Peroxisomal Proliferator–Activated Receptor-γ Upregulates Glucokinase Gene Expression in β-Cells

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Insulin is the most important molecule among the regulators in glucose homeostasis. Glucose is the primary physiological stimulus for the regulation of insulin secretion in β-cells, the process that requires glucose sensing. The glucose-sensing apparatus of β-cells consists of glucose transporter isotype 2 (GLUT2) and glucokinase (GK), which play a critical role in glucose-stimulated insulin secretion (GSIS) (1). β-Cell–specific knockout of GLUT2 or GK results in infantile death because of severe hyperglycemia (2,3). Adenovirus-mediated expression of GLUT2 and GK in IL cells results in gaining of glucose sensitivity (4). Thus, GLUT2 and GK are important in glucose sensing of β-cells. However, GLUT2, being a low-affinity, high-capacity glucose transporter, is believed to play a more permissive role in glucose sensing, allowing rapid equilibration of glucose across the plasma membrane. GK traps glucose in β-cells by phosphorylation (5) and is the flux-controlling enzyme for glycolysis in β-cells (4). Thus, it serves as the gatekeeper for metabolic signaling, suggesting that GK rather than GLUT2 is directly responsible for the insulin secretion in response to increasing blood glucose levels (5).

Thiazolidinediones (TZDs) are a new class of antidiabetic agents that act by improving insulin sensitivity in various animal models of obesity and diabetes (6–9). The biological effects of TZDs are exerted by binding to and activating peroxisomal proliferator–activated receptor-γ (PPAR-γ). There is a strong correlation between TZD–PPAR-γ interaction and the antidiabetic action of TZDs; the relative potency of TZDs for binding to PPAR-γ and activation of PPAR-γ in vitro correlates perfectly with their antidiabetic potency in vivo (10). Patients with a dominant-negative mutation in the PPAR-γ gene show severe hyperglycemia, which provides a genetic link between PPAR-γ and type 2 diabetes (11). TZDs stimulate adipocyte differentiation, preferentially generating smaller adipocytes that are more sensitive to insulin and produce lower levels of free fatty acids, tumor necrosis factor-α, and leptin (10,12). TZDs are also known to restore the functions of β-cells, reduce intracellular fat deposition, and relieve β-cells from a lipotoxic environment (13,14). There are reports that TZDs improve glucose-sensing ability in isolated islets of diabetic ZDF rats and increase GLUT2 expression in β-cells (8,13,15). These data suggest that the expression of genes involved in glucose sensing of pancreatic β-cells may be modulated by PPAR-γ. However, the molecular targets of TZDs involved in their action on the physiological regulation of insulin secretion are yet to be identified (16).

We previously reported the presence of peroxisomal proliferator response element (PPRE) in the rat GLUT2 promoter and suggested its possible significance to explain the role of PPAR-γ in restoring GSIS (15). However, the general belief that GK may be more important than GLUT2 in the glucose sensing of β-cells led us to explore the presence of PPRE in the β-cell–specific GK (βGK) gene. In this report, we identify a PPRE in the βGK gene because of severe hyperglycemia (2,3). Adenovirus-mediated expression of GLUT2 and GK in IL cells results in gaining of glucose sensitivity (4). Thus, GLUT2 and GK are important in glucose sensing of β-cells. However, GLUT2, being a low-affinity, high-capacity glucose transporter, is believed to play a more permissive role in glucose sensing, allowing rapid equilibration of glucose across the plasma membrane. GK traps glucose in β-cells by phosphorylation (5) and is the flux-controlling enzyme for glycolysis in β-cells (4). Thus, it serves as the gatekeeper for metabolic signaling, suggesting that GK rather than GLUT2 is directly responsible for the insulin secretion in response to increasing blood glucose levels (5).

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and show that this element is responsible for the upregulation of GK expression by PPAR-γ in β-cells.

**RESEARCH DESIGN AND METHODS**

**Materials.** Troglitazone was a gift from Sankyo (Tokyo, Japan). WY14643 was a gift from Sankyo (Tokyo, Japan). RGKP-9-cis retinoic acid (2 mmol/l) was purchased from Cayman Chemical (Ann Arbor, MI), and Troglitazone was a gift from Sankyo (Tokyo, Japan). WY14643 was induced for 4 h with 1 mmol/l isopropyl-β-D-thiogalactopyranoside. Poly(dI-dC) (1 µg) was added to each reaction to suppress nonspecific binding. Anti–PPAR-γ serum (2 µg) was added to the reaction for supershift assay. The protein-DNA complexes were resolved from the free probe by electrophoresis at 4°C on a 5% polyacrylamide gel in 0.5× TBE buffer (1× TBE contains 9 mmol/l Tris, 90 mmol/l boric acid, and 20 mmol/l EDTA, pH 8.0). The dried gels were exposed to X-ray film at −70°C with an intensifying screen.

The oligonucleotides used in EMSA were as follows: RGP-43/75, 5′-AGATTGCTATTCTGCTTCTACTAACAGAAGCCG-3′; RGP-43/75m1, 5′-AGAGcccgggtGGTCCAATCTAAAGAAGCCG-3′; RGP-43/75m2, 5′-AGATTGACTGcGatCAATCTAACAGAAGCCG-3′; RGP-43/75m3, 5′-AGAGcccgggtGGTCCAATCTAAAGAAGCCG-3′; and RGP-43/75m, 5′-AGAGcccgggtGGTCCAATCTAAAGAAGCCG-3′. The PHY sequence is underlined, and mutated base pairs are shown in lowercase letters (21).

**RNA preparation, RNase protection assay, and RT-PCR.** Total RNA was isolated from β-cell lines treated with 20 µmol/l troglitazone and 1 µmol/l 9-cis retinoic acid for 24 h using TRIzol reagent by the manufacturer’s protocol (Life Technologies). Production of probes and RNase protection assay (RPA) were performed using Strip-EZ RNA probe synthesis kit and RPA III kit (Ambion, Austin, TX) according to the manufacturer’s protocol. Briefly, antisense RNA probes, which covered the +370/+686 bp region of rat βGK and the +224/+440 bp region of rat β-actin, were produced from pCRGKPS6 and pCRactin using [32P]UTP and T7 RNA polymerase, then template DNA and free nucleotides were removed by DNase I digestion and two successive ethanol precipitations. Total RNA (50 µg) and 300,000 cpm of probes were hybridized at 42°C for 24 h, then unhybridized RNA was digested by RNase A/RNase T1 mix. The remaining samples after RNase digestion were precipitated and resuspended in 10 µl gel loading buffer. Samples were incubated for 5 min at 94°C and subjected to electrophoresis on 5% denaturing polyacrylamide gel. The dried gels were exposed to X-ray film at −70°C with an intensifying screen.

For RT-PCR, first-strand cDNA was synthesized from 2 µg of total RNA in 20 µl volume using random hexamer and Superscript II reverse transcriptase (Life Technologies). Reverse transcriptase reaction mixture (1 µl) was amplified with primers specific for rat GK and β-actin in a total volume of 50 µl. Linearity of the PCR was tested by amplifying 10 ng of total RNA between amplification cycles 20 and 50. According to this amplification profile, samples were amplified for 30 cycles using the following parameters: 92°C for 30 s, 55°C for 30 s, and 72°C for 30 s. β-Actin was used as an internal control for quality and quantity of RNA. The PCR products were subjected to electrophoresis on 1.4% agarose gel, and the quantities of PCR products were analyzed by Molecular Analyst II (Bio-Rad, Hercules, CA). The PCR product was confirmed by DNA sequencing. Primers used in PCR were as follows: GK sense, 5′-GGATCCGGTACTTCTGTCACCT-3′; and β-actin sense, 5′-TGGATTTTCACTCCCAAGGAC-3′; and β-actin antisense, 5′-GACGAGGCGATCAGGGGACG-3′.

**RESULTS**

**PPAR-γ activates the βGK promoter.** GK is expressed in a cell type–specific manner by alternate promoter usage (24). To determine whether PPAR-γ can regulate GK gene expression, we cloned rat liver–specific (LGK) promoter and βGK promoter into luciferase reporter vector and tested the responsiveness to PPAR-α and PPAR-γ, respectively, in CV-1 cells. As shown in Fig. 1, PPAR-α did not activate either promoter, whereas PPAR-γ activated both. Moreover, the activation of the βGK promoter by
TRANSCRIPTIONAL ACTIVATION OF β-CELL–SPECIFIC GK BY PPAR-γ

PPAR-γ was >12-fold and that of the LGK promoter 2.8-fold, suggesting the importance of PPAR-γ in the regulation of the βGK gene. These results led us to search for the presence of PPRE in the promoter region of the βGK gene.

To identify a functional PPRE in the βGK promoter, we prepared 5‘ serial deletion constructs and tested their responsiveness to PPAR-γ in CV-1 cells. As shown in Fig. 2, pRGP-1003, pRGP-404, and pRGP+10 were activated by coexpression of PPAR-γ and RXR-α in the presence of troglitazone and 9-cis retinoic acid. However, deletion down to +100 bp (pRGP+100) resulted in loss of ligand-dependent activation. Thus, the 5‘ serial deletion study suggested that PPAR-γ, heterodimerized with RXR-α, activated the βGK promoter in a ligand-dependent manner, and the activation required the sequences between +10 and +100 bp of the βGK promoter. To localize precisely the region responsible for the transactivation by PPAR-γ, we prepared several truncated promoter-luciferase constructs lacking +10/+100 (pRGPd+10/100), +49/+73 (pRGPd+49/73), and +73/+100 (pRGPd+73/100) (Fig. 3A). The constructs pRGPd+10/100 and pRGPd+49/73 lost their responsiveness to PPAR-γ, whereas pRGPd+73/100 retained PPAR-γ responsiveness. Thus, we could narrow down the location of PPRE to the region including +49/+73 bp. In addition, the region between +34 and +80 bp was highly conserved between species, matching with the −383/−337-bp region of mice (25) and the +67/+113-bp region of humans (26) (Fig. 3B). This evolutionary conservation suggests the importance of the region in regulation of βGK gene expression.

PPAR-γ/RXR-α heterodimer binds to and activates the βGK-PPRE. Although the region containing +49/+73 bp was responsible for the transactivation of the βGK promoter by PPAR-γ, there was no conventional PPRE known as DR+1, a hexameric consensus sequence (AGGTCA) in a direct repeat spaced by one nucleotide (21). Thus, to characterize the composition of the element recognized by PPAR-γ, we constructed several mutant forms of the promoter and examined PPAR-γ responsiveness in CV-1 cells (Fig. 4A). Initially, we tested three scanning mutants of the promoter having six basepair substitutions in the region between +47 and +66 bp, named pRGP-1003m1, pRGP-1003m2, and pRGP-1003m3. These mutants were not activated by PPAR-γ/RXR-α heterodimer, indicating that the +47/+66-bp region constitutes at least part of the PPRE. Then we constructed four more mutants to map the PPRE and named them pRGP-1003m4, pRGP-1003m5, pRGP-1003m6, and pRGP-1003mT. The pRGP-1003m5 and pRGP-1003m6 constructs lost their responsiveness to PPAR-γ/RXR-α heterodimer, but pRGP-1003m4 and pRGP-1003mT retained PPAR-γ responsiveness. From these results, the βGK-PPRE could be localized at the region between +47 and +68 bp, even though there was little sequence similarity with conventional DR+1.

To confirm that the βGK-PPRE mediates PPAR-γ-dependent transactivation of the βGK promoter through DNA binding of PPAR-γ, we performed EMSA with in vitro translated PPAR-γ and RXR-α using the oligonucleotide covering +43/+75 bp of the βGK gene. As shown in Fig. 4B, PPAR-γ or RXR-α alone did not bind to the probe (lanes 1 and 2). However, incubation of the probe with

![Graph](image.png)

**FIG. 1.** Comparison of PPAR responsiveness between LGK and BGK promoters. Luciferase reporter under the control of rat βGK (pRGP-1003) or LGK (pRGL-1448) promoter was cotransfected into CV-1 cells with expression vectors of PPARs and RXR-α. Appropriate ligands for receptors were treated after transfection: 10 μmol/l WY-14643 for PPAR-α, 20 μmol/l troglitazone (TGZ) for PPAR-γ, and 1 μmol/l 9-cis retinoic acid (9-cis RA) for RXR-α. Normalized luciferase activities are shown as means ± SD of three independent experiments in triplicate and are expressed as the fold increase relative to basal activity of pRGP-1003 and pRGL-1448 in the absence of expression vectors and ligands.
PPAR-γ and RXR-α formed DNA-protein complexes (lanes 3 and 5), suggesting that PPAR-γ heterodimerized with RXR-α binds to the +43/+75-bp region of βGK gene, and the binding of PPAR-γ/RXR-α heterodimer was further confirmed by anti-PPAR-γ antibody (lane 4). In contrast, heterodimer of PPAR-γ and RXR-α did not bind to mutant probes m1, m2, or m3, which contained the same mutations as pRGP-1003m1, -m2, and -m3, respectively (lanes 6, 7, and 8). From these results, it can be concluded that the region between +47 and +68 bp is a variant form of PPRE, and transactivation of βGK promoter by PPAR-γ depends on the binding of PPAR-γ/RXR-α heterodimer.

Although CV-1 cells were suitable for testing PPAR-γ responsiveness, it would be more reasonable to examine the PPAR-γ responsiveness of the βGK promoter in β-cell lines that are known to express both GK and insulin. Therefore, we transfected the promoter reporter constructs into HIT-T15 cells. The pRGP-1003 construct resulted in only a 1.5-fold increase in coexpressions of PPAR-γ and RXR-α (Fig. 5). This marginal activation might be due to higher basal activity of the βGK promoter, which depends on β-cell–specific transcription factors in HIT-T15 cells. Thus, we transfected 5′ deletion constructs of the promoter into HIT-T15 cells to minimize the effects of β-cell–specific transcription factors. The pRGP-128 construct was transactivated by PPAR-γ more highly than

FIG. 2. 5′ deletion study of βGK promoter. Luciferase reporter constructs under the control of rat βGK promoter spanning from −1,003, −404, +10, or +100 bp to +196 bp were cotransfected into CV-1 cells with or without PPAR-γ and/or RXR-α expression vectors as indicated. The cells were incubated in the presence of appropriate ligands as indicated: 20 μmol/l troglitazone (TGZ) for PPAR-γ and 1 μmol/l 9-cis retinoic acid (9-cis RA) for RXR-α. Normalized luciferase activities are shown as means ± SD of three independent experiments in triplicate and are expressed as the fold increase relative to basal activity in the absence of expression vectors and ligands.
PRGP-1003, and the PRGP/H11001 construct, containing minimal region responsible for PPAR/H9253 response, showed the highest transactivation. To further confirm the functionality of the βGK-PPRE, we introduced the same mutations into PRGp+10 as in PRGP-1003m5 and PRGP-1003mT and named them PRGP+10m5 and PRGP+10mT, respectively.

When transfected into HIT-T15 cells, PRGP+10mT was activated by PPAR-γ, whereas PRGP+10m5 was not. These results are consistent with the results from CV-1 cells and suggest that the βGK-PPRE is functioning in both β-cell lines and non-β-cell lines.

Although we showed the functionality of the βGK-PPRE
in the promoter context, it was necessary to confirm whether the βGK-PPRE itself could respond to PPAR-γ, because its sequence was very different from the consensus sequence. To this end, we constructed pGPRE3-tk-LUC, which had three copies of the βGK-PPRE in front of the thymidine kinase minimal promoter, and tested PPAR-γ responsiveness in CV-1 and HIT-T15 cells (Fig. 6). As expected, pGPRE3-tk-LUC reporter was well activated by PPAR-γ/RXR-α heterodimer in CV-1 and HIT-T15 cells. These results indicated that the βGK-PPRE is fully functional in both the βGK promoter context and the artificial promoter context.

**Trotiglitzone increases GK expression and activity in β-cell lines.** Trotiglitzone is known to have multiple effects on many genes involved in glucose and lipid metabolism, either directly or indirectly. Thus, it was necessary to confirm the effect of troglitazone on endogenous βGK expression in vitro. Total RNA was isolated from Min6 and Ins-1 cells after 24-h incubation in the presence or absence of troglitazone and 9-cis retinoic acid, and RPA and semiquantitative RT-PCR were performed. In RPA, we used the region between +370 and +696 bp, which had a β-cell-specific exon, as a probe, and we amplified the COOH-terminal region by RT-PCR, which is conserved between LGK and βGK. As shown in Fig. 7, combined treatment of troglitazone and 9-cis retinoic acid increased endogenous βGK expression in both Min6 and Ins-1 cells. Thus, activation of the βGK promoter induced endogenous βGK transcription. However, glucose is an important regulator of GK activity, and the regulation of GK activity by glucose predominantly depends on post-transcriptional mechanisms (27). Thus, we measured GK activity to confirm that activation of βGK transcription can increase GK activity in this system. Trotiglitzone increased GK activity by 40% in Ins-1 cells and 20% in Min6 cells (Fig. 8). This result clearly shows that PPAR-γ directly increases GK expression in β-cells through the βGK-PPRE, resulting in the increase of GK activity.

**DISCUSSION**

In the present study, we demonstrate that PPAR-γ stimulates βGK gene expression and enzyme activity in β-cells. The βGK-PPRE was localized within +47/–68 bp, containing the sequence TTACCTGTTGCCTCATTACTCA by promoter analysis performed in CV-1 cells. The pRGP-1003

**FIG. 4.** Localization of the PPRE and the binding of PPAR-γ. A: DNA sequences of the wild-type and mutant versions of the promoter element were shown to define the PPRE. The consensus sequence of PPRE known as DR+1 is also shown, and mutated bases are shown in lowercase. The oligonucleotide covering the indicated region (+43/+75 bp) was used as a probe (B), and mutant oligonucleotides (RGP+43/75m1, RGP+43/75m2, and RGP+43/75m3) were also used as probes for EMSA. Luciferase reporter constructs under control of the βGK promoter or mutated promoters were transfected into CV-1 cells with or without coexpression of PPAR-γ/RXR-α. The cells were incubated for 12 h after transfection in the presence of appropriate ligands: 20 μmol/l troglitazone (TGZ) for PPAR-γ and 1 μmol/l 9-cis retinoic acid (9-cis RA) for RXR-α. Normalized luciferase activities are shown as means ± SD of three independent experiments in triplicate and are expressed as the fold increase relative to basal activity in the absence of the expression vectors and ligands. B: EMSA of RGP+43/75 using in vitro translated PPAR-γ and RXR-α. [32P]-labeled double-strand oligonucleotides (w, m1, m2, and m3) were incubated with in vitro translated PPAR-γ (3 μl) and/or RXR-α (3 μl) as indicated. Anti-PPAR-γ serum (2 μl) was added into the reaction mixture (lane 4). Ab, antibody; NS, nonspecific band; PR, shifted band by PPAR-γ/RXR-α heterodimer.
Construct was activated by PPAR-γ and RXR-α in the presence of their ligands. This transactivation disappeared upon truncation of the βGK-PPRE. The mutations introduced into the βGK-PPRE abolished the transactivation of the βGK promoter by PPAR-γ and the binding of PPAR-γ/RXR-α heterodimer to the βGK-PPRE. We also showed functionality of the βGK-PPRE in insulin-secreting HIT-T15 cells. However, the fold activation in HIT-T15 cells was much smaller than in CV-1 cells. This difference in fold activation could be explained by the tissue specificity of the βGK promoter. The basal activity of the βGK promoter in HIT-T15 cells was more than 5 times higher than that in CV-1 cells. Thus, the absolute amount of the activation in HIT-T15 cells is as much as that in CV-1 cells, in spite of the small fold activation. This higher basal activity of the βGK promoter predominantly depends on the UPE3 site, which is located in the region between −104/-95 bp and the binding site for pancreatic duodenal homeobox gene-1 (PDX-1), the most well-known β-cell-specific transcription factor (28, 29). Thus, deletion constructs were better...

**FIG. 5.** PPAR-γ responsiveness of βGK promoter in HIT-T15 cells. βGK promoter–luciferase reporter constructs were transfected into HIT-T15 cells to test their responsiveness to PPAR-γ in β-cells. The structures are given. Expression vectors of PPAR-γ and RXR-α were cotransfected, and 20 μmol/l troglitazone (TGZ) and 1 μmol/l 9-cis retinoic acid (9-cis RA) were added as indicated. Normalized luciferase activities are shown as means ± SD of three independent experiments in triplicate and are expressed as the fold increase relative to basal activity in the absence of expression vectors and ligands.
creased endogenous is reduced (30). We also showed that troglitazone in-
tant function in diabetic/H9252 GK activity in
the report that increased GK transcription could increase
important in the regulation of ever, posttranscriptional regulation is known to be
fold activation, and that
/H9253 PPAR-
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5 times (data not shown). These results sug-
gested that the transactivation of the βGK promoter by
/H9253 activated the UPE3-mutated βGK pro-
moter fragment contained the
fragment contained the
/βGK-PPRE by PPAR-
activity as well as GK expression.
resulting in increased PDX-1 expression (32). Insulin can
also regulate βGK expression (33). Taking these data
together, the role of PPAR-γ on βGK expression in vivo
can be summarized as follows: 1) increased insulin sensi-
tivity can stimulate βGK expression; 2) reduced plasma
glucose concentration can restore PDX-1 expression in
β-cells and thus increase βGK expression; and 3) PPAR-γ
can directly activate βGK expression.
Insulin resistance is the common feature of type 2
diabetes and appears years before the onset of diabetes
(34). In the prediabetic stage, β-cells secrete enough
insulin to overcome the insulin resistance and maintain
euglycemia. Once the balance of insulin secretion and
action is disrupted, the clinical phenotype of type 2
diabetes develops, with defects of β-cell function (35). In
the type 2 diabetic subject, insulin secretion is not ade-
quately stimulated by glucose, and glucose-sensing appa-
ratus is downregulated in β-cells. In maturity-onset
diabetes of the young (MODY), loss of glucose sensing is
the primary event in developing diabetes (5). Thus, failure
of the β-cells to respond appropriately to glucose may be
the central event in the development of β-cell defects,
although the precise mechanism is still obscure.

**FIG. 6.** PPAR-γ responsiveness of βGK-PPRE in heterologous promoter context. The luciferase reporter construct, containing three copies of the +44/+70 bp region of βGK gene (βGK-PPRE) in front of thymidine kinase (tk) minimal promoter, was transfected in CV-1 and HIT-T15 cells. Expression vectors of PPAR-γ and RXR-α were cotransfected, and 20 μmol/l troglitazone (TGZ) and 1 μmol/l 9-cis retinoic acid (9-cis RA) were treated as indicated. Normalized luciferase activities are shown as means ± SD of three independent experiments in triplicate and are expressed as the fold increase relative to basal activity in the absence of expression vectors and ligands.

**FIG. 7.** PPAR-γ increased endogenous βGK expression in β-cell lines. A: RTA of βGK. Total RNA (50 μg) was isolated from Min6 and Ins-1 cells cultured in the presence or absence of troglitazone (20 μmol/l) and 9-cis retinoic acid (1 μmol/l) and subjected to RPA. The protected fragment contained the β-cell-specific exon of GK. B: RT-PCR of GK. Total RNA was isolated from Min6 and Ins-1 cells. The amplified region of GK was the COOH terminus of the genes. In A and B, β-actin was used as an internal control, and detailed methods are described in RESEARCH DESIGN AND METHODS.

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pGPRE3-tk-LUC βGK-PPRE(+44/+70) X3-tk Luciferase

TGZ - + Ins-1 +

βGK

β-actin

Min6 Ins-1

TGZ - +

βGK

β-actin

Min6 Ins-1

β-actin

A: RPA of βGK. Total RNA (50 μg) was isolated from Min6 and Ins-1 cells cultured in the presence or absence of troglitazone (20 μmol/l) and 9-cis retinoic acid (1 μmol/l) and subjected to RPA. The protected fragment contained the β-cell-specific exon of GK. B: RT-PCR of GK. Total RNA was isolated from Min6 and Ins-1 cells. The amplified region of GK was the COOH terminus of the genes. In A and B, β-actin was used as an internal control, and detailed methods are described in RESEARCH DESIGN AND METHODS.
βGK has an important role in glucose homeostasis because it works as a glucose sensor for GSIS under physiological conditions. Any change of βGK activity causes alteration of the glucose threshold for insulin secretion (5). β-Cells may gradually lose their predominant position in blood glucose regulation in insulin-resistant states, for example, in obesity and late pregnancy. The glucose threshold of β-cells is usually lower, and thereby basal insulin secretion is increased, in primary islets isolated from animals with insulin resistance. Troglitazone decreases basal insulin secretion but increases GSIS in the primary islets isolated from diabetic ZDF rats (14). These troglitazone-induced changes of GSIS in isolated diabetic β-cells resemble the hyperinsulinemic pattern of compensated β-cells. The way in which troglitazone decreases basal insulin secretion levels in isolated islets is still unclear. However, considering that the first step of GSIS is glucose sensing, increased GK expression may contribute to restoration of glucose threshold and GSIS. Thus, the antidiabetic action of troglitazone might be the result of the combinatorial effects on several target tissues. Enhanced insulin sensitivity improves peripheral glucose disposal, reduces insulin secretory demand, and decreases hepatic glucose output. Enhanced glucose-sensing ability of β-cells improves the function and compensatory capacity of β-cells. Thus, troglitazone decreases blood glucose and insulin levels efficiently. Furthermore, considering that glucose is known to play important roles in the maturation of β-cells (36), the restoration of glucose-sensing ability might be involved in the functional and morphological restoration of β-cells by troglitazone.

In this study, we have characterized atypical PPRE in the βGK gene. The βGK-PPRE is uncommon in that it is located downstream of the transcription initiation site, and the sequence composition is much different from conventional PPRE. There are many reports of cis-elements located in exons and introns, but no PPRE is known to be located in exons except for the GLUT2-PPRE. The PPREs in GLUT2 and βGK are located in the first exon, and these two genes have relatively long 5’ untranslated regions. At this point, we don’t know whether the location of PPREs of the glucose-sensing apparatus has special implication.

In conclusion, our results demonstrate that troglitazone directly activates βGK expression, as it does GLUT2 expression, and PPAR-γ is the direct regulator of the glucose-sensing apparatus of β-cells, suggesting that βGK gene may be one of the sought-for molecular targets of PPAR-γ on the restoration of β-cell function in type 2 diabetic subjects. In addition, considering the contribution of PPAR-γ to the restoration of glucose-sensing ability of β-cells, the ligands of PPAR-γ may be suitable for treating MODY or early-stage type 2 diabetes.

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