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

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ABSTRACT

G protein-coupled receptor 40/free fatty acid receptor 1 (GPR40/FFA1) is highly expressed in pancreatic β cells and mediates free fatty acid-induced insulin secretion. This study examined the pharmacological effects and potential for avoidance of lipotoxicity of [(3S)-6-((2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl)methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic acid hemi-naphosphate (TAK-875), a novel, orally available, selective GPR40 agonist. Insulinoma cell lines and primary rat islets were used to assess the effects of TAK-875 in vitro. The in vivo effects of TAK-875 on postprandial hyperglycemia, fasting hyperglycemia, and normoglycemia were examined in type 2 diabetic and normal Sprague-Dawley rats, TAK-875 neither enhanced insulin secretion nor caused hypoglycemia even at 30 mg/kg. TAK-875 enhances glucose-dependent insulin secretion and improves both postprandial and fasting hyperglycemia with a low risk of hypoglycemia and no evidence of β cell toxicity.

Introduction

Insulin resistance and impaired insulin secretion are major causes of the onset and development of type 2 diabetes (Muioio and Newgard, 2008). Drugs that enhance insulin secretion, such as sulfonylureas and meglitinides, are commonly used for the treatment of type 2 diabetes. However, these drugs enhance insulin secretion by direct closure of the \( \Delta V_{ATP} \) channel independent of blood glucose levels, thereby causing hypoglycemia (Doyle and Egan, 2003). Hence, patients with diabetes would benefit from the development of a novel antidiabetic drug that has a low hypoglycemic risk and effectively improves blood glucose control.

Secretion of insulin from pancreatic β cells is stimulated by glucose and other nutrients, including free fatty acids (FFAs) (Prenkti et al., 1997; Haber et al., 2003). In isolated human and rodent islets, FFAs enhance insulin secretion in a manner that depends on glucose concentration (Gravena et al., 2006). Glucose stimulates insulin secretion from pancreatic islets by activating adenyl cyclase and increasing ATP-mediated closure of \( \Delta V_{ATP} \) channels, thereby decreasing intracellular calcium concentration (IP3) and resulting in increased insulin secretion.

The in vivo effects of TAK-875 on postprandial hyperglycemia, fasting hyperglycemia, and normoglycemia were examined in type 2 diabetic and normal Sprague-Dawley rats, TAK-875 neither enhanced insulin secretion nor caused hypoglycemia even at 30 mg/kg. TAK-875 enhances glucose-dependent insulin secretion and improves both postprandial and fasting hyperglycemia with a low risk of hypoglycemia and no evidence of β cell toxicity.

ABBREVIATIONS: FFA, free fatty acid; CHO, Chinese hamster ovary; GPR40/FFA1, G protein-coupled receptor 40/free fatty acid receptor 1; hGPR40, human GPR40; IP, inositol monophosphate; IP\(_3\), inositol 1,4,5-triphosphate; [Ca\(^{2+}\)], intracellular calcium concentration; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; TAK-875, [(3S)-6-((2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl)methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic acid hemi-naphosphate; SD, Sprague-Dawley; ZDF, Zucker diabetic fatty; ZL, Zucker lean; DDP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; ER, endoplasmic reticulum; ELISA, enzyme-linked immunosorbent assay; KRBH, Krebs-Ringer-bicarbonate HEPES buffer.

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□ The online version of this article (available at http://jpet.aspetjournals.org) contains supplemental material.
2002). Plasma concentrations of FFAs are elevated in the fasted state, and they play a role in the enhancement of the postprandial insulin response in vivo (Stein et al., 1996; Dobbins et al., 1998). GPR40, a G protein-coupled receptor highly expressed in pancreatic β cells, has been identified as a receptor for both saturated and unsaturated medium- and long-chain FFAs (Briscoe et al., 2003; Itoh et al., 2003; Kottarsky et al., 2003). In addition, Itoh et al. (2003) demonstrated that the suppression of GPR40/FFA1 mRNA with small interfering RNA inhibited the enhancement of FFA-induced insulin secretion in mouse insulinoma MIN6 cells, indicating that GPR40/FFA1 is involved in the stimulation of acute insulin secretion by FFAs. The role of GPR40/FFA1 in insulin secretion has also been confirmed by the use of selective small-molecule GPR40/FFA1 agonists (Briscoe et al., 2006; Tan et al., 2008).

In pancreatic β cells, elevation of intracellular calcium triggers insulin secretion (Prentki et al., 1997). Generally, activation of Gqα protein-coupled receptors results in phospholipase C activation, inositol 1,4,5-triphosphate (IP₃) and diacylglycerol production, and increases in intracellular calcium concentration ([Ca²⁺]ᵢ) (Taylor et al., 1991). Studies have shown that GPR40/FFA1 is coupled mainly with Gqα in rodent β cell lines, and agonist stimulation of GPR40/FFA1 with FFAs enhances [Ca²⁺]ᵢ, and insulin secretion in these cells, which can be blocked by inhibitors of Gqα signaling (Fujitani et al., 2005; Shapiro et al., 2005).

Whereas FFAs acutely stimulate insulin secretion, chronic exposure to them causes β cell dysfunction and/or cell death, so-called lipotoxicity (Haber et al., 2003; Morgan, 2009). Because endogenous ligands of GPR40/FFA1 are medium- and long-chain FFAs, it has been suggested that GPR40/FFA1 might mediate chronic toxic effects of FFAs (Steneberg et al., 2005). However, conflicting results obtained from GPR40/FFA1-deficient mice have also been reported (Latomu et al., 2007; Kebede et al., 2008; Lan et al., 2008; Alquier et al., 2009); these did not show the harmful effects of GPR40/FFA1 in pancreatic β cells. Moreover, Nagasumi et al. (2009) have reported that the overexpression of GPR40/FFA1 in pancreatic β cells of mice results in enhanced insulin secretion, improved glucose tolerance, and resistance to impairment of glucose tolerance induced by a high-fat diet. Therefore, it remains under debate whether GPR40/FFA1 agonism or antagonism would be more favorable for the treatment of type 2 diabetes and related disorders.

Measurement of Intracellular Calcium Concentration. Intracellular calcium concentration ([Ca²⁺]ᵢ) and insulin secretion in these cells were determined using an IP-One ELISA kit (Cisbio, Bedford, MA) according to the manufacturer’s instructions. In brief, CHO cells or INS-1 832/15 cells were suspended in the culture medium described above and seeded at a density of 8 × 10⁴ and 5 × 10⁴ cells/well, respectively, in 96-well plates (Nalge Nunc International, Rochester, NY), and the cells were cultured overnight. After the medium was discarded, cells were incubated at 37°C for 1 h with stimulation buffer (146 mM NaCl, 4.2 mM KCl, 0.5 mM MgCl₂, 1 mM CaCl₂, 10 mM HEPES, 5.5 mM glucose, and 50 mM LiCl, pH 7.4) in the absence or presence of stimulating agents as shown. In the experiments using INS-1 832/15 cells, glucose at 1, 3, or 10 mM concentrations was added to the glucose-free stimulation buffer. After the incubation, lysis reagent was added, the plate was incubated for another 30 min at 37°C, and intracellular IP concentration was measured. EC₅₀ values were calculated by logistic regression analysis (SAS Institute, Cary, NC).

Measurement of Intracellular Calcium Concentration. INS-1 832/15 cells were seeded at a density of 5 × 10⁴ cells/well in poly-D-lysine-coated 96-well black plates (BD Biocoat; BD Biosciences, San Jose, CA), and cultured overnight before experiments. Cells were loaded for 30 min at 37°C with 1 μM Fura-2 acetoxy-
methyl ester (Dojindo, Kumamoto, Japan) in Krebs-Ringer-bicarbonate HEPS buffer (KRKH) containing 0.025% pluronic F-127 (Invitrogen), 1 mM glucose, and 1% FBS (loading buffer), followed by washing with loading buffer without Fura-2 acetoxymethyl ester. After the washing, KRKH containing 1, 3, or 10 mM glucose and 0.1% DMSO was added, the cells were excited at 340 and 380 nm alternatively, the emission signals at 510 nm were detected every 10 s by a cooled charge-coupled device camera, and the ratio was derived using an AquaCosmos (Hamamatsu Photonics, Hamamatsu, Japan). After monitoring of the glucose-induced calcium response, the equivalent volume of KRKH containing glucose and test agents was added. After incubation, supernatants from each well were collected, and secreted insulin concentration was measured using a rat insulin ELISA (Morinaga, Tokyo, Japan) according to the manufacturer’s instruction.

Insulin Secretion and Intracellular Insulin Content after Prolonged Exposure. INS-1 833/15 cells were seeded in RPMI medium and seeded in a 96-well plate at a density of 5 x 10^3 cells/well; 1% BSA and 0.1% DMSO alone (control), palmitic acid (10, 100, and 1000 μM), oleic acid (10, 100, and 1000 μM), or TAK-875 (1, 10, and 100 μM) was added to the plate. After 72-h culture, medium was discarded, and cells were preincubated for 2 h with KRKH containing 1 mM glucose and 0.2% BSA at 37°C. After discarding of the preincubation buffer, KRKH containing glucose and stimulators as indicated was added, and the plate was incubated for 2 h at 37°C. After incubation, supernatants from each well were collected, and secreted insulin concentration was measured using a rat insulin ELISA (Morinaga, Tokyo, Japan) according to the manufacturer’s instruction.

Acute Insulin Secretion Assay. INS-1 833/15 cells were seeded at a density of 5 x 10^5 cells/well in a 96-well plate, and the cells were cultured in RPMI medium overnight before experiments. After discarding of the medium, the cells were preincubated for 2 h with KRKH containing 1 mM glucose. After discarding of the preincubation buffer, KRKH containing glucose and stimulators as indicated was added, and the plate was incubated for 2 h at 37°C. After incubation, supernatants from each well were collected, and secreted insulin content was measured using a rat insulin ELISA (Morinaga, Tokyo, Japan) according to the manufacturer’s instruction.

Comparison of TAK-875 and Endogenous Ligand Agonist Activity for GPR40/FFA1. It has been demonstrated that TAK-875 increases [Ca^{2+}], in CHO cells expressing the human or rat GPR40/FFA1 (Negoro et al., 2010), but the agonist activity has not been compared with that of endogenous ligands. Thus, we first compared the agonist activity of TAK-875 with that of an endogenous ligand, oleic acid, by measuring intracellular IP, a downstream metabolite of IP3, in CHO cells expressing human GPR40/FFA1 (CHO-hGPR40). TAK-875 (0.01–10 μM) produced a concentration-dependent increase in intracellular IP production in CHO-hGPR40 (Fig. 1A). Oleic acid (3–100 μM) produced a concentration-dependent increase in intracellular IP production in CHO-hGPR40 (Fig. 1B).
(3–100 μM) also enhanced intracellular IP production in a concentration-dependent manner, but required much higher ligand concentrations to activate the receptor in comparison with TAK-875. EC50 values for TAK-875 and oleic acid were 0.072 and 29.9 μM, respectively, demonstrating that TAK-875 is 400-fold more potent at activating hGPR40 than oleic acid. Neither TAK-875 nor oleic acid elicited an IP response in control CHO cells devoid of hGPR40 (Fig. 1B).

**TAK-875 Activates the Gq\(\tau\)-Mediated Signaling Pathway in Pancreatic β Cells.** We next examined whether TAK-875 activates the Gq\(\tau\)-mediated signaling pathway in pancreatic β cells as observed in CHO cells by measuring the ability of TAK-875 to stimulate IP production and increase \([Ca^{2+}]_i\). Rat insulinoma INS-1 cell clone 833/15 was used as a pancreatic β cell model. It has been reported that INS-1 cells express endogenous GPR40/FFA1 (Schnell et al., 2007). Prior studies confirmed that INS-1 833/15 cells highly expressed endogenous GPR40/FFA1 mRNA to an extent similar to that observed in primary rat islets (data not shown). TAK-875 (0.1–10 μM) dose-dependently augmented intracellular IP production in these cells in the presence of 10 mM glucose, whereas one of the sulfonylurea drugs, glibenclamide, did not (Table 1). Glucose concentration did not affect TAK-875-induced IP production, and almost equivalent TAK-875-induced IP production was observed in the presence of 1 and 3 mM glucose compared with 10 mM glucose.

As shown in Fig. 2A, 10 mM glucose transiently increased \([Ca^{2+}]_i\) (first peak during measurement) in INS-1 833/15 cells, indicating that the glucose-sensitive \([Ca^{2+}]_i\) response was functional in this model. Addition of vehicle (DMSO) after the first \([Ca^{2+}]_i\) peak did not elevate \([Ca^{2+}]_i\) levels. In contrast, TAK-875 (3–30 μM) concentration-dependently augmented \([Ca^{2+}]_i\) (Fig. 2, B–D). We next examined the glucose dependence of these \([Ca^{2+}]_i\) elevations by TAK-875. As shown in Fig. 3, the TAK-875 (30 μM)-induced increase in \([Ca^{2+}]_i\) was attenuated at glucose concentrations of 1 and 3 mM, compared with the response observed in 10 mM glucose (Fig. 3, A–C). In contrast, glibenclamide (10 μM) augmented \([Ca^{2+}]_i\) independent of glucose concentrations (Fig. 3, D–F).

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Intracellular Inositol Monophosphate Concentration</th>
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<tr>
<td></td>
<td>1 mM Glucose</td>
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<tr>
<td>Vehicle</td>
<td>nM</td>
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<tr>
<td>TAK-875, 0.1 μM</td>
<td>153.9 ± 14.7</td>
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<tr>
<td>TAK-875, 1 μM</td>
<td>220.9 ± 19.3</td>
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<tr>
<td>TAK-875, 10 μM</td>
<td>430.2 ± 30.9</td>
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<tr>
<td>Glibenclamide, 10 μM</td>
<td>480.0 ± 36.5</td>
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* P < 0.025 vs. control (vehicle with each respective glucose concentration) by one-tailed Williams’ test.

![Fig. 2. TAK-875 exhibits concentration-dependent augmentation of intracellular calcium concentrations in INS-1 833/15 cells. Changes in the intracellular calcium concentration in INS-1 833/15 cells after initial challenge with 10 mM glucose and subsequent challenge with 10 mM glucose and vehicle (DMSO) or TAK-875 are shown. Fura-2-loaded INS-1 833/15 cells were treated with 10 mM glucose alone. After treatment with glucose, the cells were stimulated with DMSO (A) or 3, 10, or 30 μM TAK-875 (B, C, and D, respectively) in the presence of 10 mM glucose. The traces shown are averages of the ratio of fluorescent intensity excited at 340 and 380 nm (average of 30 cells). Bars indicate the presence of glucose, vehicle, or TAK-875 in the buffer solution.](https://example.com/figure2.png)
Prolonged Agonist Stimulation of GPR40/FFA1 by TAK-875 Does Not Cause β Cell Dysfunction. The effects of prolonged exposure to TAK-875 on β cell function were examined in cytokine-sensitive INS-1 832/13 cells instead of INS-1 833/15 cells, a cytokine-resistant clone (Collier et al., 2006). The endogenous ligands for GPR40/FFA1, palmitic acid and oleic acid (Itoh et al., 2003), were used as comparators. Before the experiment, we confirmed that TAK-875 (6.25–100 μM), palmitic acid (62.5–1000 μM), and oleic acid (62.5–1000 μM) showed agonist activities in CHO cells expressing human or rat GPR40/FFA1 in the presence of 1% BSA, corresponding to the BSA concentration to be used in the prolonged exposure experiments (Supplemental Fig. 1). In addition, we observed that palmitic acid, oleic acid, and TAK-875 dose-dependently augmented insulin secretion in INS-1 832/13 in the presence of 10 mM glucose and 1% BSA (Supplemental Fig. 2). These results indicate that TAK-875 sufficiently stimulates GPR40/FFA1 within this dose range, compared with palmitic acid and oleic acid.

In INS-1 832/13 cells, 72-h exposure to palmitic acid (1000 μM) together with 1% BSA resulted in a significant reduction in the insulin secretory response to 20 mM glucose (Fig. 5A). Under the same conditions, neither oleic acid (10–1000 μM) nor TAK-875 (1–100 μM) significantly altered glucose-stimulated insulin secretion in these cells. Intracellular insulin content was significantly reduced after 72-h exposure to palmitic acid (1000 μM) or oleic acid (1000 μM), and the deleterious effect was particularly pronounced for palmitic acid compared with oleic acid (p ≤ 0.05 by Aspin-Welch test) (Fig. 5B). In contrast, prolonged exposure to TAK-875 (100 μM) did not affect intracellular insulin content.

Prolonged Agonist Stimulation of GPR40/FFA1 by TAK-875 Does Not Cause Induction of a Marker of Apoptosis in Pancreatic β Cells. It is well known that chronic exposure to FFAs in pancreatic β cells causes not only impairment of their function but also cell apoptosis (Haber et al., 2003; Morgan, 2009). To clarify the effects of prolonged exposure to TAK-875 on apoptotic events in β cells, INS-1 832/13 cells were treated with TAK-875, palmitic acid, or oleic acid in the presence of 1% BSA for 72 h, and subsequent caspase 3/7 activity was measured. In these cells, 72-h exposure to palmitic acid (62.5–1000 μM) and oleic acid (62.5–1000 μM) caused dose-dependent enhancement of caspase 3/7 activity, and statistically significant effects were observed at doses above 250 μM palmitic acid and 500 μM oleic acid (Fig. 5, C and D). In contrast, TAK-875 (6.25–100 μM) did not show any effect on caspase 3/7 activity under the same conditions (Fig. 5E).

TAK-875 Augments Insulin Secretion and Improves Glucose Tolerance during the Oral Glucose Tolerance Test in Type 2 Diabetic Rats. Next, we performed an oral
Glucose tolerance test in type 2 diabetic N-STZ-1.5 rats (Portha et al., 1989) to examine the effects of TAK-875 on impaired postprandial glucose tolerance. Single oral administration of TAK-875 (1–10 mg/kg) to these rats 1 h before an oral glucose load resulted in a potent and dose-dependent reduction of glucose excursion (Fig. 6, A and B). The effects on plasma glucose levels were probably mediated through the compound’s effects on insulin, because plasma insulin levels, especially during the early phase of the oral glucose tolerance test, increased simultaneously and dose-dependently with TAK-875 (Fig. 6, C and D).

Glucose-Lowering Effects of TAK-875 on Normal and Elevated Fasting Plasma Glucose. Because TAK-875 showed strictly glucose-dependent insulinotropic effects in vitro (Fig. 4), we speculated that TAK-875 might enhance insulin secretion and reduce blood glucose only when blood glucose levels are elevated. To clarify the hypothesis, the effects of TAK-875 on fasting normoglycemia and hyperglycemia were examined in normal SD rats and ZDF rats, respectively. Two insulin secretagogues that act on the K$_{ATP}$ channel, nateglinide and the sulfonylurea glibenclamide, were included in these studies as comparators. As shown in Fig. 7, A and B, nateglinide (50 mg/kg) lowered plasma glucose levels below normal fasting levels in SD rats by increasing plasma insulin. Likewise, glibenclamide (10 mg/kg) gradually decreased plasma glucose levels below normal fasting levels with a significant increase in plasma insulin levels. In contrast, TAK-875 at 30 mg/kg, which is a 3- to 10-fold higher dose compared with the dose that improved glucose tolerance in diabetic rats (Fig. 6), did not alter fasting glucose levels in SD rats with normal glucose homeostasis (Fig. 7A). Likewise, TAK-875 did not significantly alter insulin secretion in SD rats with normal fasting glucose levels (Fig. 7B).

The effects of TAK-875, glibenclamide, and nateglinide on fasting hyperglycemia were evaluated in male ZDF rats. As shown in Fig. 7C, fasting plasma glucose levels before drug administration were significantly elevated in ZDF rats compared with the normal ZL rats. In ZDF rats, oral administration of TAK-875 (10 mg/kg) increased plasma insulin levels (Fig. 7D) and lowered plasma glucose levels (Fig. 7C), whereas nateglinide (50 mg/kg) and glibenclamide (10 mg/kg) did not show statistically significant change.

**Discussion**

GPR40/FFA$_1$ is highly expressed in pancreatic islets in mice, rats, and humans (Briscoe et al., 2003; Itoh et al., 2003; Tomita et al., 2006). Although it has been reported that GPR40/FFA$_1$ is expressed not only in pancreatic β cells (insulin-positive cells) but also in α cells (glucagon-positive cells) in mice (Flodgren et al., 2007), expression in insulin-positive cells is dominant in rats and humans (Itoh et al., 2003; Tomita et al., 2006). In this study, we focused on the function of GPR40/FFA$_1$ in pancreatic β cells and examined the events caused by pharmacological activation of the receptor by using in vitro and in vivo rat models. Our data indicate that TAK-875 is a potent agonist for GPR40/FFA$_1$ and activates the phospholipase C pathway, presumably via Gq in pancreatic β cells. This mechanism of insulinotropic action by TAK-875 is novel among insulinotropic drugs, including sulfonylureas, meglitinides, dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) analogs, and is distinct from those of glucose-dependent insuli-
notropic polypeptide and G protein-coupled receptor 119 ago-
nists (Drucker, 2007; Winzell and Ahre n, 2007).

We found that TAK-875 enhanced \([\text{Ca}^{2+}]_i\) in a glucose-
dependent manner in INS-1 cells. Because the increase in
\([\text{Ca}^{2+}]_i\) is related to enhanced insulin secretion in \(\beta\) cells (Prentki et al., 1997), this mechanism may explain the glu-
cose-dependent insulinotropic effects of TAK-875 through
GPR40/FFA1. The phenomenon was consistent with other
reports in which enhancement of \([\text{Ca}^{2+}]_i\) in rat primary
\(\beta\) cells by stimulation of GPR40/FFA1 with oleic acid depends
on glucose concentration (Fujiwara et al., 2005). A specific
receptor for IP3 is present in the endoplasmic reticulum (ER),
and the interaction of IP3 with the IP3 receptor triggers
calcium release from the ER (Berridge, 1993). TAK-875 also
enhanced intracellular IP production, thus, an increase in
\([\text{Ca}^{2+}]_i\) with TAK-875 may, at least in part, result from
calcium release from the ER. The interesting observation is
that IP production by TAK-875 was not glucose-dependent,
whereas enhancement of \([\text{Ca}^{2+}]_i\) and insulin release strictly
depended on glucose concentration. Similar effects have been
shown in Gq-coupled muscarinic receptors stimulated with
the agonist carbachol, in which IP production occurs regard-
less of glucose concentration, whereas insulin release is glu-
cose concentration-dependent in rat islets (Zawalich et al.,
1989). Thus, one explanation is that these events might be
common phenomena among Gq-coupled receptors. Another
explanation is that the difference may be caused by the
different type of assay used: real-time and transient mea-
surement of intracellular calcium versus measurement of
cumulative IP, which is the degradation product of IP3. Fur-
ther analysis will be necessary to clarify how GPR40/FFA1-
mediated signals interact with glucose in pancreatic \(\beta\) cells.

Postprandial and fasting hyperglycemia caused by insuffi-
cient insulin secretion in response to blood glucose is ob-

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**Fig. 5.** Prolonged stimulation of GPR40/FFA1 with TAK-875 does not cause \(\beta\) cell dysfunction and initiate apoptotic signaling in INS-1 832/13 cells.

A, insulin secretion capacity in response to high glucose concentration after 72-h exposure to palmitic acid, oleic acid, or TAK-875 in INS-1 832/13 cells. INS-1 832/13 cells were treated with palmitic acid (10, 100, or 1000 \(\mu\)M), oleic acid (10, 100, or 1000 \(\mu\)M), or TAK-875 (1, 10, or 100 \(\mu\)M) for 72 h, and subsequent insulin secretory capacities in response to 20 mM glucose were examined. Empty bars, vehicle alone; filled bars, palmitic acid, oleic acid, or TAK-875. Data shown are mean ± S.D. (\(n = 3\)). **, \(p \leq 0.01\) by Aspin-Welch test. #, \(p \leq 0.025\) versus control (20 mM glucose stimulation) by one-tailed Williams’ test. B, intracellular insulin content in INS-1 832/13 cells after 72-h exposure to palmitic acid (1000 \(\mu\)M), oleic acid (1000 \(\mu\)M), or TAK-875 (100 \(\mu\)M). Data shown are mean ± S.D. (\(n = 3\)). **, \(p \leq 0.05\) and ***, \(p \leq 0.01\) versus vehicle control by Dunnett’s test. C to E, caspase 3/7 activity in INS-1 832/13 cells after 72-h exposure to palmitic acid (62.5–1000 \(\mu\)M) (C), oleic acid (62.5–1000 \(\mu\)M) (D), or TAK-875 (6.25–100 \(\mu\)M) (E). Data shown are mean ± S.D. (\(n = 3\)). #, \(p < 0.025\) versus control by one-tailed Williams’ test.
served in patients with type 2 diabetes. Our results indicate that TAK-875 directly acts on pancreatic β cells but augments insulin secretion only when blood glucose levels are elevated. Indeed, in vivo oral administration of TAK-875 (3–10 mg/kg) in type 2 diabetic rats improved both postprandial and fasting hyperglycemia. In terms of future studies, it will be of interest to determine whether chronic exposure to TAK-875 in vivo improves type 2 diabetes. Especially, male

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**Fig. 6.** TAK-875 improves postprandial hyperglycemia in type 2 diabetic rats. Male N-STZ-1.5 rats were fasted overnight and orally given vehicle or TAK-875 (1, 3, and 10 mg/kg). One hour later, all animals received an oral glucose load (1 g/kg), and plasma glucose and insulin were monitored for 2 h. A, time-dependent change of plasma glucose. B, area under the curve of plasma glucose (0–120 min). C, time-dependent change of plasma insulin. D, area under the curve of plasma insulin (pre-30 min). Data are mean ± S.D. (n = 6). #, p < 0.025 versus control by one-tailed Williams’ test.

**Fig. 7.** TAK-875 improves fasting hyperglycemia without affecting fasting normoglycemia. A and B, effects of TAK-875 on fasting normoglycemia. Male SD rats were fasted overnight and orally given vehicle, TAK-875 (30 mg/kg), nateglinide (50 mg/kg), or glibenclamide (10 mg/kg). Plasma glucose (A) and insulin levels (B) were monitored over 3 h. C and D, effects of TAK-875 on fasting hyperglycemia. Male ZDF and age-matched ZL rats were fasted overnight and orally given vehicle, TAK-875 (10 mg/kg), nateglinide (50 mg/kg), or glibenclamide (10 mg/kg). Plasma glucose (C) and insulin levels (D) were monitored over 6 h. Data are mean ± S.D. (n = 6). *, p ≤ 0.05 and **, p ≤ 0.01 versus control by Dunnett’s test. #, p < 0.05 versus control by Steel test.
ZDF rats, in which the single oral dose of TAK-875 improved fasting hyperglycemia, exhibit severe type 2 diabetes with age-dependent decline of plasma insulin levels and β cell mass (Pick et al., 1998). Thus, future studies will focus on the effects of multiple doses of TAK-875 on pancreatic β cell function, apoptosis, and islet morphology in this rat model. On the other hand, oral administration of high doses of TAK-875 (30 mg/kg) in normal fasted rats did not induce hypoglycemia. Oral administration of TAK-875 results in rapid absorption of the compound (Negoro et al., 2005), indicating that the absence of hypoglycemic events and the minor insulinitropic effects observed in normal rats receiving high doses of TAK-875 may not be caused by the low plasma concentration of the compound. Rather, these results suggest that TAK-875 may present a low risk of hypoglycemia, an adverse effect common to sulfonylureas and meglitinides.

Although GPR40/FFA₁ has been considered a possible lipotoxicity mediator (Steneberg et al., 2005), a number of experimental observations do not support a central role for GPR40/FFΑ₁ in lipotoxicity (Latour et al., 2007; Kebede et al., 2008; Lan et al., 2008; Alquier et al., 2009; Nagasumi et al., 2009). In our experiments, prolonged agonist stimulation to TAK-875 for 72 h in INS-1 cells, at the dose range in which sufficient agonist activity was observed, did not affect subsequent glucose-stimulated insulin secretion, insulin content, or caspase 3/7 activity, whereas FFAs did affect these parameters. In addition, we did not observe any correlation between these events and agonist activity for GPR40/FFΑ₁. Our results, therefore, suggest that chronic toxic events induced by FFAs may be independent of GPR40/FFΑ₁, and chronic activation of GPR40/FFΑ₁ by TAK-875 may not lead to either β cell dysfunction or apoptosis. FFAs may induce toxicological effects by other mechanisms, such as long-chain fatty acyl-coenzyme A accumulation, ceramide synthesis, and ER stress induction (Haber et al., 2003; Morgan, 2009).

Currently, GLP-1 analogs and DPP-4 inhibitors are in clinical use. GLP-1 analogs are glucose-dependent insulinitropic agents, showing excellent efficacy for the treatment of diabetes with a low risk of hypoglycemia. However, these drugs are peptides and currently require administration via injection (Mikhail, 2008). On the other hand, DPP-4 inhibitors are orally available small-molecule insulinitropic drugs, with an excellent safety profile. However, the indirect insulinitropic effects dependent on endogenous GLP-1 and/or glucose-dependent insulinitropic polypeptide may limit the efficacy in some patients.

Combination therapy with anti diabetic drugs is often used for the treatment of type 2 diabetes. Our results indicate that TAK-875 is a glucose-dependent insulinitropic agent with a low risk of hypoglycemia. These novel features may allow the use of TAK-875 in combination with insulin sensitizers (metformin and thiazolidines) and α-glucosidase inhibitors, with a reduced risk of hypoglycemic events. In addition, because TAK-875 has novel insulinitropic effects, the combination with insulin secretagogues such as sulfonylureas, DPP-4 inhibitors, and GLP-1 analogs may potentiate their glucose-lowering effects.

In conclusion, our results indicate that the GPR40/FFΑ₁ agonist TAK-875 has the potential to be a highly effective drug that warrants further investigation for the treatment of type 2 diabetes.


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