PRP Pharmacology Research & Perspectives
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ORIGINAL ARTICLE

A selective GPR40 (FFAR1) agonist LY2881835 provides immediate and durable glucose control in rodent models of type 2 diabetes

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Keywords
FFAR1, GPCR, GPR40, hyperglycemia, insulin, type 2 diabetes.

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Funding Information
This work was financially supported by Eli Lilly and Company.

Received: 5 October 2016; Accepted: 11 October 2016

Pharma Res Per, 4(6), 2016, e00278, doi: 10.1002/prp2.278

doi: 10.1002/prp2.278

Abstract

LY2881835 is a selective, potent, and efficacious GPR40 agonist. The objective of the studies described here was to examine the pharmacological properties of LY2881835 in preclinical models of T2D. Significant increases in insulin secretion were detected when LY2881835 was tested in primary islets from WT mice but not in islets from GPR40 KO mice. Furthermore, LY2881835 potentiated glucose stimulated insulin secretion in normal lean mice. Acute administration of LY2881835 lowered glucose during OGTTs in WT mice but not in GPR40 KO mice. These findings demonstrate that LY2881835 induces GPR40-mediated activity ex vivo and in vivo. LY2881835 was administered orally at 10 mg/kg to diet-induced obese (DIO) mice (an early model of T2D due to insulin resistance) for 14 days. Statistically significant reductions in glucose were seen during OGTTs performed on days 1 and 15. When a study was done for 3 weeks in Zucker fa/fa rats, a rat model of insulin resistance, normalization of blood glucose levels equivalent to those seen in lean rats was observed. A similar study was performed in streptozotocin (STZ)-treated DIO mice to explore glucose control in a late model of T2D. In this model, pancreatic insulin content was reduced ~80% due to STZ-treatment plus the mice were insulin resistant due to their high fat diet. Glucose AUCs were significantly reduced during OGTTs done on days 1, 7, and 14 compared to control mice. In conclusion, these results demonstrate that LY2881835 functions as a GPR40-specific insulin secretagogue mediating immediate and durable glucose control in rodent models of early- and late-stage T2D.

Abbreviations

DIO, diet-induced obese; EBSS, Earle's Balanced Salt Solution; ECL, electrochemiluminescence; FBS, fetal bovine serum; GPCR, G-protein-coupled receptors; HEK293, human embryonic kidney; IPGTT, intraperitoneal glucose tolerance test; IP, intraperitoneal; KO, knockout; LY2881835, (3S)-3-(4-{[4-(1′H-spiro [indene-1, 4′-piperidin]-1′-ylmethyl) benzyl] oxy} pheny l) hex-4-ynoic acid; OGTT, oral glucose tolerance test; PKC, protein kinase C; RFUs, relative fluorescence units; STZ, streptozotocin; T2D, type 2 diabetes; WT, wild type.

Introduction

G-protein-coupled receptor 40 (GPR40), also known as free fatty acid receptor 1 (FFAR1), is a member of a family of lipid-activated receptors (Briscoe et al. 2003; Brown et al. 2005; Itoh et al. 2003) that is highly expressed in the pancreas and enteroendocrine cells (Edfalk et al. 2008; Tomita et al. 2006) with lower levels expressed in the brain (Nakamoto et al. 2012). GPR40 mediates medium and long chain fatty acid stimulated insulin secretion in

the presence of elevated glucose levels (Briscoe et al. 2003; Itoh et al. 2003). It is predominantly coupled with the G protein α-subunit of the Gq family (Gαq). The molecular mechanisms of GPR40-mediated signal transduction have been studied in insulinoma cell lines and primary pancreatic β-cells. Activation of Gαq-protein-coupled receptors triggers an increase in phospholipase C (PLC). The latter induces an inositol 1, 4, 5-triphosphate (IP_3)-mediated intracellular calcium mobilization and protein kinase C (PKC) activation which are known to be linked to enhanced insulin secretion in pancreatic β-cells (Fujiwara et al. 2005; Prentki et al. 1997; Zawalich and Zawalich 1996). This glucose-dependent insulin secretion property has established GPR40 agonists as attractive therapeutics for the treatment of T2D.

Preclinical and clinical evidence also validates GPR40 as a drug target to treat T2D

Deletion of GPR40 impairs glucose-induced insulin secretion in mice (Alquier et al. 2009). In recent years numerous GPR40 agonists have been reported to stimulate insulin secretion in a glucose-dependent manner and attenuate hyperglycemia in rodent models of diabetes (Briscoe et al. 2006; Brown et al. 2012; Christiansen et al. 2008; Lin et al. 2011; Tan et al. 2008; Tsujihata et al. 2011). GPR40 is expressed in human islets (Feng et al. 2006; Fujiwara et al. 2005). Several human GPR40 single-nucleotide polymorphisms have been associated with insulin secretion. Ogawa et al. suggested that the His211Arg polymorphism may contribute to a variation in insulin secretory capacity. Another variant, Gly180Ser, demonstrated reduced response in vitro to fatty acids and also diminished insulin secretory capacity in human carriers (Ogawa et al. 2005; Vettor et al. 2008). Furthermore, clinical data from TAK-875 have provided human validation for the target. Oral administration of TAK-875 for 12 weeks in individuals with T2D resulted in HbA1c reduction superior to historical data with DPPIV inhibitors and comparable with that of sulfonylureas with lower incidents of hypoglycemia (Araki et al. 2012; Burant et al. 2012).

In this report, we describe the preclinical pharmacological characterization of a novel synthetic GPR40 agonist, LY2881835. LY2881835 stimulates glucose-dependent insulin secretion in mouse islets in a GPR40 specific manner. In vivo, LY2881835 improved glucose tolerance in normal, DIO- and STZ-treated mice and fatty Zucker rats. The efficacy persisted after daily dosing of LY2881835 for 14 or 21 days in these models. These data support the potential utility of LY2881835 for the treatment of T2D.

Materials and Methods

Compounds

(3S)-3-(4-{[4-(1′H-spiro[indene-1,4′-piperidin]-1′-ylmethyl) benzyl]oxy}phenyl)hex-4-ynoic acid (LY2881835) was designed and synthesized at Lilly Research Laboratories as described in patent WO 2011046851. A formulation of 0.5% methylcellulose with 0.25% Tween-80 was used for all in vivo studies.

Animals

Male ICR mice and Zucker fa/fa rats were purchased from Envigo (Indianapolis, IN). Male diet-induced obese (DIO) mice and knockout (KO) mice were purchased from Taconic (Hudson, NY). The strains used are given in the specific Methods section below. All animals were singly housed in rooms using a 12-h light/dark cycle (light on 0600) and had ad libitum access to food and water unless otherwise stated. All in vivo experiments were performed in compliance with the policies of the Animal Care and Use Committee of Eli Lilly and Company, in conjunction with the American Association for the Accreditation of Laboratory Animal Care-approved guidelines.

In vitro GPR40 receptor binding

Crude cell surface membranes were prepared from human embryonic kidney 293 (HEK293) cells stably transfected with full-length recombinant human GPR40 cDNA (DiscoveRx, Fremont, CA), using differential centrifugation methods. 10 μL of compound diluted in 100% DMSO and 90 μL of Assay Buffer (50 mmol/L Tris-HCl, pH 7.5, 5 mmol/L CaCl2, 5 mmol/L MgCl2, 0.1% w/v fatty acid-free BSA) were added to a deep 96-well polypropylene assay plate (Beckman Coulter). 200 μL of [^{3}H]-TAK-875 (52 Ci/mmol, Quotient Bioresearch Radiochemicals Ltd. Irvine, CA, USA; 5 nmol/L final concentration) and 200 μL of hGPR40 membranes (5 μg/well), both diluted in Assay Buffer, were added to the assay plate, followed by a 1 min shake and a 2 h incubation at room temperature (22°C).

Assays were terminated by filtration through GF/C glass fiber filtermats (Perkin Elmer, Waltham, MA, USA) presoaked in 50 mmol/L Tris-HCl, pH 7.5, using a Mach III cell harvester (TomTec, TomTec, Hamden, CT, USA). Filtermats were washed two times with 5 mL of ice-cold 50 mmol/L Tris-HCl, pH 7.5 buffer, dried 1 h in a convection oven at 60°C and embedded with Meltilex A solid scintillant (Perkin Elmer). Radioactivity was determined as counts per minute (CPM) using a Trilux Microbeta plate scintillation counter (Perkin Elmer). The equilibrium

dissociation constant (K_i) was calculated from the relative IC_{50} value based upon the equation $K_i = IC_{50}/(1 + L/Kd)$, where IC_{50} is the concentration of added compound that results in 50% inhibition of [^{3}H]-TAK-875 binding, L equals the concentration of radioligand used in the experiment and K_d equals the equilibrium binding affinity constant of the radioligand, determined from saturation analysis ([^{3}H]-TAK-875 K_d = 6.2 Nmol/L). Reported values for K_i are shown as geometric means ± the standard error of the mean (SEM). Geometric means are calculated by the equation GeoMean = 10(average (log K_{i1} + log K_{i2} + . . .log K_{iN})/square root of the number of replicates, N).

Calcium flux assay

HEK293-hGPR40 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) plus F12 medium in 3:1 ratio supplemented with 10% fetal bovine serum (FBS) and 800 μg/mL geneticin at 37°C and 5% CO_2. Agonist assays were performed using a Calcium 4 Dye assay kit (Molecular Devices) in the presence (0.1%) of fatty acid-free bovine serum albumin (BSA) in assay buffer. Receptor activation was measured as an increase in intracellular calcium using the Fluorescence Imaging Plate Reader (FLIPR) technology. Maximum change in fluorescence over the base line was used to determine agonist response. EC_{50} values were calculated using Excel Fit software (version 4; IDBS) by plotting concentration versus relative fluorescence units (RFUs). Percent efficacy was calculated based on maximal response exhibited by compound compared to the natural ligand, linoleic acid at 100 μmol/L. Reported values for EC_{50} are shown as geometric means ± the standard error of the mean (SEM).

Human, rat and mouse β-arrestin assays

Human osteosarcoma (U2OS) cells expressing mouse, rat, or human GPR40 were developed by DiscoveRx. These cells coexpress the Prolink (PK)-tagged GPR40 and the Enzyme Acceptor (EA)-tagged beta-arrestin fusion proteins. If activation of GPR40 stimulates beta-arrestin (β-arrestin) recruitment, it would force complementation of the beta galactosidase (β-gal) enzyme fragments, forming a β-gal enzyme that generates a chemiluminescent signal using the DiscoveRx PathHunter detection kit. Cells were incubated overnight at 5000 cells/well in 384-well plates in culture media containing 1% FBS. Serial diluted compounds in DMSO (2-fold dilutions to generate 20 concentrations) were step-down diluted in culture media containing 1% FBS and added to cells with a final top concentration starting of 100 μmol/L. After addition of compounds, cells were incubated for 90 min at 37°C in 5% CO_2 incubator, and DiscoveRx kit detection reagents were added. Measurement of the electrochemiluminescent (ECL) signal was ascertained with the Envision reader, after 1-h incubation at room temperature. Data were fit to a four-parameter-fit logistics to determine EC_{50} values, and percent stimulation was measured versus maximum response to a reference GPR40 agonist at 1 μmol/L. Reported values for EC_{50} are shown as geometric means ± the standard error of the mean (SEM).

Insulin secretion from isolated mouse islets

Pancreatic islets were isolated from 2- to 3-month-old male wild-type C57BL/6 (WT) or GPR40 KO mice. Purified islets (Histopaque-1100 gradient [Sigma-Aldrich, St. Louis, MO, USA], 18 min at 750x gravity) were cultured overnight in RPMI-1640 medium (Invitrogen, Waltham, MA, USA) containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, and preconditioned by a 30 min incubation in Earle's Balanced Salt Solution (EBSS) supplemented with 0.1% BSA and 2.8 mmol/L glucose. Subsequently, islets were incubated for 90 min in EBSS (Invitrogen) supplemented with 0.1% BSA, 2.8 or 11.2 mmol/L glucose and increasing levels of compound (6 batches of 4 islets per condition). Linoleic acid and GLP-1(7–36) amide (Bachem) were used as positive controls. Insulin levels in medium were measured using an internally developed ECL insulin assay.

Compound exposure and IPGTT in lean mice

The exposure study was performed in ICR mice from Envigo. The animals received oral administration of LY2881835 at 10 mg/kg. EDTA-plasma was obtained by retro orbital bleeding at 0.5, 1, 2, 4, and 8 h post compound administration. LY2881835 concentration was determined by LC-MS.

Intraperitoneal glucose tolerance test (IPGTT) was performed in 9–10-week-old male ICR. The day before an IPGTT (approximately 1600), animals were fasted in clean cages. The following morning (approximately 0800), animals were randomized using fasting glucose and body weight. Mice were orally administered LY2881835 at 0.3, 1, 3, or 10 mg/kg, or vehicle 60 min prior to an intraperitoneal (IP) injection of glucose (2 g/kg). Blood glucose levels were determined using a glucometer from tail bleeds taken at 0, 15, 30, and 60 min after the glucose challenge. Average of two readings is reported at each time point. An area under curve (AUC) of glucose-time during the IPGTT until 60 min after glucose administration was calculated. Also, blood samples (12–15 μL) were collected into serum tubes at 0, 3, 6, and 15 min post glucose injection for insulin measurement. Serum tubes were centrifuged at 3000*g* for 5 min and serum transferred into

96-well plates for insulin analysis using a Mesoscale rat/mouse insulin assay. An AUC of insulin-time during the IPGTT until 15 min after glucose administration was calculated.

OGTT in WT and KO mice

Two- to three-month-old chow-fed male WT or GPR40 KO or GPR120 KO mice were used. The night before an OGTT (approximately 1600), animals were fasted in clean cages. The following morning (approximately 0800), animals were randomized using fasting glucose and body weight. Mice were orally administered LY2881835 at 30 mg/kg, or vehicle 60 min prior to an oral gavage of glucose (2 g/kg). Blood glucose levels were determined using a glucometer from tail bleeds taken at 0, 15, 30, 60, and 120 min post the glucose challenge. Average of two readings is reported at each time point.

Glycemic control in DIO mice

Five- to six-month-old male DIO mice from Taconic were used in this study. After 2 weeks acclimation to the facility, the mice are randomized to treatment groups (n = 7/group) using their body weight and fasting blood glucose levels. Mice were orally administered LY2881835 at 10 mg/kg or vehicle once a day for 15 days. OGTTs (as described) were performed on days 1 and 15 with blood samples taken at 0, 15, 30, 60, and 120 min after the glucose challenge. Average of two readings is reported at each time point.

Glycemic control in fatty zucker fa/fa rats

Two- to three-month-old male Zucker fa/fa rats from Envigo were used in this study. After two weeks acclimation to the facility, rats were randomized to treatment groups (n = 6/group) based on their body weight and blood glucose levels. Rats were orally administered LY2881835 at 1 mg/kg or vehicle once a day for 21 days. OGTTs (as described) were performed on days 1 and 21 with blood samples taken at 0, 10, 20, 40, and 60 min after the glucose challenge. Average of two readings is reported at each time point. A group of age matched lean rats were included in the study.

Glycemic control in STZ-treated DIO mice

Five- to six-month-old male DIO mice from Taconic were injected intraperitoneally (IP) with 50 mg/kg of streptozotocin (STZ) twice in a week. Two weeks post the STZ injection, animals with glucose levels between 180 to 300 mg/dL at 9 AM were selected for the study. The day before the study, animals were randomized into groups based on body weight and glucose levels. LY2881835 was administered orally once a day for 16 days at 30 mg/kg. A DPP IV inhibitor, sitagliptin at 3 mg/kg was included as a positive control. Sitagliptin was administered 60 min and LY2881835 at 30 min prior to the start of an OGTT. OGTTs were performed on days 1, 7, and 14 with blood samples taken at 0, 15, 30, 60, and 120 min after the glucose challenge. Average of two readings is reported at each time point. A group of age matched non-STZ-treated mice were included in the study. At the completion of 16 day treatment the pancreas were collected to determine pancreatic insulin contents.

Statistical analysis for in vivo studies

Data are expressed as mean ± standard error (SEM). Blood glucose values were analyzed using ANOVA with repeated measures with Log2 transformation to stabilize variation. Dunnett comparison was carried out to assess the treatment effect compared to vehicle. AUCs were analyzed using one-way ANOVA followed by Dunnett comparison. Significance is denoted at $P < 0.05$.

Results

LY2881835 is a structurally novel, potent, efficacious, and selective agonist at GPR40

LY2881835 is a novel spiro [indene-1, 4′-peperdine] (Fig. 1) identified through hypothesis driven structural modifications to endogenous FFAs, focused on breaking planarity and reducing lipophilicity. LY2881835 exhibits good binding affinity in membranes expressing recombinant human GPR40 (hGPR40). The binding affinity for LY2881835 is K_i = 4.7 nmol/L (Table 1). It is a 164 nmol/L partial agonist (62%) on hGPR40 when tested in a calcium flux assay (FLIPR). Moreover, LY2881835 is a potent full agonist in β-arrestin

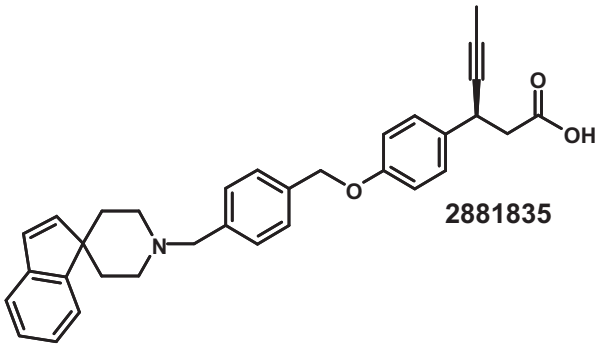


Figure 1. Chemical structure of the GPR40 agonist (3S)-3-(4-{[4-(1′H-spiro [indene-1, 4′-piperidin]-1′-ylmethyl) benzyl] oxy} pheny l) hex-4-ynoic acid (LY2881835).

Table 1. In vitro pharmacology of the GPR40 agonist LY2881835 at human (hGPR40), mouse (mGPR40), or rat (rGPR40) receptor.

hGPR40 binding affinity $K_i \pm$ SEM (nmol/L)	hGPR40 Ca^{2+} flux $EC_{50} \pm$ SEM (nmol/L) (max efficacy)	hGPR40 β-arrestin $EC_{50} \pm$ SEM (nmol/L) (max efficacy)	mGPR40 β-Arrestin $EC_{50} \pm$ SEM (nmol/L) (max efficacy)	rGPR40 β-Arrestin $EC_{50} \pm$ SEM (nmol/L) (max efficacy)
4.7 ± 3.5 (N = 3)	164 ± 94 (62%) (N = 7)	8.7 ± 6.3 (137%) (N = 19)	0.85 ± 0.66 (133%) (N = 18)	2.0 ± 1.6 (124%) (N = 9)

recruitment assays using human, mouse, and rat GPR40 (Table 1). LY2881835 demonstrates selectivity against other closely related receptors (GPR43, GPR120, GPR119, TGR5) plus the peroxisome proliferator-activated receptors, PPARα, β/δ and γ. LY2881835 was also examined in a broad range of assays representing nuclear hormone receptors, other G-protein-coupled receptors(GPCRs) and enzyme targets. No positive signals were detected in any assay system not expressing GPR40 (data not shown). These data confirm a high level of selectivity of LY2881835 for GPR40.

LY2881835 stimulates GPR40-mediated insulin secretion in mouse primary islets

Primary islets isolated from GPR40 WT and KO mice were employed to examine the effects of LY2881835 on insulin secretion and to evaluate whether the effects were GPR40 mediated. Initial studies with LY2881835 using primary islets from WT mice demonstrated that enhanced insulin secretion was observed when islets were cultured with LY2881835 in the presence of 11.2 mmol/L glucose but not when cultured with 2.8 mmol/L glucose (data not shown). As shown in Figure 2A, a statistically significant increase in insulin secretion was demonstrated when LY2881835 was tested in primary islets from WT mice in the presence of 11.2 mmol/L glucose. Enhanced insulin secretion was absent when LY2881835 was tested using the same conditions in primary islets from GPR40 KO mice (Fig. 2B). In addition, a reduction in insulin secretion was seen in islets incubated with linoleic acid as expected since linoleic acid is a natural ligand for GPR40. These data confirm that LY2881835 stimulates glucose-dependent insulin secretion through GPR40.

LY2881835 achieves sustained plasma concentrations and stimulates insulin secretion in lean mice

Before extensive in vivo, characterization studies could be designed, it was important to understand the concentration time course of LY2881835. Therefore, LY2881835 was administrated orally at 10 mg/kg to lean mice, and plasma samples were collected for determination of compound concentration over a time course of 8 h. Plasma concentrations peaked and remained elevated at 15, 30,

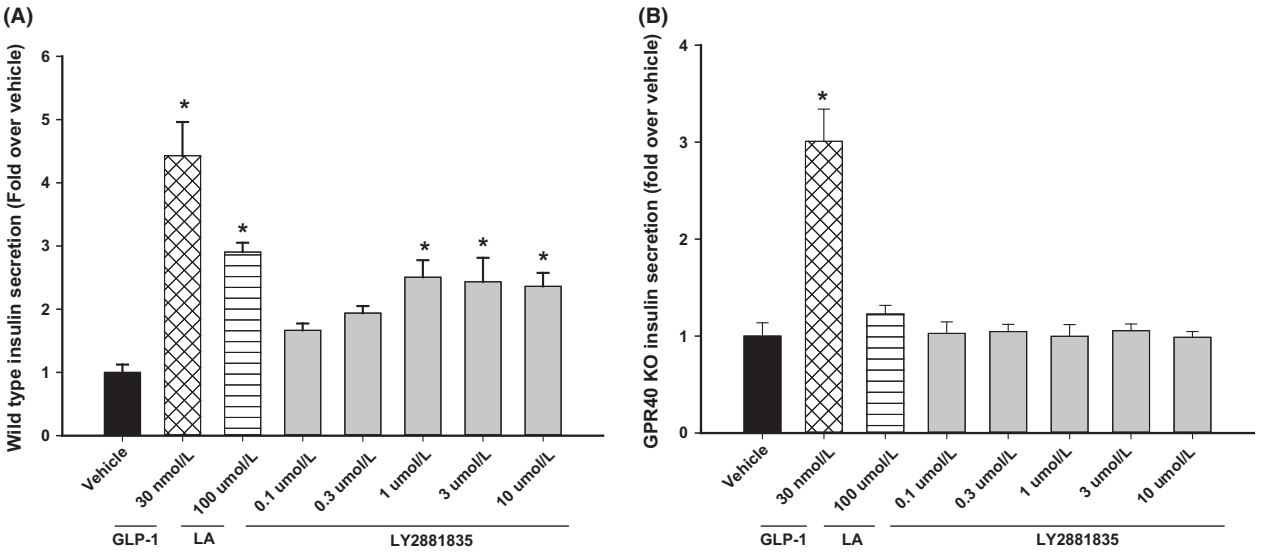


Figure 2. Effect of LY2881835 on insulin secretion in primary mouse islets. (A) Enhanced insulin secretion in WT primary islets in the presence of 11.2 mmol/L glucose. LA, linoleic acid, an endogenous GPR40 ligand. (B) No insulin secretion with LY2881835 or LA in primary islets isolated from GPR40 KO mice. *$P < 0.05$ versus vehicle. Data were analyzed using ANOVA with Dunnett's comparison.

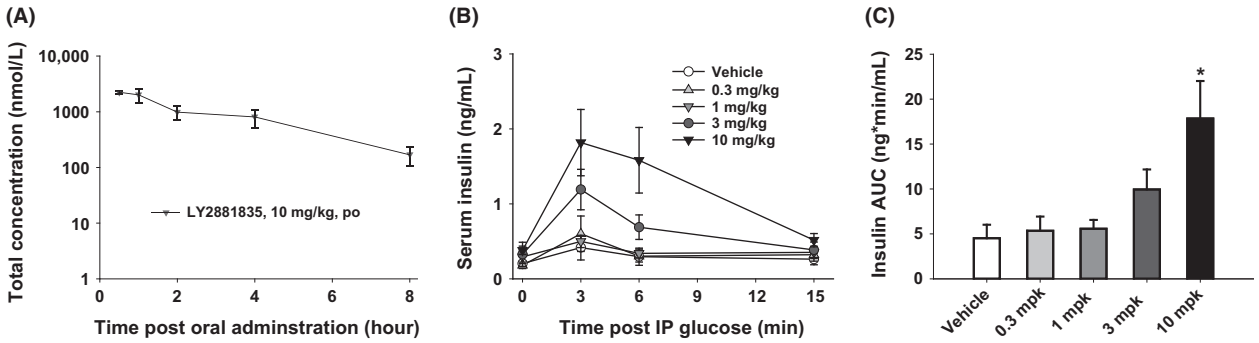


Figure 3. Plasma exposure profile of LY2881835 plus effect on glucose-dependent insulin secretion in normal mice. Total (A) circulating concentrations of LY2881835 were determined in lean mice after a single oral dose (10 mg/kg). A dose-dependent enhancement of insulin secretion (B) and depicted by AUC (C) were detected during an IPGTT in lean mice. Data were expressed as mean ± SEM with six animals in each group. $*P < 0.05$ versus vehicle. Insulin AUCs were analyzed using ANOVA with Dunnett's comparison. IPGTT, intraperitoneal glucose tolerance test.

and 60 min followed by a slight decrease in levels throughout the 8 h study (Fig. 3A).

To evaluate the effects on insulin secretion in vivo, LY2881835 was administered orally at 0.3, 1, 3, and 10 mg/kg to normal ICR mice. Sixty min after compound administration, an IPGTT was performed. Insulin levels were measured at 0, 3, 6, and 15 min. LY2881835 showed a clear dose-dependent enhancement in insulin secretion (Fig. 3B). Compared to the vehicle-treated mice, statistically significant enhancement in insulin AUC was seen at the 10 mg/kg dose group (Fig. 3C). These findings support that LY2881835 stimulates glucose-dependent insulin secretion in vivo.

LY2881835 lowers glucose in a GPR40-dependent manner in vivo

To evaluate specificity in vivo, LY2881835 was administered orally at 30 mg/kg to GPR40 WT or KO mice. Sixty min after compound administration, an OGTT was performed. Compared to the vehicle-treated mice, significant glucose lowering was seen in WT mice (Fig. 4A) but not in GPR40 KO mice (Fig. 4B). Glucose lowering efficacy in closely related GPR120 KO mice was similar to that observed in WT mice (Fig. 4C).

LY2881835 inhibits postprandial glucose excursion in DIO mice

The DIO mouse model is a model of early T2D due to insulin resistance. LY2881835 was administered orally once daily at 10 mg/kg to DIO mice for 2 weeks with OGTTs performed on days 1 and 15. Significant reductions in glucose levels were demonstrated during OGTTs on both days indicating that desensitization of the receptor did not occur with chronic activation. Fasting glucose levels were also significantly reduced at time 0 of the OGTTs (60 min after LY2881835 was administered), but no evidence of hypoglycemia was observed (Fig. 5A and B).

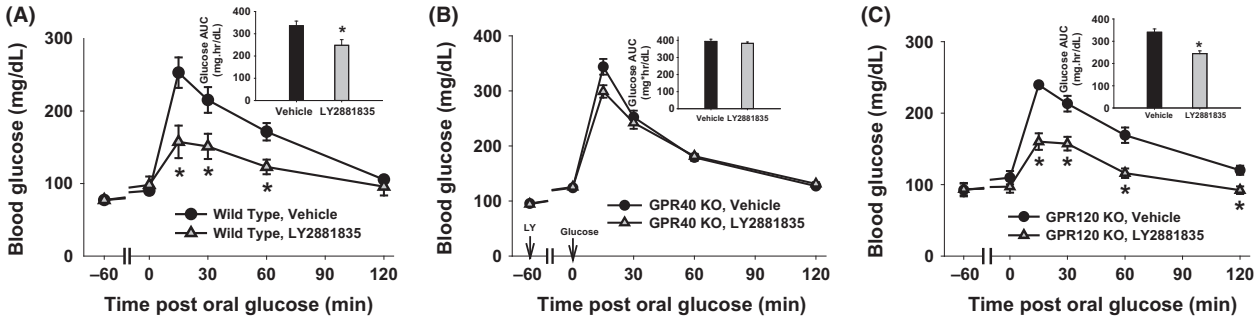


Figure 4. Glucose levels during OGTTs in WT or GPR40 KO mice after 30 mg/kg LY2881835 administration. (A) LY2881835 significantly reduced postprandial glucose levels in WT mice. (B) LY2881835 had no effect on glucose levels in GPR40 KO mice. (C) LY2881835 significantly reduced postprandial glucose levels in GPR120 KO mice. $*P < 0.05$ versus vehicle with eight animals per group. Data were analyzed using ANOVA with repeated measures.

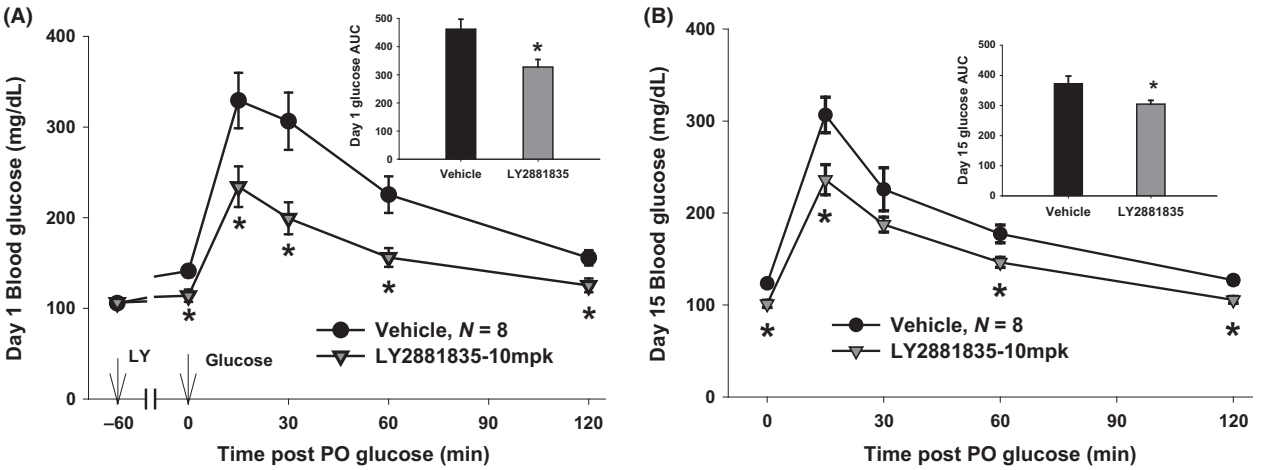


Figure 5. Significantly lowered fasting glucose and postprandial glucose levels during OGTTs performed on days 1 and 15 after oral administration of LY2881835 at 10 mg/kg to diet-induced obese mice. Mice were treated with vehicle or 10 mg/kg LY2881835 60 min prior to the oral glucose challenge A. OGTT on day 1. (B) OGTT on day 15. $*P < 0.05$ versus vehicle with eight animals per group. Data were analyzed using ANOVA with repeated measures.

LY2881835 inhibits postprandial glucose excursion in Zucker fa/fa rats

The Zucker fa/fa rat is another model of early T2D due to significant insulin resistance. LY2881835 was administered orally once daily for 21 days to Zucker fa/fa rats at 1 mg/kg. Rosiglitazone, a PPARγ agonist, was included in this study at 3 mg/kg to serve as a positive control. OGTTs were performed on days 1 and 21 with blood samples taken at 0, 10, 20, 40 and 60 min after the oral glucose challenge. Blood glucose was significantly lowered to levels seen in lean control rats during OGTTs performed on days 1 (Fig. 6A) and 21 (Fig. 6B) confirming efficacy and durability of LY2881835 in this model.

LY2881835 inhibits glucose excursion in STZ-treated DIO mice

The STZ-treated DIO mouse model is a model of later-stage T2D due to insulin resistance combined with reduced insulin capacity in pancreatic islet beta cells. LY2881835 was administered orally once a day for 14 days to STZ-treated DIO mice at 30 mg/kg. A DPP-IV

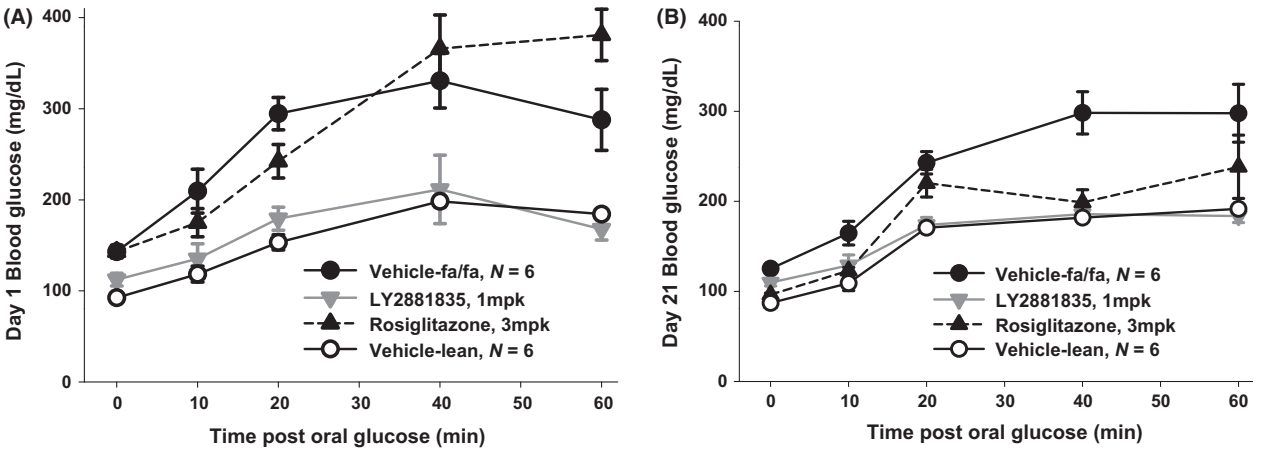
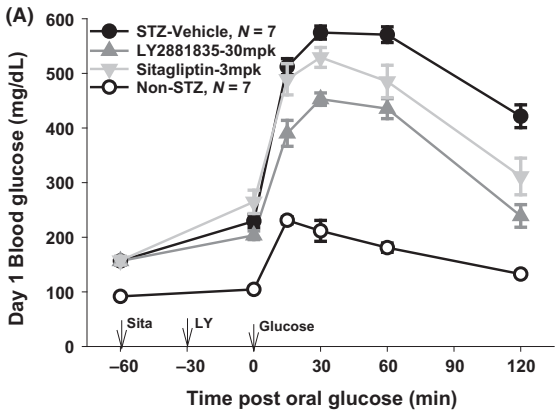
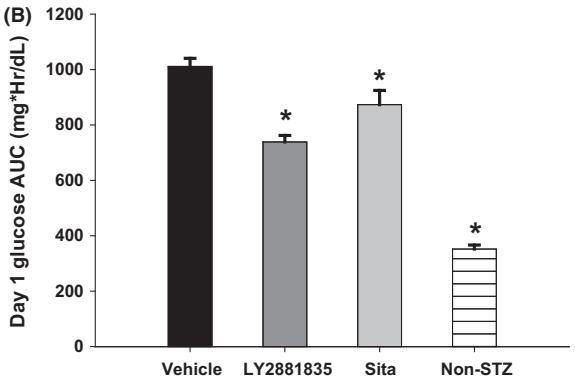
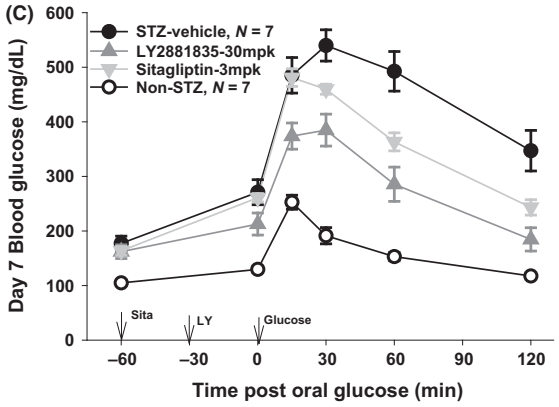
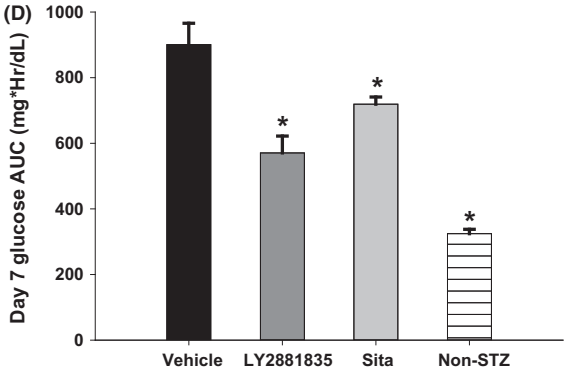


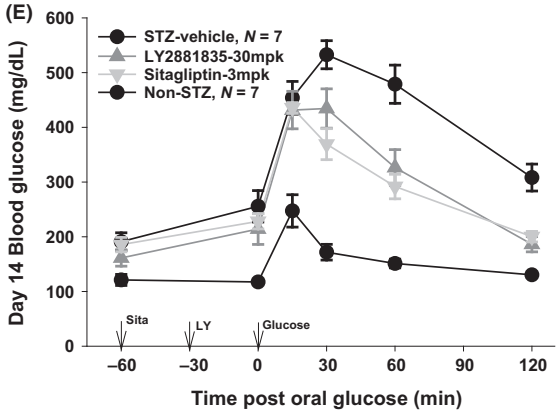
Figure 6. Effect of chronic administration of LY2881835 on glucose tolerance in Zucker fa/fa rats. Vehicle or LY2881835 at 1 mg/kg was orally administered once a day for 3 weeks. (A) Reductions in glucose levels during the OGTT after the initial treatment (Day 1). (B) Reductions in glucose levels during the OGTT after 21 day treatments (Day 21).

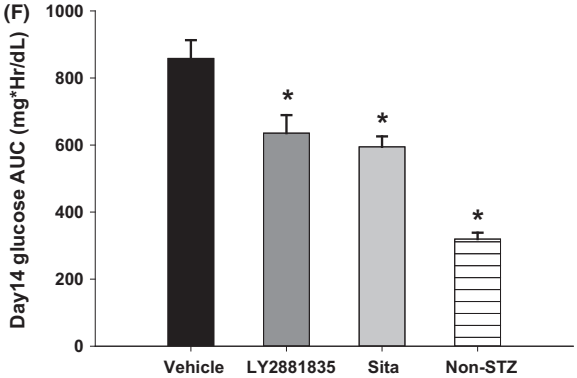












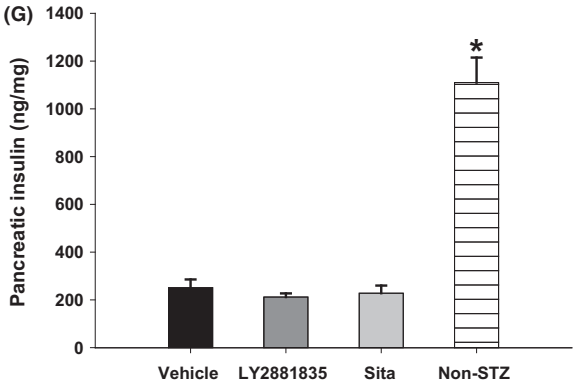


Figure 7. Both LY2881835 and sitagliptin, a DPP-IV inhibitor, significantly lowered glucose levels during an OGTT in STZ-treated diet-induced obese mice. Vehicle or LY2881835 at 30 mg/kg or sitagliptin at 3 mg/kg was orally administered once a day for 16 days. (A–B) Glucose and AUC during OGTT on day 1. (C–D) Glucose and AUC during OGTT on day 7. (E–F) Glucose and AUC during OGTT on day 14. (G) Pancreatic insulin content on day 16. $*P < 0.05$ versus vehicle control with 7 animals per group. Data were analyzed using ANOVA with Dunnett's comparison. AUC, area under curve; STZ, streptozotocin.

inhibitor, sitagliptin, was included as a positive control. OGTTs were performed on days 1, 7 and 14. As shown in Figure 7A, LY2888135 lowered blood glucose after a single treatment. Compared to the vehicle-treated mice, significant reductions in glucose AUC were seen for both LY2881835 and sitagliptin groups (Fig. 7B). Results are similar for additional OGTTs performed on days 7 and 14 (Fig. 7C–F). Compared to nontreated animals, pancreatic insulin content was reduced ~80% due to STZ-treatment. Two weeks of treatment with LY2881835 or sitagliptin did not change insulin content (Fig. 7G).

Discussion

Over the past several decades, the prevalence of diabetes has continued to rise thus becoming a primary health and economic burden globally. Uncontrolled hyperglycemia leads to several conditions that impact both the morbidity and mortality of these individuals (World Health Organization, 2016). However, currently available oral treatments for T2D are either poor in efficacy or associated with undesired adverse effects such as hypoglycemia and weight gain. Therefore, weight neutral therapies effective in glucose control with low adverse side effects are highly desirable. GPR40 agonists possess the characteristics to be one such therapy. In this study, we investigated a structurally novel, efficacious and selective GPR40 agonist in multiple preclinical models.

Since GPR40 is a Gαq-coupled GPCR, the compound was initially evaluated in a calcium flux assay using Fluorescence Imaging Plate Reader (FLIPR) technology. LY2881835 is a partial agonist in the FLIPR assay. During extensive testing of GPR40 agonists, it became apparent that activity in FLIPR assays did not correlate with in vivo glucose lowering. Therefore, activity in β-arrestin assays was explored and determined to be a more accurate predictor of in vivo efficacy. LY2881835 is a potent full agonist when tested in β-arrestin assays using human, mouse, or rat GPR40. The potency of LY2881835 in the hGPR40 β-arrestin assay correlates with its binding affinity (Table 1). LY2881835 confirms the well-documented fact that GPR40 agonists stimulate glucose-dependent insulin secretion (Fig. 3). LY2881835 did not stimulate insulin secretion in islets from GPR40 KO mice (Fig. 2B). Glucose improvement during an OGTT was absent in GPR40 null mice compared to that seen in wild-type mice (Fig. 4A and B) and in closely related GPR120 null mice (Fig. 4C). These findings confirm that LY2881835 is a highly selective GPR40 agonist.

Studies were also performed in three different preclinical models to address potential durability concerns during early and late T2D. First, a single dose of LY2881835 improved fasting glucose and postprandial hyperglycemia in DIO mice, an early model of T2D due to insulin resistance. The antidiabetic activity was persistent throughout the duration of treatment, 15 days (Fig. 5). When LY2881835 was administered to an insulin-resistant rat model, Zucker fa/fa rats, for 21 days, continued glucose lowering efficacy was demonstrated (Fig. 6). Results with rosiglitazone in this study were as expected for an insulin sensitizer, with no glucose lowering during the OGTT on day1 followed by significant glucose lowering during the OGTT on day 21. These data demonstrate the immediate onset of therapeutic activity with a GPR40 agonist compared to that not seen with a PPARγ agonist (Fig. 6A). The third model, STZ-treated DIO mice, represents late-stage T2D by combining insulin resistance with severely impaired insulin secretion. Results from this study clearly demonstrate that immediate and durable glucose lowering efficacy is achieved with LY2881835 in a late-stage model of T2D. Although Tanaka et al. reported recently that administration of a GPR40 agonist to ob/ob mice for 4 weeks significantly increased pancreatic insulin concentrations (Tanaka et al. 2013), we saw no change in pancreatic insulin content after 16 days of treatment. This discrepancy may reflect the animal models, the duration of treatment, or the GPR40 agonists used in the studies. In summary, no tachyphylaxis was demonstrated after repeat administration of LY2881835 to multiple preclinical models suggesting that this mechanism will be effective in treating both early- and late-stage T2D. In addition, these finding suggest that treatment with a GPR40 agonist will provide the distinct advantage of immediate glucose lowering with equal or greater efficacy compared to that achieved with a PPARγ agonist.

In conclusion, we have discovered and characterized a novel GPR40 agonist, LY2881835. LY2881835 functions as a GPR40-specific insulin secretagogue mediating immediate and durable glucose control in rodent models of early and late T2D. These findings suggest that a GPR40 agonist may provide effective glucose control in diabetics with insulin resistance and substantially reduced β-cell function.

Acknowledgements

This work was financially supported by Eli Lilly and Company. The authors thank Hsiu-Chiung Yang and Travis Shockley for insulin analysis; Han Wu for statistical assistance; and Tim Jones for compound exposure values.

Author Contribution

Chen and Miller participated in research design and wrote or contributed to the writing of the manuscript. Kahl, Song, Riley, Hu, and Peng conducted the experiments. Kahl, Montrose, Bokvist, Maiti, and Hamdouchi contributed new reagents or analytical tools. Chen, Song, and Riley performed data analysis.

Disclosure

None declare.

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