

Rosiglitazone (BRL 49653) Enhances Insulin Secretory Response via Phosphatidylinositol 3-Kinase Pathway

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To elucidate the direct effect of rosiglitazone (RSG), a new thiazolidinedione antihyperglycemic agent, on pancreatic insulin secretion, an in situ investigation by rat pancreatic perfusion was performed. At a basal glucose concentration of 6 mmol/l, RSG (0.045–4.5 μ mol/l) stimulated insulin release in a dose-dependent manner. In addition, 4.5 μ mol/l RSG potentiated the glucose (10 mmol/l)-induced insulin secretion. Both the first and second phases of glucose-induced insulin secretion were significantly enhanced by RSG, by 80.7 and 52.4%, respectively. The effects of RSG on insulin secretion were inhibited by a phosphatidylinositol 3-kinase (PI3K) inhibitor, LY294002. In contrast, the glucose-stimulated insulin secretion was not affected by LY294002. The potentiation effect of RSG on glucose-stimulated insulin secretion, in both the first and second phases, was significantly blocked by LY294002. These results suggest that RSG has a direct potentiation effect on insulin secretion in the presence of 10 mmol/l glucose, mediated through PI3K activity. The inability of LY294002 to inhibit glucose-induced insulin secretion suggests that different pathways are responsible for glucose and RSG signaling. *Diabetes* 50:2598–2602, 2001

Defects in insulin secretion from the pancreatic β -cells and insulin action on the target cells are two major causes for type 2 diabetes (1). In regard to their role in diabetes treatment, thiazolidinediones (TZDs), a novel class of antidiabetic agents that can produce a potent insulin-sensitizing activity in vivo (2), are effective in reducing blood glucose, insulin, and triglyceride levels in both insulin-resistant animal models and type 2 diabetic patients (3–5). Although their mechanism of action is not entirely understood, it is well recognized that TZDs are high-affinity ligands for the peroxisome proliferator-activated receptor (PPAR)- γ , a member of the nuclear receptor family (6). The relative

potency that various TZDs show in binding and activating PPAR- γ in vitro correlates with their antidiabetic action in vivo (7,8), thus indicating that PPAR- γ mediates the insulin-sensitizing effect of these molecules. Rosiglitazone (RSG) (i.e., BRL 49653) is a relatively new antidiabetic agent of the TZD class that enhances sensitivity to insulin in the liver, adipose tissue, and muscle, resulting in an improvement of insulin-mediated glucose disposal (9).

There have been many studies that have focused on the mechanism behind the insulin-sensitizing effect of TZDs. TZDs have been shown to modulate several processes that increase sensitivity to insulin, including effects on the number, phosphorylation state, and kinase activity of the insulin receptors. They have also been shown to affect hepatic glucose metabolism (10). Recently, Rieusset et al. (11) reported that the stimulation of PPAR- γ by RSG induced mRNA expression of the p85 α subunit of the phosphatidylinositol 3-kinase (PI3K) and of the uncoupling protein-2 genes in human adipocytes. Because PI3K is a major component of insulin action on glucose transport and intracellular metabolism, the induction of its expression might explain, at least in part, the insulin-sensitizing effect of TZDs.

In fatty rats, pathological changes in the pancreatic islet, such as islet β -cell hyperplasia (12), elevated pancreatic insulin content (13,14), and a disseminated distribution of α -cells in the islet (12), have been described. These adaptive changes could be ameliorated or prevented by RSG treatment (15). Up to now, there have only been a few studies investigating the effect of enhancing insulin secretory capacity by TZDs. The TZD compound troglitazone (CS-045) has a triglyceride-lowering effect on the fat-laden islets of Zucker diabetic fatty (ZDF) rats as well as on the normal islets (16). In addition, Unger and colleagues (17–19) have reported a close relationship between the accumulation of islet fat and the appearance of β -cell abnormalities and hyperglycemia. They also demonstrated that a troglitazone-mediated reduction in the triglyceride content of fat-laden ZDF islets improved β -cell function in vitro (20) and prevented hyperglycemia in vivo. Functional rescue was also observed, reflected by the normal glucose-stimulated insulin response, which was absent in the untreated controls (21). The above evidence that TZDs protect β -cells from lipoapoptosis in rodents implies that part of the therapeutic action of TZDs in human type 2 diabetes may be the result of the prevention of β -cell loss and the restoration of the insulin secretory capacity (21). Recently, Brown et al. (22) reported that GW1929, a novel N-aryl tyrosine activator of PPAR- γ , restored the glucose-

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ANOVA, analysis of variance; AUC, area under the curve; PI3K, phosphatidylinositol 3-kinase; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisomal proliferator response element; RIA, radioimmunoassay; RSG, rosiglitazone; TZD, thiazolidinedione.

induced biphasic insulin secretion from isolated perfused pancreata after oral administration for 14 days in ZDF rats. Administration of GW1929 also resulted in the preservation of pancreatic islet morphology and β -cell insulin content (22). However, these effects of PPAR- γ activators on the islets were secondarily attributed to the amelioration of glucose toxicity. In the present study, we explored the direct effect of RSG on glucose-induced insulin secretion by using an isolated rat pancreas perfusion system and by examining the potential signaling mechanism of stimulatory effect by RSG.

RESEARCH DESIGN AND METHODS

Animals and chemicals. Male Sprague-Dawley rats obtained from the Animal Center of the National Science Council of the Republic of China and weighing between 250 and 350 g were used in these experiments. The rats were kept at room temperature ($\sim 25^{\circ}\text{C}$) in plastic cages under a 12-h cycle of light. The rats were given free access to tap water and were fed ad libitum with commercial diet (Fwusow; Sha-Lu, Taichung, Taiwan). After an overnight fast (>12 h), the rats were anesthetized with a 65 mg/kg intraperitoneal injection of pentobarbital sodium (MTC Pharmaceuticals, Cambridge, Canada). Access to the pancreata was gained through a ventral midline incision, and the animals' celiac arteries and portal veins were cannulated with polyvinyl tubings of 0.625 and 1.2 mm (internal diameter), respectively. The rats were maintained at 37°C throughout the experiments. Krebs-Ringer bicarbonate buffer, supplemented with 10 mmol/l *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 6 mmol/l glucose, 0.1% dextran, and 0.2% bovine serum albumin, was used as the perfusate (basal medium). This solution was continuously aerated with 95% O_2 /5% CO_2 , and the pH value was maintained at 7.4. All chemicals were purchased from Sigma Chemical (St. Louis, MO), except for RSG and LY294002, which were kindly provided by SmithKline Beecham Pharmaceuticals (Essex, U.K.) and Eli Lilly (Indianapolis, IN), respectively.

Pancreatic perfusion. Using a method of Grodsky and Fanska (23), the *in situ* rat pancreatic perfusion with an open system was performed at 37°C . The pancreatic perfusion was maintained at a flow rate of 1 ml/min, which did not cause noticeable edema or impair insulin release. After the cannulation of the celiac artery and the portal vein and the establishment of the rat pancreatic perfusion preparation, the first 10 min of perfusion were considered as an equilibration period. Subsequently, the effluent fluid was collected every minute from the cannula of the portal vein for 50 or 55 min. In experiment 1, after a baseline period of 15 min, the perfusate containing RSG (0.045, 0.45, 1.5, or 4.5 $\mu\text{mol/l}$) was administered for 30 min, followed by the basal medium for 10 min. In experiment 2, after a baseline period of 10 min, the medium containing a PI3K inhibitor (LY294002 at 3.9 $\mu\text{mol/l}$) was administered for 5 min and was followed by LY294002 (3.9 $\mu\text{mol/l}$)/RSG (4.5 $\mu\text{mol/l}$) for another 30 min and a basal medium washout for the last 10 min. In experiment 3, after a baseline period of 10 min, the perfusate containing 10 mmol/l glucose with or without RSG (4.5 $\mu\text{mol/l}$) was administered for 30 min, followed by a basal medium washout for the last 10 min. In experiment 4, after a baseline period of 10 min, the medium containing LY294002 (3.9 $\mu\text{mol/l}$) was administered for 5 min as pretreatment and was followed by 10 mmol/l glucose with or without RSG (4.5 $\mu\text{mol/l}$) for another 30 min and a basal medium washout for the last 10 min. The collected effluent fluid was kept at 4°C and subsequently assayed within 12 h for insulin by using radioimmunoassay (RIA), as previously described by Hale and Randle (24). Rat insulin was used as standard for the RIA.

Data expression and statistical analysis. Data of effluent insulin concentrations were expressed as a percentage of the baseline level (mean of 12 baseline values) in means \pm SE. Data were analyzed by using analysis of variance (ANOVA) to determine the significance of treatment and time. The treatment multiplied by time interaction was used as an error term to determine the effect of treatment. The significance of treatment was determined from the conservative *F* value. Tukey's highly significant difference test was used to determine the differences between treatments for which the ANOVA indicated a significant ($P < 0.05$) *F* ratio. For analyzing the first (from 2 to 7 min) and second (from 8 to 30 min) phases of insulin secretion during glucose perfusion, the areas under the curve (AUCs) of the percentage increase over baseline (Figs. 2 and 5) were calculated and compared using Student's *t* tests. $P < 0.05$ was considered statistically significant.

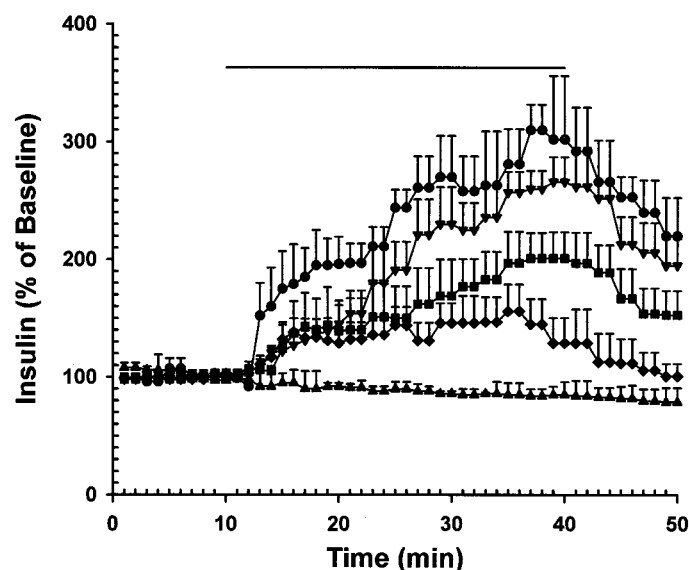


FIG. 1. Effect of RSG on insulin secretion. In pancreatic perfusion experiments (see RESEARCH DESIGN AND METHODS), an equilibration period of 15 min preceded time 0. After a baseline period of 10 min, RSG (0.045–4.5 $\mu\text{mol/l}$) was administered for 30 min, followed by the basal medium perfusion. The horizontal line indicates the presence of RSG. Values are means \pm SE ($n = 4$). Baseline effluent concentrations (means \pm SE) of insulin were $4,320 \pm 361$, $5,450 \pm 213$, $4,930 \pm 526$, $5,496 \pm 226$, and $4,205 \pm 283$ $\mu\text{g/ml}$ for the basal control group (\blacktriangle) and 0.045 (\blacklozenge), 0.45 (\blacksquare), 1.5 (\blacktriangledown), and 4.5 (\bullet) $\mu\text{mol/l}$ for the RSG group, respectively.

RESULTS

Dose-dependent effect of RSG on insulin secretion.

To demonstrate the direct effect of RSG on insulin secretion, various concentrations of RSG (0.045, 0.45, 1.5, and 4.5 $\mu\text{mol/l}$) within basal media with 6 mmol/l glucose were infused via *in situ* pancreatic perfusion. As shown in Fig. 1, insulin secretion was induced by RSG in a time- and dose-dependent manner ($P < 0.01$ vs. control). Insulin secretion was observed within 2 min but reached a statistical significant level at 15 min (RSG 4.5- $\mu\text{mol/l}$ group) of continuous perfusion of RSG. This effect of RSG on insulin secretion still did not reach a plateau before the washout period. The induction of insulin secretion was monophasic, which was different from the typical glucose-induced biphasic insulin secretion (Fig. 2). Insulin secretory response declined immediately after discontinuation of RSG perfusion (Fig. 1).

Potential of glucose-induced insulin secretion by RSG.

To evaluate the effect of RSG on glucose-induced insulin secretion, glucose alone (10 mmol/l) and glucose containing RSG (4.5 $\mu\text{mol/l}$) were perfused through isolated rat pancreata. As shown in Fig. 2, 10 mmol/l glucose induced a biphasic response in insulin secretion, with an early peak at 2–7 min (first phase) and a continuous increase thereafter (second phase). After the administration of RSG in glucose perfusate, both the glucose-induced first and second phases of insulin secretion were further enhanced compared with those found in the perfusion experiments with glucose alone ($P < 0.01$). Insulin secretory response declined immediately after discontinuation of glucose and RSG perfusion (Fig. 2).

To address the effect of RSG on different phases of insulin secretion after glucose stimulation, we compared the AUCs of the percentage increase over baseline of the

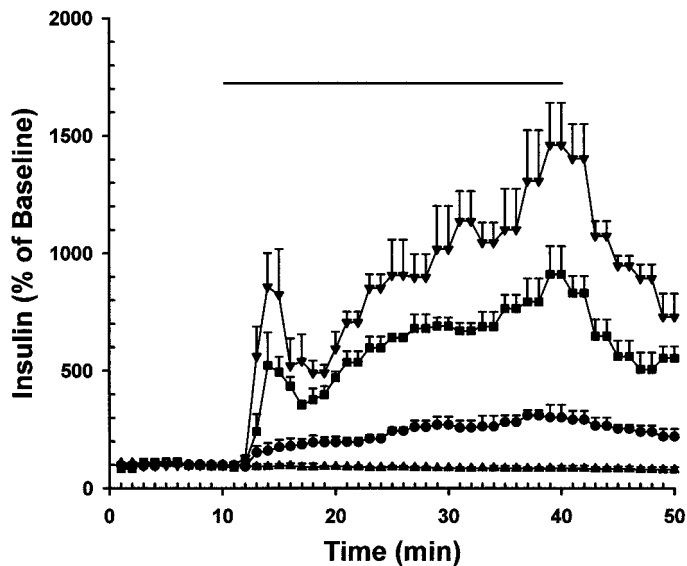


FIG. 2. Potentiation of glucose-induced insulin secretion by RSG. After a baseline period of 10 min, glucose (10 mmol/l) with or without RSG (4.5 μ mol/l) was administered for 30 min (as indicated by the horizontal line), followed by basal medium perfusion. Values are means \pm SE ($n = 4$). Baseline effluent concentrations of insulin were $4,320 \pm 361$, $4,205 \pm 283$, $4,556 \pm 400$, and $4,136 \pm 198$ pg/ml for the basal control (\blacktriangle), RSG (\bullet), glucose (\blacksquare), and glucose + RSG groups (\blacktriangledown), respectively.

first and second phases of insulin secretion. As shown in Table 1, both the first and second phases of insulin secretion were enhanced by simultaneous RSG perfusion (by 80.7 and 52.4%, respectively).

Role of PI3K pathway in RSG effect on insulin secretion. To determine the possible signaling pathways leading to the effect of RSG on insulin secretion, a PI3K inhibitor, LY294002 (25,26), was included in the perfusate. As shown in Fig. 3, the addition of LY294002 at 3.9 μ mol/l decreased RSG-potentiated insulin release dramatically ($P < 0.01$), indicating a possible role of the PI3K pathway in the acute effect of RSG. In contrast, both the first and second phases of insulin secretion after glucose stimulation were not affected by the PI3K inhibitor (Fig. 4). As depicted in Fig. 5, the administration of LY294002 blocked the potentiation of glucose-induced insulin secretion by RSG and yielded a time-response curve that was almost superimposed to that produced by the perfusion experiments with glucose alone (Fig. 4). Both the first and second phases of insulin secretion were reduced by LY294002 (by 34.3 and 33.0%, respectively) (Table 2).

TABLE 1
Effect of RSG on the glucose-induced first and second phases of insulin secretion

	First phase (2–7 min)	Second phase (8–30 min)
Glucose (10 mmol/l)	$1,456.2 \pm 162.1^*$	$18,251.4 \pm 650.5$
Glucose (10 mmol/l) + RSG (4.5 μ mol/l)	$2,630.7 \pm 300.4^\dagger$	$27,815.9 \pm 1,387.4^\dagger$
Mean increase over glucose effect	80.7%	52.4%

Data are means \pm SE, unless otherwise indicated. *AUC of the percentage increase over baseline. $^\dagger P < 0.01$ vs. glucose group (Student's t test).

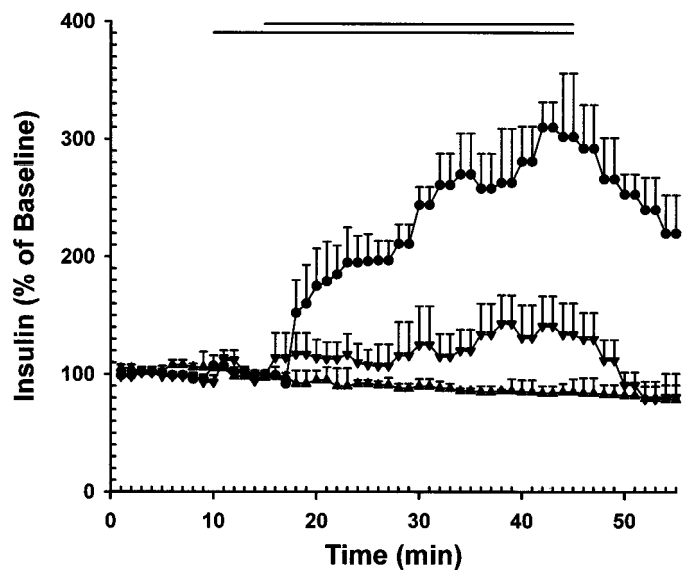


FIG. 3. Effect of LY294002 on basal and RSG-induced insulin secretion. After a baseline period of 10 min, LY294002 was administered at 3.9 μ mol/l for 5 min before RSG (4.5 μ mol/l)/LY294002 (3.9 μ mol/l) was perfused for 30 min (as indicated by the horizontal lines). Values are means \pm SE ($n = 4$). Baseline effluent concentrations of insulin were $4,320 \pm 361$, $4,205 \pm 283$, and $4,268 \pm 448$ pg/ml for the basal control (\blacktriangle), RSG (\bullet), and LY294002 + RSG (\blacktriangledown) groups, respectively.

DISCUSSION

Previous studies have reported that troglitazone, a member of the TZD family, can protect β -cells from lipopoptosis and restore the insulin-secretory capacity of β -cells (20,21). In addition, a novel N -aryl tyrosine activator of PPAR- γ (GW1929) can restore glucose-induced biphasic insulin secretion in the perfused pancreas of ZDF rats after oral administration for 14 days (22). However, these effects of PPAR- γ activators on the islets are secondarily attributed to the amelioration of glucose toxicity and/or

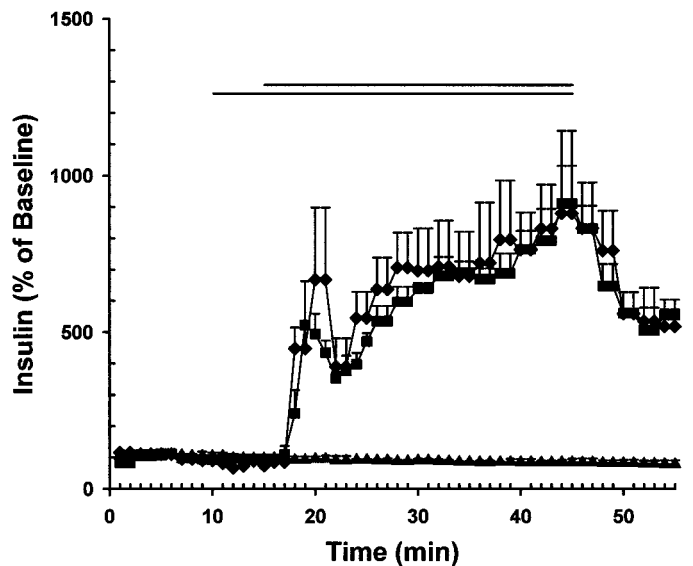


FIG. 4. Effect of LY294002 on glucose-induced insulin secretion. After a baseline period of 10 min, glucose (10 mmol/l) was perfused for 30 min with or without LY294002 (3.9 μ mol/l) (as indicated by the horizontal lines). Values are means \pm SE ($n = 4$). Baseline effluent concentrations of insulin were $4,320 \pm 361$, $4,556 \pm 400$, and $5,605 \pm 573$ pg/ml for the basal control (\blacktriangle), glucose (\blacksquare), and LY294002 + glucose (\blacklozenge) groups, respectively.

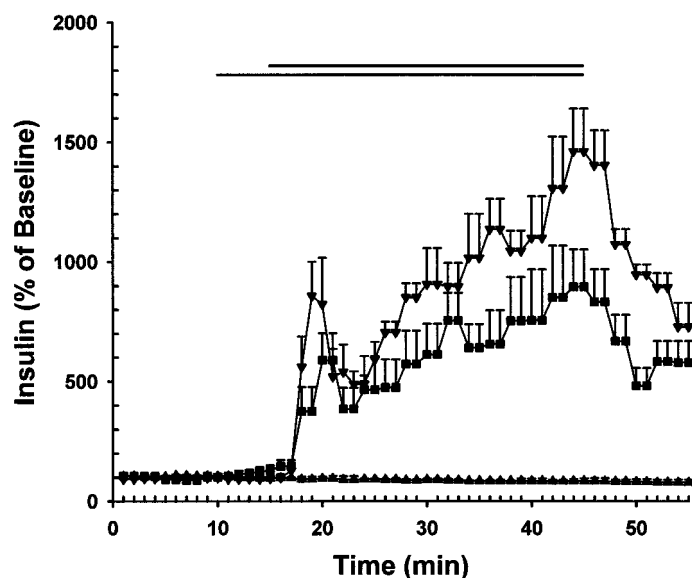


FIG. 5. Effect of LY294002 on potentiation of RSG on glucose-induced insulin secretion. After a baseline period of 10 min, glucose (10 mmol/l)/RSG (4.5 μ mol/l) was perfused with or without LY294002 (3.9 μ mol/l) (as indicated by the horizontal lines). Values are means \pm SE ($n = 4$). Baseline effluent concentrations of insulin were $4,320 \pm 361$, $4,205 \pm 283$, $4,136 \pm 198$, and $5,975 \pm 550$ pg/ml for the basal control (\blacktriangle), RSG, glucose + RSG (\blacktriangledown), and LY294002 + glucose + RSG (\blacksquare) groups, respectively.

lipotoxicity (22). In the present study, we first detected the direct effect of RSG on glucose-induced insulin secretion by using an isolated rat pancreas perfusion system. We found that insulin secretion was induced by RSG in a time- and dose-dependent manner in the presence of 6 or 10 mmol/l glucose, indicating that RSG can potentiate glucose-stimulated insulin secretion. In the absence of glucose, perfusion of RSG alone did not stimulate insulin secretion (data not shown). Moreover, the effect of RSG on insulin secretion was blocked by a PI3K inhibitor, LY294002, indicating an involvement of PI3K in RSG-induced insulin secretion.

The direct effect of troglitazone on insulin secretion had been observed in the isolated islets and in a β -cell line, HIT (27). Masuda et al. (27) reported that 1 μ mol/l troglitazone stimulated insulin release, whereas at a higher dose (100 μ mol/l), troglitazone inhibited insulin release from these cells. To explore the mechanisms by which troglitazone triggers insulin release, a putative membrane binding site was postulated due to a noncompetitive displacement of the binding of [3 H]glibenclamide by troglitazone on the β -cell membrane. However, the insulinotropic effect of troglitazone was different from that of glibenclamide because 1 μ mol/l troglitazone increased insulin secretion but failed to reduce ATP-sensitive K^+ -channel activity (27). Thus, the signaling pathway of the insulinotropic effect of

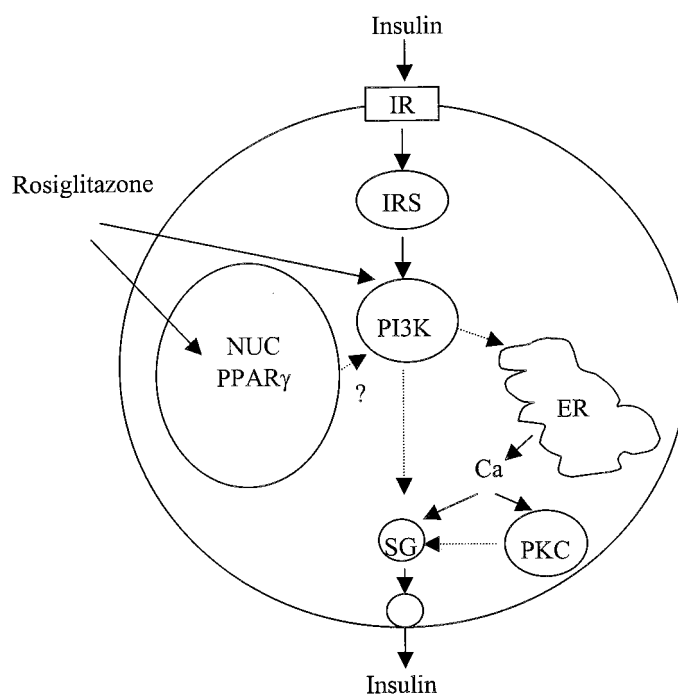


FIG. 6. Illustration for showing the pathways leading to insulin release in the pancreatic β -cells. ER, endoplasmic reticulum; IR, insulin receptor; IRS, insulin receptor substrate; NUC, nucleus; SG, secretory granules. The pathway from insulin stimulation to insulin secretion was depicted according to Aspinwall et al. (28).

troglitazone on pancreatic islets and HIT cells is still unclear. Our study also showed that RSG could induce insulin secretion and potentiate both the first and second phases of glucose-stimulated insulin secretion with only short-term administration. Importantly, we found that a PI3K inhibitor inhibited both the direct and potentiation effects of RSG on insulin secretion. Treatment with this inhibitor did not reduce the glucose-induced insulin secretion. These results unequivocally indicated that the direct and potentiation effects of RSG on insulin secretion are mediated by the PI3K pathway (Fig. 6). The doses that we used in our present study were quite comparable with the blood concentrations after a single dosing of RSG at 1–8 mg. Interestingly, a recent study showed that insulin-induced insulin secretion also involved insulin receptors, insulin receptor substrate-1, and PI3K activation, which led to insulin secretion via an increase in intracellular calcium concentration (28,29).

Although PPAR- γ proteins, members of the nuclear receptor family, are expressed in the rat islet and in a β -cell line, MIN6 (L-M.C., M-W.L., unpublished observations), it is unlikely that the activation of these proteins mediates the potentiation effect of RSG on the first phase of glucose-induced insulin secretion, because the onset of

TABLE 2

Effect of LY294002 on the glucose-induced first and second phases of insulin secretion after glucose stimulation with RSG treatment

	First phase (2–7 min)	Second phase (8–30 min)
Glucose (10 mmol/l) + RSG (4.5 μ mol/l)	2,630.7 \pm 300.4*	27,815.9 \pm 1,387.4
Glucose (10 mmol/l) + RSG (4.5 μ mol/l) + LY294002 (3.9 μ mol/l)	1,729.3 \pm 231.2†	18,642.4 \pm 2067.9†
Mean decrease (%) over glucose + RSG effect	34.3	32.9

Data are means \pm SE, unless otherwise indicated. *AUC of the percentage increase over baseline. † $P < 0.01$ vs. glucose + RSG group (Student's t test).

the RSG-induced increase is too fast to be attributable to a genomic action. Because the second-phase secretion was potentiated by RSG within 30 min of exposure, it is likely that even the stimulation of this phase was not an effect at the genomic level. However, a recent study reported that a peroxisomal proliferator response element (PPRE) exists in the rat GLUT2 promoter. In HIT-T15 cells, promoter activity of the rat GLUT2 gene was increased by troglitazone and 9-*cis* retinoic acid, and mutations of GLUT2-PPRE resulted in a reduction of the promoter activity. In addition, they also observed an increase in the GLUT2 transcription by troglitazone and 9-*cis* retinoic acid in rat islets (30). Therefore, the potentiation effect of RSG on the second phase of glucose-induced insulin secretion might be explained by the increased expression of GLUT2 on pancreatic β cells, which play a key role in β -cells for glucose signaling to insulin secretion and biosynthesis (31). In a longer-term treatment, troglitazone was found to enhance the expression of p85 α , a subunit of PI3K (11). Therefore, it is possible for a potentiation of RSG on the second phase of glucose-induced insulin secretion via an increase in PI3K protein synthesis and/or GLUT2 expression. Further studies are needed to elucidate the mechanism(s) involved in this pathway.

In conclusion, RSG has a potentiation effect on insulin secretion in the presence of glucose, which is mediated by the PI3K pathway and is clearly distinct from that of glucose-stimulated insulin secretion. From this point of view, TZD derivatives could become a promising category of therapeutic agents for type 2 diabetes, in addition to their well-known insulin-sensitizing effect on the peripheral tissues.

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