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Sulfonylureas uncouple glucose-dependence for GPR40-mediated enhancement of insulin secretion from INS-1E cells

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ABSTRACT

Activation of GPR40 is reported to enhance insulin secretion in the presence of glucose. We determined whether sulfonylureas could replace glucose for GPR40-mediated enhancement of insulin secretion and investigated underlying mechanisms using INS-1E cells. GW9508, a specific agonist of GPR40, significantly enhanced insulin secretion in the presence of high concentrations of glucose. In contrast, sulfonylureas increased insulin secretion in the absence of glucose. In the presence of sulfonylureas, activation of GPR40 significantly enhanced insulin secretion. The L-type calcium channel (LTCC) activator S-(−)-Bay K8644 also concentration-dependently increased insulin secretion in the absence of glucose. In the presence of 10 μmol/L S-(−)-Bay K8644, GW9508 significantly increased insulin secretion. On the other hand, the LTCC blocker nifedipine significantly inhibited insulin secretion mediated by either glucose, glipizide or glucose plus GW9508. Thus, sulfonylureas could replace glucose to support GPR40-mediated enhancement of insulin secretion, whereas blockage of LTCC reduced both glucose and sulfonylurea-mediated insulin secretion.

1. Introduction

G-protein-coupled receptor 40 (GPR40), a class A G-protein-coupled receptor, belongs to the free fatty acid receptor family (Rayasam et al., 2007; Milligan et al., 2006; Stoddart et al., 2008). The natural ligands of GPR40 include medium- and long-chain free fatty acids (Itoh et al., 2003; Briscoe et al., 2003). It has been reported that GPR40 is preferentially expressed in pancreatic islets and β-cell lines (Itoh et al., 2003; Tomita et al., 2006) and that the level of gene expression in human pancreatic islets appears to be higher than that of sulfonylurea receptor type 1 (SUR1) or glucagon-like peptide-1 receptor (GLP-1R) (Tomita et al., 2005, 2006). It was demonstrated that activation of GPR40 increased insulin secretion and reduced blood glucose in mice and rats (Latour et al., 2007; Tan et al., 2008; Doshi et al., 2009). Activation of GPR40 also significantly enhanced glucose-dependent insulin secretion from rodent pancreatic islets and β-cell lines (Itoh et al., 2003; Tan et al., 2008; Briscoe et al., 2006; Salehi et al., 2005; Shapiro et al., 2005). Similarly, treatment with long-chain free fatty acids, natural ligands of GPR40, significantly increased glucose-dependent insulin secretion from human pancreatic islets (Gravena et al., 2002). However, no amplification of insulin secretion was observed after activation of GPR40 in the presence of 0–5 mmol/L glucose (Tan et al., 2008; Zhang et al., 2007; Shapiro et al., 2005).

The mechanism underlying the glucose-dependence for GPR40 activation-induced enhancement of insulin secretion is not completely understood. High concentrations of glucose are known to stimulate insulin secretion through an intracellular pathway that involves an increase of the intracellular ATP/ADP ratio and closure of K_{ATP} channels, followed by membrane depolarization, leading to the activation of L-type calcium channels (LTCCs) and an increase of intracellular calcium $[Ca^{2+}]_i$ (MacDonald et al., 2005; Proks and Lippiat, 2006; Straub and Sharp, 2002). Therefore the glucose-dependence of GPR40 activation-induced enhancement of insulin secretion may partially rely on the activation of LTCC. To test this hypothesis, we investigated whether activation of GPR40 in INS-1E cells by the small-molecule agonist GW9508 could increase insulin secretion in the presence of a sulfonylurea under glucose-free condition, because sulfonylureas are known to activate LTCCs through the inhibition of SUR1 followed by closure of K_{ATP} channels (Barg, 2003; Proks and Lippiat, 2006; Rendell, 2004; Mears, 2004). Experiments were also performed using S-(−)-Bay K8644, a specific LTCC activator, and nifedipine, an LTCC antagonist. The novel finding in this study is that the GPR40 agonist GW9508 enhanced insulin secretion in the absence of glucose when either a sulfonylurea or an LTCC agonist was present. The results suggest that GPR40-mediated

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enhancement of insulin secretion depends on the activation of LTCC.

2. Materials and methods

2.1. Reagents and materials

Primers for rat GPR40, SUR1, and GLP-1R were designed using Beacon Designer 7.0 (PREMIER Biosoft International, Palo Alto, CA). Primers were synthesized by Operon Biotechnologies, Inc. (Huntsville, AL 35805). GW9508 was synthesized by Asinex (Winston-Salem, NC) (Briscoe et al., 2006). Glipzide, glybenclamide, tolbutamide and exendin-4 were purchased from Sigma. Adenosine deaminase was purchased from Roche Molecular Biochemicals (Nutley, NJ). All reagents for cell culture were purchased from Invitrogen. Rat insulin ELISA assay kits were obtained from Millipore (formerly Linco). Fetal bovine serum was purchased from Mediatech (Manassas, VA). RNA miniprep kits were purchased from Stratagene (La Jolla, CA), iScript Reverse Transcription kits were purchased from Bio-Rad Laboratories (Hercules, CA), and SYBR Green PCR kits were obtained from Applied Biosystems (Foster City, CA). FLIPR Calcium 4 assay kits were purchased from Molecular Devices (Sunnyvale, CA). HitHunter cAMP XS assay kits were purchased from GE Healthcare (Piscataway, NJ). All general reagents were purchased from Sigma.

2.2. Cell culture

INS-1E cells were cultured in RPMI 1640 medium supplemented with 11 mM glucose, 10% fetal bovine serum (FBS), 1% penicillin/streptomycin/glutamine, 10 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid), 1 mM sodium pyruvate, 50 μM β-mercaptoethanol (optional). Cells were subcultured every 3–4 days.

2.3. Insulin secretion assay

INS-1E cells were seeded in 96-well plates at density of 5×10^4 cells/well and cultured at 37 °C in room air/5% CO_2 for 2–3 days. The cell medium was replaced with glucose-free RPMI 1640 containing 1% FBS for 1 h. Cells were then stimulated with agents in Krebs-Ringer buffer (129 mmol/L NaCl, 4.8 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 5 mmol/L $NaHCO_3$, and 10 mmol/L HEPES, pH 7.4) containing 0.1% BSA (fatty acid free) for 1 h in the presence of 0 mmol/L (vehicle) or 5–20 mmol/L glucose. Supernatants (100 μL) were harvested from all wells to determine insulin levels. Insulin concentration was measured using an ELISA kit.

2.4. Ca^{2+} mobilization assay

INS-1E cells were seeded in 96-well plates at a density of 8×10^4/well and cultured overnight. Calcium 4 reagent (100 μL) was added to each well and incubated for 1 h. Cells were treated with drugs and fluorescence activity was measured using a FlexStation. $[Ca^{2+}]_i$ concentration is reported as relative fluorescent units (maximum increase of relative fluorescence units above basal). Data are presented as mean ± SEM.

2.5. Measurement of cAMP production

Cultured INS-1E cells were detached with PBS containing 2 mM EDTA. Cells were pelleted at 750 × *g* and resuspended in Opti-MEM I with 1% FBS. ADA (1 U/ml) was added to eliminate adenosine. Cells were loaded into 96-well plates (~5000 cells/well) and incubated with agonists for 30 min at 37 °C. The cAMP production was measured using a HitHunter cAMP XS Assay kit according to the manufacturer's instructions.

2.6. Real-time RT PCR

Total RNA was extracted using a RNA miniprep kit. cDNA was then synthesized using an iScript Reverse Transcription kit. Real-time PCR was performed using SYBR Green PCR reagents on Stratagene Mx3000P (Stratagene, La Jolla, CA). Relative mRNA levels were calculated based on the Ct values (threshold cycle time) and reported relative to the levels of rat β-actin.

3. Results

3.1. INS-1E cells express GPR40 and secrete insulin in response to glucose

Results of Real-time RT-PCR showed that INS-1E cells, a pancreatic β-cell line, express GPR40 mRNA at a level similar to that of SUR1 (Fig. 1A). Stimulation of INS-1E cells increased insulin secretion in a glucose-concentration-dependent manner (Fig. 1B). Therefore INS-1E cells are an appropriate model system to look for potential interactions of glucose concentration, GPR40 and SUR1 on the regulation of insulin secretion.

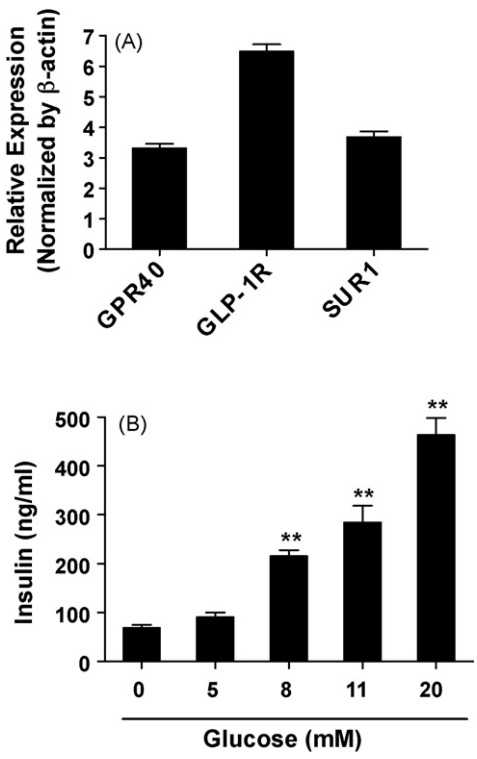

Fig. 1. INS-1E cells express GPR40 and secrete insulin in response to glucose in a concentration-dependent manner. (A) Relative expression levels of mRNA in INS-1E cells for rat G-protein-coupled receptor 40 (GPR40), glucagon-like peptide-1 receptor (GLP-1R) and sulfonylurea receptor type 1 (SUR1), normalized to the expression level of β-actin. Data shown are mean ± SD of values from 6 replicate samples. The experiment was repeated two times with similar results. (B) Glucose-concentration-dependently increased insulin secretion from INS-1E cells. Data shown are mean ± SD of values from six independent experiments each with triplicate samples. $^{**}p < 0.01$ compared to 0 glucose.

3.2. Activation of GPR40 enhanced glucose-dependent insulin secretion from INS-1E cells

Because long-chain free fatty acids are involved non-selectively in many biological pathways, we chose to explore GPR40 activation using GW9508, a selective and potent small-molecule agonist of GPR40 (Briscoe et al., 2006). As expected, GW9508 concentration-dependently enhanced insulin secretion in the presence of high glucose (Fig. 2A). In the presence of 20 mmol/L glucose, 1 and 10 μmol/L GW9508 increased insulin secretion by 21.8 ± 4.8 and 50.8 ± 4.9%, respectively (Fig. 2A). The effect of 10 μmol/L GW9508 to increase insulin secretion appeared to be greater (in absolute terms) in the presence of 20 than in the presence of 5 mmol/L glucose (Fig. 2B). In the absence of glucose, GW9508 did not significantly increase insulin secretion (Fig. 2B).

3.3. Sulfonylureas uncouple glucose-dependence for GPR40-mediated enhancement of insulin secretion by INS-1E cells

Glipizide, a sulfonylurea, increased insulin secretion in the absence of glucose (Fig. 3A). The effects of 3 and 30 μmol/L glipizide on insulin secretion were significantly increased when 10 μmol/L GW9508 was also present (Fig. 3A). To test whether the GPR40-mediated increase of insulin secretion in the presence of glipizide is also seen with other sulfonylureas, we determined the effects of two additional sulfonylurea ligands (tolbutamide and glyben-