Chronic Exposure to SCO-267, an Allosteric GPR40 Full Agonist, Is Effective in Improving Glycemic Control in Rats

Ryokichi Koyama, Mitsugi Ookawara, Masanori Watanabe, and Yusuke Moritoh

Research Division, SCOHIA PHARMA, Inc., Fujisawa, Kanagawa, Japan

Received September 17, 2020; accepted January 15, 2021

ABSTRACT

Full agonist-mediated activation of free fatty acid receptor 1 (FFAR1/GPR40) alleviates diabetes in rodents. Considering that diabetes is a chronic disease, assessment of treatment durability of chronic exposure to a GPR40 full agonist is pivotal for treating patients with diabetes. However, the physiologic significance of chronic in vitro and in vivo exposure to GPR40 full agonists is largely unclear. Here, we evaluated the in vitro and in vivo effects of chronic treatment with SCO-267, a GPR40 full agonist, on signal transduction and glucose control. In vitro experiments showed that SCO-267 is an allosteric full agonist for GPR40, which activates the Gq, Gs, and G12/13 pathways and β-arrestin recruitment. The calcium signal response was largely sustained in GPR40-overexpressing CHO cells even after prolonged incubation with SCO-267. To evaluate the in vivo relevance of chronic exposure to SCO-267 full agonists, SCO-267 (1 and 10 mg/kg) was administered once daily to neonatally streptozotocin-induced diabetic rats for 15–33 days, and glucose control was evaluated. After 15 days of dosing followed by the drug washout period, SCO-267 improved glucose tolerance, most likely by increasing insulin sensitivity in rats. After 33 days, repeated exposure to SCO-267 was highly effective in improving glucose tolerance in rats. Furthermore, chronic exposure to SCO-267 increased pancreatic insulin content. These results demonstrated that even after chronic exposure, SCO-267 effectively activates GPR40 in cells and rats, suggesting the clinical application of SCO-267 in treating chronic diseases including diabetes.

SIGNIFICANCE STATEMENT

GPR40 is a validated therapeutic target for diabetes. This study showed that even after chronic exposure, SCO-267, an allosteric GPR40 full agonist, effectively activates GPR40 in cells and rats; these results suggest a durable efficacy of SCO-267 in patients.

Introduction

Free fatty acid receptor 1 (FFAR1/GPR40) is a G protein-coupled receptor (GPCR) that is endogenously activated by medium-to-long chain fatty acids (Briscoe et al., 2003; Itoh et al., 2003). The receptor potentiates the secretion of glucose-dependent insulin from pancreatic β cells and stimulates the secretion of incretins such as glucagon-like peptide 1 (GLP-1) from intestinal endocrine cells (Mancini and Poitout, 2013; Pais et al., 2016). Fasiglifam, a partial agonist of GPR40, which improves glucose control mainly by stimulating insulin secretion (Tsujihata et al., 2011), showed a glucose-lowering effect in clinical studies on patients with type 2 diabetes mellitus (T2DM) (Burant et al., 2012; Kaku et al., 2016). The results of these clinical trials indicate that GPR40 is a promising therapeutic target for T2DM. Since the report of the superior glucose-lowering efficacy of a full GPR40 agonist, AM-1638, over a partial GPR40 agonist (Lin et al., 2012), various synthetic full GPR40 agonists have been investigated as new drug candidates (Li et al., 2016, 2020). These full agonists bind to the allosteric binding site of the receptor independent of binding sites for endogenous ligands or fasiglifam (Lin et al., 2012, 2016; Lu et al., 2017; Ho et al., 2018). Furthermore, in contrast to the partial agonists activating the Gq signal, these full agonists activate not only the Gq signal but also the Gs and G12/13 signals (Hauge et al., 2014) and G12/13 signals (Rives et al., 2018), which may explain its robust incretin stimulation and maximal efficacy in preclinical models (Defossa and Wagner, 2014). Based on these observations, GPR40 full agonists have been suggested as a novel strategy to treat diabetes (Li et al., 2018).

Considering that diabetes is a chronic disease with metabolic dysfunctions, the durability of drug efficacy is highly important (Kahn et al., 2006), and this is also the case with full agonists for GPR40. Generally, chronic agonist exposure causes GPCR desensitization and internalization, and the response is reduced (Drake et al., 2006; Kelly et al., 2008). These effects occur within a few minutes to hours, depending on the GPCR and agonist ligands. For example, relaxin family peptide receptor 1 demonstrates prolonged agonist-induced cAMP response by poor internalization and a lack of β-arrestin interaction (Callander et al., 2009). In addition, the neuropeptide FF-activated proto-oncogene Mas can be restimulated...
in calcium response, whereas the receptor activated by non-peptide ligands cannot be stimulated (Tirupula et al., 2014). A durable glucose control effect of GPR40 partial agonists in preclinical models has been reported (Chen et al., 2016, 2020), and patients with T2DM treated with fasiglifam continued to exhibit reduced HbA1c for 52 weeks (Kaku et al., 2016). However, the in vitro and in vivo effect of chronic exposure to GPR40 full agonist on downstream signaling of GPR40 is still unclear. Therefore, evaluating the downstream signaling of GPR40 upon chronic treatment with GPR40 full agonists is of importance when considering the application of this class of compounds for treating chronic metabolic diseases in clinical settings.

The present study was conducted to reveal the effect of chronic exposure to SCO-267, a GPR40 full agonist, on downstream signaling of GPR40 in vitro and in vivo. The signal transduction and allosteric properties of SCO-267 were evaluated using a recombinant expression system. In addition, the chronic effect of SCO-267 was investigated with respect to the Goα signal in cell models. Finally, the chronic effects of SCO-267 on glycemic control were evaluated in a rat model.

Materials and Methods

Materials. SCO-267, fasiglifam, and AM-1638 were obtained from SCOHIA PHARMA (Fujisawa, Japan). γ-Linolenic acid was purchased from Sigma-Aldrich (Tokyo, Japan). For in vitro studies, compounds were dissolved in dimethyl sulfoxide, except for γ-linolenic acid, which was dissolved in ethanol. For in vivo studies, compounds were suspended in 0.5% methylcellulose solution (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

Myo-Inositol 1 Phosphate Homogeneous Time-Resolved Fluorescence Assay for Goα Signaling. CHO dihydrofolate reductase–deficient cells stably expressing human FFAR1 (mRNA for GPR40) with different receptor mRNA expression levels were established previously (Yabuki et al., 2013). The mRNA copy number in high (clone 104) and low (clone 2) FFAR1-expressing cells was quantified by quantitative polymerase chain reaction as reported previously (Yabuki et al., 2013). These cells were cultured in minimum essential medium-α (Thermo Fisher Scientific, Waltham, MA) supplemented with 10% dialyzed FBS (GE Healthcare, Chicago, IL), 100 U/ml penicillin-streptomycin (FUJIFILM Wako), and 10 mM HEPES solution (FUJIFILM Wako) and were tested for mycoplasma contamination before the experiment. The day before the assay, human FFAR1-expressing CHO cells were plated at 5000 cells per well in poly-d-lysine–coated 384-well white plates. After culturing overnight, the cells were treated with compounds in stimulation buffer [included in the IP-One homogeneous time-resolved fluorescence (HTRF) assay kit; PerkinElmer, Waltham, MA] containing 0.01% fatty acid–free bovine serum albumin (BSA) at varying concentrations and incubated at 37°C for 30 minutes. Intracellular myo-inositol 1 phosphate (IP1) level was measured using the IP-One HTRF Assay Kit (PerkinElmer) according to the manufacturer’s protocol. HTRF signals were detected using the EnVision multimode plate reader (PerkinElmer). For the desensitization assay, CHO cells expressing high levels of human FFAR1 (clone 104) were pretreated with the compounds in culture medium for 4 hours at 37°C. To remove excess compound, the cells were washed twice with Dulbecco’s phosphate-buffered saline and treated with compounds at 37°C for 30 minutes. Intracellular cAMP level was determined using the HTRF cAMP Goα Dynamic Kit (PerkinElmer) according to the manufacturer’s instructions. HTRF signals were detected using EnVision (PerkinElmer). Raw data or corrected data were analyzed using Prism 7, and a four-parameter logistic fit equation was used to determine EC50 and DC50 for the desensitization analysis.

Serum Response Factor Response Element Reporter Gene Assay for Goα Signaling. CHO cells expressing high levels of human FFAR1 (clone 104) were transfected with pGL4.34 (E1350, Promega, Madison, WI, USA), which contains a luciferase gene with serum response factor response element (SRF-RE) in response to serum response factor through the Goα12/13-RhoA–mediated pathway, using Lipofectamine 3000 (Thermo Fisher Scientific). The transfected cells were plated at 15,000 cells per well in poly-d-lysine–coated 384-well white plates. After culturing for 4 hours, the culture medium was replaced with assay medium [minimum essential medium-α containing 10 mM HEPES (pH 7.5) and 2% FBS] before overnight incubation at 37°C in the presence of 5% CO2. The cells were stimulated with drugs in the assay medium for 6 hours at 37°C, and luciferase activity was measured using EnVision (PerkinElmer) with the Steady-Glo luciferase assay system (Promega). Raw data were analyzed using Prism 7, and a four-parameter logistic fit equation was used to determine EC50.

β-Arrestin Recruitment Assay. The PathHunter β-arrestin assay (DiscoverX, Fremont, CA) was used to assess β-arrestin recruitment activity. PathHunter HEK293 cells stably expressing human GPR40, obtained from Takeda Pharmaceutical Company Limited (Tokyo, Japan; mycoplasma tested), were added into poly-d-lysine–coated 384-well white plates at 10,000 cells per well in Dulbecco’s modified Eagle’s medium supplemented with 10% dialyzed FBS, 0.05 mg/ml hygromycin B (FUJIFILM Wako), 0.25 mg/ml gentamicin (FUJIFILM Wako), and 100 U/ml penicillin-streptomycin. After overnight incubation at 37°C in the presence of 5% CO2, the medium was replaced with Opti-MEM I (Thermo Fisher Scientific) containing 0.1% fatty acid–free BSA. Thereafter, compound stimulation was performed for 4 hours at 37°C, followed by incubation with the PathHunter Detection Reagent Solution at 22–26°C for 1 hour. Luminescence was measured using EnVision (PerkinElmer). Raw data were analyzed using Prism 7, and a four-parameter logistic fit equation was used to determine EC50.

Animals. Male neonatally streptozotocin-induced (N-STZ) diabetic rats were developed by subcutaneous administration of 120 mg/kg streptozotocin (STZ) to Wistar Kyoto rats (RABICs, LTD., Kanagawa, Japan) at 1.5 days after birth. Saline-injected rats were used as normal control rats. N-STZ rats have been reported to show dysfunction of insulin secretion and action, which is similar to the pathology of human T2DM (Porterba et al., 2007). All animals were housed in rooms under a 12-hour light/dark cycle (light on at 7:00 AM) and had ad libitum access to standard laboratory chow diet (CE-2; CLEA Japan, Inc., Tokyo, Japan) and tap water. The care of the animals and use of the experimental protocols were approved by the
Institutional Animal Care and Use Committee of Shonan Health Innovation Park accredited by the American Association for Accreditation of Laboratory Animal Care. For animal experiments, 0.5% methylcellulose was used as the vehicle. All blood samples used in the present study were obtained via the tail vein of the animals.

Subchronic Study of SCO-267 for Evaluating Insulin and Glucose Tolerance. Twenty-five-week-old N-STZ rats were randomized into groups based on body weight, fasting glucose level, and glycosylated hemoglobin (n = 6). The animals were orally administered either SCO-267 (1 and 10 mg/kg) or vehicle once a day for 15 days, followed by a washout period of 3 days. The first day of treatment was designated as day 1. The insulin tolerance test (ITT) and oral glucose tolerance test (OGTT) were performed on day 18 after overnight fasting (17 hours). In the ITT, insulin (0.25 IU/kg; Novo-Nordisk, Bagsvaerd, Denmark) was injected subcutaneously, and plasma glucose level was determined at the indicated time points. In the OGTT, glucose (1.5 g/kg) was orally administered, and blood glucose and insulin levels were determined at the indicated time points. The plasma level of SCO-267 was determined before administering glucose in the OGTT.

Chronic Study of SCO-267 for Evaluating Glucose Tolerance. Twenty-five-week-old N-STZ rats were fasted for 18 hours. The rats were then randomized into groups (n = 6) based on body weight, fasting glucose levels, and glycosylated hemoglobin. The average body weight of N-STZ rats and normal rats was 379 ± 7 and 442 ± 16 g, respectively. The rats were then orally administered test materials (SCO-267, 1 and 10 mg/kg; glibenclamide, 10 mg/kg) or vehicle 80 minutes before oral glucose loading (1.5 g/kg). The first treatment day was designated as day 1. Glibenclamide was orally administered, and blood glucose and insulin levels were determined at the indicated time points. The plasma level of SCO-267 was determined before administering glucose in the OGTT.

Results

SCO-267 Is a Full Agonist for GPR40 Activating the Gαq, Gαs, and Gα12/13 Pathways and β-Arrestin Recruitment. To assess the functional desensitization of GPR40 by SCO-267, the effect of pretreatment with SCO-267 on reactivation of the receptor was examined using the IP1 assay. Chronic exposure to SCO-267 at 37°C for 4 hours at less than 10 nM concentration did not cause signal loss compared with the restimulation response in the control (Fig. 3A). When the cells were pretreated with 1 µM SCO-267, the reactivation response remained at approximately 70%. The desensitization potency of SCO-267 (DC50 = 45 nM) was approximately 300 times higher than that of AM-1638.

SCO-267 Activates Downstream Signaling After Chronic Exposure in Cells. To assess the functional desensitization of GPR40 by SCO-267, the effect of pretreatment with SCO-267 on reactivation of the receptor was examined using the IP1 assay. Chronic exposure to SCO-267 at 37°C for 4 hours at less than 10 nM concentration did not cause signal loss compared with the reactivation response in the control (Fig. 3A). When the cells were pretreated with 1 µM SCO-267, the reactivation response remained at approximately 70%. The desensitization potency of SCO-267 (DC50 = 45 nM) was approximately 300 times higher than that of AM-1638. To compare the rate of desensitization with GLP-1 agonism, which has been demonstrated to be effective in clinical settings when chronically exposed (Buse et al., 2004), the effect of extendin-4 on the reactivation of the GLP-1 receptor was examined using the cAMP assay. When the cells were pretreated with 100 nM...
extendin-4, the restimulation response remained at approximately 70% (Fig. 3B). The desensitization potency of extendin-4 (DC50 = 100 pM) was approximately four times higher than its EC50. In contrast, the residual response to chronic exposure to fasiglifam, which showed durable efficacy in a 52-week clinical study (Kaku et al., 2016), at 30 μM was approximately 30% (Supplemental Fig. 1).

**SCO-267 Improves Insulin Sensitivity in N-STZ Rats.**

To explore the effect of chronic exposure to SCO-267 on glucose tolerance and insulin sensitivity, SCO-267 was administered to diabetic N-STZ rats for 15 days, and the glucose tolerance and insulin sensitivity were evaluated after the drug washout period (Fig. 4). The plasma level of SCO-267 (1 and 10 mg) in rats after the drug washout period (day 18) was 0.22 and 0.35 ng/ml, respectively. The unbound SCO-267 concentration calculated using the rat plasma protein binding activity (Ueno et al., 2019) was 1.4 (1 mg/kg SCO-267) and 2.3 pM (10 mg/kg SCO-267), both of which are unlikely to activate GPR40. In fact, the insulin level was not increased in N-STZ rats subchronically treated with SCO-267 upon glucose loading (Fig. 4A), and this confirmed the complete removal of SCO-267. In contrast, N-STZ rats subchronically treated with SCO-267 (10 mg/kg) showed improved glucose tolerance (Fig. 4B). In addition, the ITT revealed that N-STZ rats subchronically treated with SCO-267 (10 mg/kg) showed increased insulin sensitivity (Fig. 4C).

**SCO-267 Exerts Sustained Glucose-Lowering Effect After Administration in N-STZ Rats.** To explore whether chronic exposure to SCO-267 is effective in improving glycemic control in vivo, glucose tolerance was evaluated after the first and repeated dosing of SCO-267 (1 and 10 mg/kg) in N-STZ rats. In this experiment, food intake levels were lower and body weight was decreased in the 10 mg/kg SCO-267 dose group (Fig. 5, A and B). In the OGTT, after the first dose, SCO-267 significantly increased insulin secretion and improved glucose tolerance, which were superior to those in normal rats (Fig. 5, C and D). As shown in Fig. 5E, the plasma level of SCO-267 was 28.8 ± 1.5 and 24.2 ± 2.3 ng/ml before the 33rd dose of SCO-267 upon glucose loading (Fig. 1A), and this confirmed the complete removal of SCO-267. In contrast, N-STZ rats subchronically treated with SCO-267 (10 mg/kg) showed improved glucose tolerance (Fig. 4B). In addition, the ITT revealed that N-STZ rats subchronically treated with SCO-267 (10 mg/kg) showed increased insulin sensitivity (Fig. 4C).

### Table 1

**Pharmacological potencies of SCO-267**

| Test Material          | Gαq (IP1) high FFAR1 expression EC50 [95% CI] nM | Gαq (IP1) low FFAR1 expression | Gαs (cAMP) | Gα12/13 (SRF-RE reporter activity) | β-Arrestin (β-arrestin recruitment) |
|------------------------|------------------------------------------------|-------------------------------|------------|-------------------------------------|-----------------------------------|
| SCO-267                | 0.093 [0.035–0.14]                                | 0.91 [0.52–1.2]               | 12 [5.8–30] | 2.1 [1.1–3.7]                      | 0.12 [0.032–0.31]                 |
| AM-1638                | 1.2 [1.1–1.5]                                     | 26 [19–35]                   | 30 [22–42] | 1.7 [0.61–3.8]                     |                                   |
| Fasiglifam             | 2.6 [1.9–3.4]                                     | >10,000                      | >30,000    | 120 [42–1500]                      | 8.5 [4.4–15]                      |
| γ-LA                   | N.A.                                             | >150,000                     | >150,000   | >150,000                           |                                   |

Data are representative of two experiments performed in three or four technical replicates. γ-LA, γ-linolenic acid; CI, confidence interval; N.A., not applicable.
10 mg/kg SCO-267 (time = 0) and after 24 hours. In the OGTT after the 33rd dosing (day 33), SCO-267 still increased insulin secretion and improved glucose tolerance, which were superior to those in normal rats (Fig. 5, F and G). Glibenclamide showed a trend of improvement in glucose tolerance after the first dose and impaired glucose tolerance after the 33rd dose (Fig. 5, C and D, F and G). In addition to the sustained glucose-lowering effect, SCO-267 increased pancreatic insulin level at the end of the study (Fig. 5H).

**Discussion**

In this study, SCO-267, a GPR40 full allosteric agonist, was effective in activating downstream signaling after chronic exposure in vitro and in vivo. The in vitro experiments showed that SCO-267 activated the Gi, Gαs, and G12/13 pathways and β-arrestin recruitment and binds to a site different from that of fasiglifam and endogenous ligand with positive cooperativity. The in vitro desensitization analysis using GPR40-overexpressing cells showed that GPR40 can be activated by SCO-267 after 4 hours of exposure to SCO-267. Our experiment using N-STZ rats showed that SCO-267 treatment of 15 days improved glucose tolerance by increasing insulin sensitivity. A 33-day repeated dose study, in which treatment of 15 days improved glucose tolerance by increasing insulin sensitivity. A 33-day repeated dose study, in which the first dose and impaired glucose tolerance after the 33rd dose (Fig. 5, C and D, F and G). In addition to the sustained glucose-lowering effect, SCO-267 increased pancreatic insulin level at the end of the study (Fig. 5H).

In the IP1 accumulation assay, the E_max of SCO-267 was as high as that of AM-1638, a well studied GPR40 full agonist, in CHO cells expressing low levels of human FFAR1, indicating that SCO-267 is a GPR40 full agonist. In addition, SCO-267 showed positive cooperativity with fasiglifam or γ-linolenic acid in the IP1 accumulation assay, indicating that SCO-267 is allosteric with either fasiglifam or the endogenous ligand. These results demonstrated that SCO-267 is an allosteric full agonist of GPR40.

SCO-267 was efficacious in activating downstream signaling even after chronic exposure in human GPR40-expressing CHO cells, similar to exendin-4. Pretreatment of cells with SCO-267 for 4 hours at high concentrations (≥100 nM) caused only 30% loss of restimulation response, similar to that of exendin-4. The loss rate of the re-stimulation response was higher with fasiglifam, which showed a 70% loss of restimulation response. These findings indicate that SCO-267–mediated chronic activation of GPR40 may not be efficacious in desensitizing downstream signaling, which is likely an important characteristic of an agonistic drug candidate. Typically, GPCRs undergo internalization and desensitization upon chronic exposure to agonists through phosphorylation by G protein–coupled receptor kinase and β-arrestin recruitment (Kelly et al., 2008). It has been reported that GPR40 undergoes rapid linoleic acid–induced internalization through arrestin-3 and GPCR kinase 2, and the internalized receptors are recycled to the cell surface via recycling endosomes in GPR40-overexpressed HEK293 cells (Qian et al., 2014). These recycled receptors on the cell surface may allow the restimulation response of SCO-267.

In the chronic dosing study in rats, the plasma SCO-267 concentration immediately before and 24 hours after the 33rd dose of 10 mg/kg SCO-267 was 28.8 and 24.2 ng/ml, respectively. In a previous study, N-STZ rats dosed with SCO-267 (0.3 mg/kg, E_max = 22.7 ng/ml) potently stimulated insulin secretion and improved glucose tolerance (Ueno et al., 2019).

**Fig. 2.** Effects of increasing concentrations of fasiglifam or γ-linolenic acid on the dose-response curve of SCO-267. The IP1 accumulation assay of SCO-267 in the presence of fasiglifam (A) or γ-linolenic acid (B) at various concentrations using CHO cells expressing low levels of human FFAR1 (clone 2). Representative graphs of two independent experiments are shown. The data are presented as means ± S.D. of two technical replicates. γ-LA, γ-linolenic acid.
This suggests that the plasma level of exposure achieved by 10 mg/kg SCO-267 was high enough to activate GPR40 throughout the day in our chronic dosing study in N-STZ rats. Even under these conditions, the sustained efficacy of SCO-267 on the glucose-lowering effect, which was superior to that in normal rats, was observed upon drug dosing. The continuous glucose-lowering effect of exendin-4 was confirmed in patients with T2DM after 30 weeks of treatment (Drucker et al., 2008). In addition, the effect of fasiglifam has been confirmed in rats treated for 6 weeks (Ito et al., 2013) and in patients with T2DM after 52 weeks of treatment (Kaku et al., 2016). Taken together with the present in vitro observations, in which SCO-267 showed equal or less desensitization to exendin-4 and fasiglifam, SCO-267 may induce similar durability of therapeutic efficacy in patients.

Notably, after the drug washout period, N-STZ rats treated with SCO-267 for 15 days showed increased insulin sensitivity. In the present study, food intake and body weight were lowered in SCO-267–treated N-STZ rats. Hence, increased insulin sensitivity may be the indirect result of weight loss. In addition, GLP-1 stimulation by SCO-267 may have contributed to the increased insulin sensitivity. Our previous data showed that SCO-267 stimulated GLP-1 in N-STZ rats (Ueno et al., 2019). GLP-1 is known to promote peripheral glucose uptake and reduce hepatic glucose production partially through the central nervous system (Sandoval and D’Alessio, 2015). Further studies are required to investigate the mechanism of SCO-267 dosing on increased insulin sensitivity.

STZ treatment causes abnormalities in insulin secretion and β-cell function (Bonner-Weir et al., 1981). Interestingly, chronic exposure of N-STZ rats to SCO-267 significantly increased the pancreatic insulin level. Hyperglycemia induces glucotoxicity, which results in β-cell dysfunction (Kaiser et al., 2003). This may have been caused by a decrease in glucotoxicity via the glucose-lowering activity of SCO-267. Furthermore, it has been reported that vincamine, a monoterpenoid indole alkaloid, which activates GPR40, protected STZ-treated INS-832/13 cells, a rat insulinoma cell line, through GPR40 activation (Du et al., 2019) and that CNX-011-67, a GPR40 agonist, reduces inflammation-induced apoptosis of NIT1 cells, a mouse pancreatic β-cell line (Verma et al., 2014).
Overall, SCO-267 may improve β-cell function via a direct GPR40-mediated effect.

In the present study, we were unable to determine the components of GPR40 and hormones that are important for the in vivo observations with SCO-267, which showed a sustained improvement in glucose tolerance in diabetic rats. Thus, future studies using specific antagonists/inhibitors and gene knockout models are needed. In addition, we evaluated only SCO-267 as a GPR40 full agonist, and it is uncertain if the current findings are universal to GPR40 full agonists. Thus, other GPR40 full agonists should be investigated in future studies.

In conclusion, even after chronic exposure, SCO-267 effectively activates GPR40 in cells and rats. In diabetic rats, chronic exposure to SCO-267 was highly effective in improving glucose tolerance. These findings suggest that sustained exposure to SCO-267 likely induces a durable glucose-lowering effect without tachyphylaxis in patients with diabetes.

Acknowledgments

We thank Kaori Nakanishi and Ryoko Yamao for conducting the in vitro experiment.

Authorship Contributions

Participated in research design: Koyama, Ookawara, Watanabe, Moritoh.
Conducted experiments: Koyama, Ookawara.
References

Bonner-Weir S, Trent DF, Honey RN, and Weir GC (1981) Responses of neonatal rat islets to streptozotocin: limited B-cell regeneration and hyperglycemia. *Diabetes* 30:64–69.

Briscoe CP, Tadayon M, Andrews JL, Benson WG, Chambers JK, Edlert MM, Ellis GC, Kidby, Stacey VK, Gent AS, Minnick DT, et al. (2003) The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J Biol Chem* 278:11303–11311.

Brown SP, Dransfield PJ, Vimalratanaha, Miao X, Zhu L, Patapporong Y, Sun Y, Liu J, Luo J, Zhang J, et al. (2012) Discovery of AM-1638: a potent and orally bioavailable GPR40/FFA1 full agonist. *ACS Med Chem Lett* 3:726–730.

Burrant CF, Viswanathan P, Marcink J, Cao C, Vakilynejad M, Xie B, and Leifke E (2012) TAK-875 is a potent allosteric GPR40 agonist that serves as a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet* 379:1403–1411.

Buse JB, Henry RR, Han J, Kim DD, Fineman MS, and Baron AD; Exenatide-113 DURATION-1 Study Group (2008) Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. *Diabetes Obes Metab* 10(Suppl 1):S37–S48.

Burant CF, Viswanathan P, Marcink J, Cao C, Vakilynejad M, Xie B, and Leifke E (2012) TAK-875 is a potent allosteric GPR40 agonist that serves as a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet* 379:1403–1411.

Callander GE, Thomas WG, and Bathgate RA (2009) Prolonged RXFP1 and RXFP2 signaling can be explained by poor internalization and a lack of beta-arrestin recruitment. *J Am Coll Physiol Cell Physiol* 296:C1058–C1066.

Chen Y, Ren Q, Zhou Z, Deng L, Hu L, Zhang L, and Li Z (2020) HWL-088, a new potent free fatty acid receptor 1 (FFAR1) agonist, improves glucolipid metabolism and acts additively with metformin in ob/ob diabetic mice. *Br J Pharmacol* 177:2236–2290.

Chen Y, Song M, Riley JP, Hu CC, Peng X, Scheuner D, Bokvist K, Maiti P, Kahl SD, Kaku K, Enya K, Nakaya R, Ohira T, and Matsuno R (2016) Long-term safety and efficacy of fasiglifam (TAK-875), a G-protein-coupled receptor 40 agonist, in combination with metformin in patients with type 2 diabetes. *Diabetes Care* 27:2628–2635.

Callagher GE, Thomas WG, and Bathgate RA (2009) Prolonged RXFP1 and RXFP2 signaling can be explained by poor internalization and a lack of beta-arrestin recruitment. *J Am Coll Physiol Cell Physiol* 296:C1058–C1066.

Chen Y, Song M, Riley JP, Hu CC, Peng X, Scheuner D, Bokvist K, Maiti P, Kahl SD, Montrose-Hafradze C, et al. (2016) A selective GPR40 (FFAR1) agonist LY2881835 provides immediate and durable glucose control in rodent models of type 2 diabetes. *Pharmacol Res Perspect* 4:e00278.

Deibel K, Pott R, and Wagner M (2014) Recent developments in the discovery of FFA1 agonist receptors as novel oral treatment for type 2 diabetes mellitus. *Bioorg Med Chem Lett* 24:2991–3000.

Druck YR, Benzaquen M, and Lefkovitz RJ (2006) Trafficking of G protein-coupled receptors. *Circ Res* 99:570–582.

Drucker DJ, Shobayashi T, and Goldstein A (2018) Fatty acid receptor FFA1/GPR40 a decade later: how much do we know? *Trends Endocrinol Metab* 30:140–148.

Du Y, Song J, Lu X, Shi X, Xu X, Lu J, Lv J, Huang X, Chen J, Wang H, et al. (2019) Free fatty acids improve glucose homeostasis in type 2 diabetes mice. *J Endocrinol* 240:195–214.

Hauge M, Vestmar MA, Husted AS, Ekberg JP, Wright MJ, Di Salvo J, and Husted AS (2008) Ally E, Bailey CP, and Henderson L (2008) Agonist-selective mechanisms of GPCR desensitization. *Br J Pharmaco* 153(Suppl 1):S379–S388.

Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Byrne N, Wang J, et al. (2018) Free fatty acid receptor 1 (FFAR1) as an emerging therapeutic target for type 2 diabetes mellitus: recent progress and prevailing challenges. *Med Res Rev* 38:425–425.

Li Z, Xu A, Jiang W, and Qian H (2018) Free fatty acid receptor 1 (FFAR1) as an emerging therapeutic target for type 2 diabetes mellitus: recent progress and prevailing challenges. *Med Res Rev* 38:425–425.

Lin DC, Guo Q, Luo J, Zhang J, Nguyen K, Chen M, Tran T, Dransfield PJ, Brown SP, Hovee J, et al. (2012) Identification and pharmacological characterization of multiple allosteric binding sites on the free fatty acid 1 receptor. *Mol Pharmacol* 82:843–859.

Li Z, Byrne N, Wang J, Brignone G, Brown FK, Chobanian HR, Colletti SL, Di Salvo J, Thomas-Fowlkes B, Guo Y, et al. (2017) Structural basis for the cooperative allosteric activation of the free fatty acid receptor 4. *Nat Struct Mol Biol* 24:570–577.

Mancini AD and Poutov V (2013) The fatty acid receptor 4 promoter activity: a decade later: how much do we know? *Trends Endocrinol Metab* 24:389–407.

Pais R, Gribble FM, and Reimann F (2016) Stimulation of incretin secreting cells. *Ther Adv Endocrinol Metab* 7:23–44.

Portba H, Movassat J, Cazin-Tournier, Baille D, Giroux M, Serradas P, Drolz M, and Kergoat M (2007) Neonatal streptozotocin-induced (n-STZ) diabetic rats: a family of type 2 diabetes models, in *Animal Models of Diabetes*, pp 223–250, Elsevier, Amsterdam.

Qian J, Wu C, Chen X, Li X, Ying G, Jia L, Ma Q, Li G, Shi Y, Zhang G, et al. (2014) Differential requirements of arrestin-3 and clathrin for ligand-dependent and -independent internalization of human G protein-coupled receptor 40. *Cell Signal* 26:2412–2423.

Rives ML, Rady B, Swansson N, Zhao S, Qi J, Arnould E, Bakaj I, Mancini A, Breton B, Lee SF, et al. (2018) GPR40-mediated Galphai2 activation by allosteric full agonists highly efficacious at potentiating glucose-stimulated insulin secretion in human islets. *Mol Pharmacol* 93:581–591.

Sandoval DA and D’Alessio DA (2015) Physiology of proglucagon peptides: role of GPR40 and GLP1 in health and diabetes. *J Endocrinol* 224:333–346.

Srivastava A, Yano J, Hirozane Y, Kefala G, Grusvitz F, Snell G, Lane W, Ivetac A, Aertgeerts K, Nguyen J, et al. (2014) High-resolution structure of the human GPR40 receptor bound to allosteric agonist TAK-875. *Nature* 513:124–127.

Tirupula KC, Denoyer R, Speth RC, and Karruk SS (2014) Atypical signaling and functional desensitization response of MAS receptor to peptide ligands. *PLoS One* 9:e103520.

Tsuchiya Y, Ito R, Suzuki M, Harada A, Negoro N, Yasaki T, Momose Y, Takeuchi K (2011) TAK-875, an orally available G protein-coupled receptor 40/ free fatty acid receptor 1 agonist, enhances glucose-dependent insulin secretion and improves both postprandial and fasting hyperglycemia in type 2 diabetic rats. *J Pharmacol Exp Ther* 335:229–237.

Ueno H, Ito R, Abe SI, Oikawa M, Miyashita H, Ogino M, Miyamoto Y, Yoshihara T, Kobayashi A, Tsugita Y, et al. (2019) SGO-267, a GPR40 full agonist, improves glycemic and weight control in rat models of diabetes and obesity. *J Pharmacol Exp Ther* 370:172–181.

Verma MK, Sadasivuni MK, Yatesesh AN, Neelima K, Murdula S, Reddy M, Smitha R, Bivasw S, Chandravanshi B, Pallavi PM, et al. (2014) Activation of GPR40 attenuates chronic inflammation induced impact on pancreatic β-cells health and function. *BMC Cell Biol* 15:24.

Yabuuchi K, Komatsu H, Tsugita H, Maeda T, Ito R, Ito M, Matsushita-Nagasumi K, Sakuma K, Miyawaki K, Ikeshi N, Takeuchi N, et al. (2013) A novel antidiabetic drug, fasiglifam/TAK-875, acts as an ago-alsosteric modulator of FFA1. *PLoS One* 8:e76280.