior findings (3–5), body weight and nonfasting glycemia were similar in Px and sham rats at all time points.

Isolated islets showed no difference in glucose-induced insulin secretion between Px and sham rats at 5 days postsurgery (Fig. 1A) compared with 14 days when it was increased in Px rats (Fig. 1B). An opposite time profile was found for β-cell proliferation (Fig. 1C), as it was threefold increased 5 days postsurgery (P = 0.012) and back to normal at 14 days (P = NS). Our prior study (7) of 60% Px rats 4 weeks postsurgery showed even greater β-cell hyperproliferation than noted herein. These results confirmed that 5 days post-Px was at the peak of the β-cell hyperproliferation, whereas 14 days was during the transition phase to enhanced β-cell function.

PPAR-γ expression in Px β-cells. Because of the proposed importance of lipid signaling in β-cell adaptation (30,31), we examined mRNA expression of a large number of lipid metabolism–related regulatory enzymes and signaling factors by RT-PCR in isolated islets from 5-day Px and sham rats (Table 1). No significant differences were noted except for PPAR-γ; its mRNA level in Px islets was nearly twice that of shams (P < 0.05). Immunoblots of whole-islet extracts revealed a modest (30%) increase in PPAR-γ protein level in 5-day Px versus sham islets and a doubling at 14 days, as opposed to no difference at 2 days (Fig. 2A). Study of nuclear and cytoplasmic proteins of 14-day postsurgery islets confirmed that the increased PPAR-γ in Px islets was localized within the nuclei (180 ± 11% of shams, P < 0.001), as there was virtually no cytoplasmic signal in either group (Fig. 2B).

Enhanced nuclear Pdx-1 and Nkx6.1 in β-cells of Px rats. To gain insight into the β-cell functional adaptation post-Px, we performed immunofluorescence and semi-quantitative image analysis for nuclear expression of the β-cell transcription factors Pdx-1 and Nkx6.1 at 14 days postsurgery (sections were censored for insulin to confirm these transcription factors were β-cell specific). Pdx-1 nuclear staining was increased in Px β-cells (168 ± 14% of shams, P < 0.004) in a uniform pattern with intense staining visible in most or all of the β-cells (Fig. 2C and D). Immunoblots of nuclear and cytoplasmic proteins from 14-day postsurgery islets confirmed the increased level of nuclear Pdx-1 in Px rats (210 ± 9% of shams, P = 0.006) (Fig. 2B). Nkx6.1 staining intensity also was increased (Px β-cells 137 ± 10% of shams, P = 0.015), although with more heterogeneity, as a marked increase was observed in the nuclei of some Px β-cells and no apparent change in others (Fig. 2E and F). Similar studies performed 2 and 5 days postsurgery found no differences in nuclear Nkx6.1 or Pdx-1 staining between Px and sham β-cells (data not shown).

Troglitazone effects in INS cells. Our findings in the Px rats caused us to investigate regulatory effects of PPAR-γ signaling in β-cells by coculturing 832/12 INS-1 cells with the PPAR-γ agonist troglitazone. Troglitazone (10 μmol/l) reduced the proliferation rate and cell number within 2 and 3 days, respectively (Fig. 3A and B). Glucose-induced insulin secretion was unchanged in 3-day troglitazone-treated INS-1 cells (Fig. 3C).

![Figure 1](image1.png)

**FIG. 1.** Glucose-induced insulin secretion in isolated islets from 60% Px (■) and sham-operated (□) rats studied on the 5th (4; n = 8) and 14th (B; n = 4) postsurgical days. C: β-Cell proliferation in 60% Px (■) and sham-operated (□) rats studied on the 2nd, 5th, and 14th postsurgical days (n = 3–5). Pancreas sections were stained for Ki-67 and insulin to mark proliferating β-cells. β-Cell proliferation was subnormal at 2 days post-Px (P < 0.05), peaked at 5 days (P < 0.012), and was back to normal at 14 days. *P < 0.05. NS, not significant.

**TABLE 1**

Comparative RT-PCR analysis of mRNA expression in isolated islets of sham-operated and 60% Px rats 5 days postsurgery

| Gene                     | Shams (%) | Px (%) |
|--------------------------|-----------|--------|
| Carnitine                | 100 ± 9   | 88 ± 3 |
| Fatty-acyl synthase      | 100 ± 6   | 94 ± 8 |
| Lactate dehydrogenase-A  | 100 ± 6   | 119 ± 3|
| Malonyl-CoA dehydrogenase| 100 ± 4   | 87 ± 2 |
| PPAR-α                   | 100 ± 25  | 111 ± 25|
| PPAR-γ                   | 100 ± 18  | 181 ± 25|
| Pyruvate carboxylase     | 100 ± 8   | 78 ± 3 |
| SOCS3                    | 100 ± 13  | 95 ± 25|
| SREBP-1c                 | 100 ± 8   | 87 ± 9 |
| UCP-2                    | 100 ± 3   | 100 ± 3|

Data are means ± SE. Values were calculated from individual samples from eight sham and eight Px rats, except for sterol regulatory element–binding protein-1c (SREBP-1c) and PPAR-α, where four animals were used for each group. Quantitative mRNA levels (after normalization of the indicated gene to α-tubulin or cyclophilin B) were obtained by densitometry and are expressed as percent of sham results. Statistical significance was found for PPAR-γ (P < 0.05). SOCS3, suppressor of cytokine signaling 3.
Immunofluorescence studies performed in 3-day troglitazone-treated and vehicle-treated INS-1 cells showed no effect of troglitazone on the intensity of PPAR-\(\gamma\)/H9253 staining (data not shown). In contrast, there was a threefold increase in nuclear Pdx-1 (\(P < 0.001\); Fig. 4A) and a doubling of nuclear Nkx6.1 immunostaining (\(P < 0.015\); Fig. 4B). Glucokinase and GLUT2 were investigated because of their known transcriptional regulation in β-cells by Pdx-1 (21,22). Both were increased in troglitazone-treated cells by 1.7- and 1.5-fold, respectively (glucokinase, \(P < 0.001\); GLUT2, \(P < 0.015\)) (Fig. 4C and D).

**RNA interference-mediated suppression of PPAR-\(\gamma\) in INS cells.** We further investigated PPAR-\(\gamma\) regulation of Pdx-1 and Nkx6.1 expression in INS-1 cells using RNA interference (RNAi) for PPAR-\(\gamma\). RNAi was administered at 0 and 48 h, followed by troglitazone or vehicle for 3 days (72–144 h). PPAR-\(\gamma\) mRNA was lowered to 29 ± 1% of control at 72 h (\(P < 0.01\); Fig. 5A) and 21 ± 2% of control treated cells by 1.7- and 1.5-fold, respectively (glucokinase, \(P < 0.001\); GLUT2, \(P < 0.015\)) (Fig. 4C and D).

**FIG. 2.** Immunoblots of isolated islets and immunofluorescence confocal microscopy images of representative pancreas section from 60% Px and sham-operated rats. A: PPAR-\(\gamma\) immunoblot of whole-islet extracts obtained on the 2nd, 5th, and 14th postsurgical days. Each lane was loaded with 60 μg islet protein from a single rat (samples are from two time-matched Px and two sham rats). B: Immunoblots of fractionated cytoplasm (C) and nuclear protein (N) from islets obtained on the 14th postsurgical day, probed for PPAR-\(\gamma\) (top), Pdx-1 (middle), and transcription factor IID (bottom to establish equivalent loading). Each lane contains 50 μg protein. Blots are representative images from three separate experiments. C and D: Representative islets exhibiting β-cells from 14-day postsurgery sham and Px rats stained for Pdx-1. E and F: Representative islets stained for Nkx6.1. A higher level of nuclear Nkx6.1 signal is present in some, but not all, β-cells of the Px rat. Imaging parameters for each factor were identical between the animal groups. Brightly autofluorescent erythrocytes mark the vascular beds in E and F.

**FIG. 3.** Effect of the PPAR-\(\gamma\) agonist troglitazone on INS-1 cell proliferation and glucose-induced insulin secretion. INS-1 cells were treated with 10 μmol/l troglitazone (■) or DMSO (□) for the number of days indicated. A: Proliferation was measured on fixed cells by immunocytochemistry using the proliferation marker Ki-67 as described in the RESEARCH DESIGN AND METHODS. B: Number of INS-1 cells expressed as the fold increase in cell number compared with the count at day 0. Results in A and B are means ± SE of three separate experiments. C: Glucose-induced insulin secretion (\(n = 4\)) in INS-1 cells incubated for 3 days with the PPAR-\(\gamma\) agonist troglitazone (■) or DMSO (□). *P < 0.05 for the time-matched troglitazone vs. DMSO-exposed cells.
at 120 h ($P < 0.015$). PPAR-γ protein lagged somewhat, being $47 \pm 2\%$ of control at 96 h ($P = 0.002$; Fig. 5B) and $24 \pm 3\%$ of control at 120 h ($P < 0.001$).

In vehicle-treated cells, RNAi knockdown of PPAR-γ (Fig. 6A and D) lowered Pdx-1 mRNA level to $23 \pm 2\%$ of control ($P < 0.001$) and protein level to $36 \pm 1\%$ of control ($P = 0.004$; data not shown). Nkx6.1 mRNA level was lowered to $36 \pm 2\%$ of control ($P < 0.001$), although its protein level did not significantly change (data not shown), implying a turnover time that exceeded the experiment's duration. Consistent with the troglitazone results and the known pattern of transcriptional regulation by Pdx-1 in β-cells (21,22), glucokinase (42 ± 1\% of control, $P < 0.001$), GLUT2 (45 ± 4\% of control, $P = 0.005$), and proinsulin II (53 ± 3\% of control, $P = 0.005$) mRNA levels were decreased, whereas uncoupling protein (UCP)-2, proinsulin I, and PPAR-α were unchanged ($P = NS$).

Additional evidence for PPAR-γ regulation of Pdx-1 and Nkx6.1 gene expression was found by comparing troglitazone- and vehicle-treated INS cells. The mRNA levels of Pdx-1 (1.7-fold) and Nkx6.1 (1.9-fold) were both increased by troglitazone. PPAR-γ RNAi lowered this augmenting effect but did not eliminate it, which could reflect the residual PPAR-γ protein or a non–PPAR-γ action of troglitazone.

DISCUSSION

PPAR-γ is expressed in islet β-cells (32,33). It is important to determine its actions because of the use of PPAR-γ agonists, the thiazolidinedione class of pharmaceuticals, in human diabetes. Also, there are reports of preserved β-cell function (and consequently prevention of diabetes) using these agents in Hispanic women with prior gestational diabetes who were at high risk for permanent type 2 diabetes (34,35), as well as in individuals with impaired glucose tolerance (36). The best-documented effect of PPAR-γ in β-cells is to curtail proliferation (16,17). A prosurvival effect also has been reported based on an in vitro study of human islets with the PPAR-γ agonist rosiglitazone (18). There are many studies of diabetic rodents treated with thiazolidinediones showing prevention of β-cell apoptosis (37), although it is not known whether that benefit was direct or secondary to lowered glucose tolerance.
glycemia from enhanced tissue insulin sensitivity. Finally, variable effects of PPAR-γ agonists have been reported for insulin secretion, as subsequently detailed. Thus, the physiologic and pharmacologic effects of β-cell PPAR-γ signaling remain largely unknown from these diverse findings.

The current study began by studying 60% Px rats at the switchover period (2 weeks postsurgery), between the regeneration and hyperfunction stages post-Px, finding enhanced nuclear expression of PPAR-γ, Pdx-1, and Nkx6.1. This grouping is of considerable interest, as the known independent effects—antiproliferation and prosurvival for PPAR-γ (16–18) and prodifferentiation for Pdx-1 and Nkx6.1 (21–25,27)—are sufficient to explain how the transition might occur. However, this study provides no evidence as to whether any of these factors have a regulatory role in the post-Px β-cell adaptation; clarification of this issue will require comparable studies in mice with genetically modified β-cell expression of these various factors. Instead, the important aspect of these findings is that they caused us to investigate PPAR-γ activation in INS-1 cells, resulting in the novel observation that PPAR-γ transcriptionally regulates pdx-1 and Nkx6.1. The evidence is the troglitazone treatment and siRNA results in the current study; in addition, we recently identified a PPAR-γ response element in the 5′ regulatory element of pdx-1 by showing PPAR-γ binding and transcriptional regulation using electrophoretic mobility shift assay, mutational studies, and chromatin immunoprecipitation (38).

Thus, our investigation of PPAR-γ signaling in INS-1 cells not only confirmed the lowered proliferation reported by others (16,17), but also discovered a prodifferentiation effect in terms of augmented mRNA expression of Pdx-1, Nkx6.1, proinsulin, glucokinase, and GLUT2. Our results do not distinguish whether the last two are Pdx-1 mediated (21,22), a result of direct regulation by PPAR-γ (39,40), or both. Regardless, combined antiproliferation and prodifferentiation properties are a well-known feature of PPAR-γ action in many tissues (12–15), with PPAR-γ agonists being investigated for use as antineoplastic therapy (41,42). β-Cells (at least in vitro) can now be added to that list.

An observation that seems at odds with a role for PPAR-γ activation in the post-Px β-cell adaptation was the increased PPAR-γ mRNA and slightly enhanced protein level in 5-day Px islets, when β-cell proliferation was increased threefold. One possible explanation is the time lag between PPAR-γ activation and inhibition of β-cell proliferation (2 days) that was noted in our study of INS-1 cells and troglitazone. Also, the magnitude of the PPAR-γ hyperexpression at 5 days was much less than at 15 days post-Px, when the hyperproliferation had ceased, and the fold increase required to initiate a biologic effect is unknown.

An unresolved issue is how PPAR-γ signaling affects β-cell function. Antiproliferation (16,17) and prosurvival effects (18) are well established. One would also expect an increase in glucose-induced insulin secretion from the observed gene expression increases for glucokinase, proinsulin, Pdx-1, and Nkx6.1 in INS-1 cells. In support of this are the findings in isolated islets from mice with a β-cell–specific knockout of PPAR-γ that observed the expected enhancing effect of PPAR-γ stimulation on glucose-induced insulin secretion, which was lost in these mice (16).

In contrast, many studies have assessed isolated islets or clonal β-cells after hours to several days of exposure to PPAR-γ agonists (typically thiazolidinediones), often with PPAR-γ overexpression. A wide variety of effects are reported, including a reduction (17,43–47), no change (16,48,49), and increases in glucose-induced insulin secretion (50,51), which may reflect the imperfect specificity for PPAR-γ of these agents. This may also account for our failure to see an effect of 3 days’ exposure to troglitazone on glucose-induced insulin secretion in INS-1 cells. Moreover, we were unable to assess the PPAR-γ siRNA system, as preliminary studies showed a reduction in glucose responsiveness of mock-transfected INS-1 cells. Thus, the most direct evidence supports an augmenting effect of activated PPAR-γ signaling in β-cells on glucose-induced insulin secretion despite our having failed to confirm this using our in vitro systems.

Of note, overexpression studies for PPAR-γ in β-cells have reported an opposite conclusion, namely inhibition of glucose-induced insulin secretion, often with other findings that disagree with the results of the current study (45–47). Parton et al. (45) reported lowering of glucose-induced insulin secretion in isolated islets with a 10-fold increase in PPAR-γ protein from adenoviral overexpression, along with a 30% reduction in Pdx-1 mRNA level, which is at variance with the PPAR-γ–induced increase in Pdx-1 transcription in our study. Their effect was magnified slightly when the PPAR-γ overexpression was combined with a 24-h incubation with a PPAR-γ agonist, but, importantly, there was no reduction in Pdx-1 mRNA when the PPAR-γ agonist was used alone. Ito et al. (46) reported lowered glucose-induced insulin secretion in rat islets with adenoviral PPAR-γ overexpression that they ascribed to induction of UCP-2 expression. In contrast, we observed no change in UCP-2 mRNA level with PPAR-γ RNAi. These differences highlight that overexpression studies allow one to test how cells react to superphysiologic conditions but must be viewed with caution in terms of giving insight into normal regulation and function.

In summary, we have shown a dual action for PPAR-γ in INS-1 cells to curtail proliferation as noted by others and a newly described regulation of Pdx-1 and Nkx6.1 transcription and consequently that of GLUT2, glucokinase, and proinsulin. This combination of antiproliferation and prodifferentiation effects, while being described for the first time in β-cells, is a well-known feature of PPAR-γ action in many other cell types. Our investigation of PPAR-γ action in INS-1 began because of our study of 60% Px rats at the transition from the early on β-cell hyperplasia to the subsequent β-cell functional maturation. At that same time, β-cell nuclear expression of PPAR-γ, Pdx-1, and Nkx6.1 are all increased. Additional studies are needed to determine whether those findings are coincidental or play a regulatory role in the post-Px β-cell adaptation.

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