RESEARCH

The reversible effects of free fatty acids on sulfonylurea-stimulated insulin secretion are related to the expression and dynamin-mediated endocytosis of K_{ATP} channels in pancreatic β cells

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Abstract

Objective: Lipotoxicity-induced pancreatic β cell-dysfunction results in decreased insulin secretion in response to multiple stimulus. In this study, we investigated the reversible effects of palmitate (PA) or oleate (OA) on insulin secretion and the relationship with pancreatic β-cell ATP-sensitive potassium (K_{ATP}) channels.
Methods: MIN6 cells were treated with PA and OA for 48 h and then washed out for 24 h to determine the changes in expression and endocytosis of the K_{ATP} channels and glucose-stimulated insulin secretion (GSIS) and sulfonylurea-stimulated insulin secretion (SU-SIS).
Results: MIN6 cells exposed to PA or OA showed both impaired GSIS and SU-SIS; the former was not restorable, while the latter was reversible with washout of PA or OA. Decreased expressions of both total and surface Kir6.2 and SUR1 and endocytosis of K_{ATP} channels were observed, which were also recoverable after washout. When MIN6 cells exposed to free fatty acids (FFAs) were cotreated with 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) or dynasore, we found that endocytosis of K_{ATP} channels did not change significantly by AICAR but was almost completely blocked by dynasore. Meanwhile, the inhibition of endocytosis of K_{ATP} channels after washout could be activated by PIP2. The recovery of SU-SIS after washout was significantly weakened by PIP2, but the decrease of SU-SIS induced by FFAs was not alleviated by dynasore.
Conclusions: FFAs can cause reversible impairment of SU-SIS on pancreatic β cells. The reversibility of the effects is partial because of the changes of expression and endocytosis of Kir6.2 and SUR1 which was mediated by dynamin.

Key Words
- diabetes
- free fatty acids
- sulfonylureas
- insulin secretion
- ATP-sensitive potassium channels

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Introduction

Various factors may be responsible for β cell dysfunction, including reduced β cell mass (due to de- or transdifferentiation into other cell type) and β cell exhaustion (related to endoplasmic reticulum (ER) and oxidative stress and decreased insulin biosynthesis and secretion) (1, 2). Of these factors, ER and oxidative stress

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are well known to affect receptor-signal transduction, gene expression, and ion channel transport and induce cell apoptosis and death (3). The expression and function of receptors such as calcium-sensing receptor (4), glucose transporter proteins (5), and G protein-coupled receptor (6) are related to β cell dysfunction. Type 2 diabetes (T2D) is also linked to deficiencies in the proteins necessary for insulin exocytosis, in particular, the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins. SNARE proteins can bind to and regulate ATP-sensitive potassium (K_{ATP}) channels in β cells to mediate insulin secretion (7).

K_{ATP} channels play pivotal roles in the regulation of insulin secretion. The K_{ATP} channels consist of four inward rectifying potassium ion channel (Kir6.2) subunits and four sulfonylurea receptor 1 (SUR1) subunits, the latter of which are also sulfonylurea receptors (8). Following an increase in the ATP/ADP ratio, surface K_{ATP} channels close which triggers the opening of voltage-dependent Ca^{2+} channels, eliciting an influx of Ca^{2+}. The rise in intracellular Ca^{2+} then stimulates the exocytosis of insulin-containing secretory granules. The density of the K_{ATP} channel subunits (Kir6.2 and SUR1) on the β cell membranes may have an important role in the physiology of insulin secretion (9). SUR1 and Kir6.2 are synthesized in the ER and assembled in the Golgi apparatus before transportation to the cell membrane. It is generally believed that secretion of insulin in response to high glucose depends on increased intracellular ATP. However, Han *et al*. suggested that internalization of K_{ATP} channels could be induced by high glucose and that the change in surface density of these channels plays a greater role in regulating β cell excitability than ATP-dependent gating (10).

Surface channel numbers of K_{ATP} channels at the plasma membrane are influenced by regulation in the secretory pathway, internalization, recycling, or degradation of surface channels (11). The importance of the endocytic trafficking mechanism was first recognized in studies on mutant channels involved in insulin secretion diseases (12). Recent studies have found that K_{ATP} channel trafficking is also regulated by cellular energy status via AMP-activated protein kinase (AMPK), which plays vital roles in translocation of K_{ATP} channels to the surface membrane (13, 14). Most of these studies have focused on the role of K_{ATP} channels in β cells exposed to glucotoxicity. Palmitic acid is also reported to correlate with K_{ATP} channel trafficking and dysfunction in β cells (15). However, the mechanism and regulation of K_{ATP} channels during lipotoxicity require further investigation.

In this study, we explored the changes in expression and endocytosis of the SUR1 and Kir6.2 subunits in MIN6 cells under chronic exposure to free fatty acids (FFAs). We also described the insulin secretion stimulated by high glucose and sulfonylureas (glibenclamide and glimepiride) related to the distribution of SUR1, providing a new perspective for both potential mechanisms of β cell-dysfunction and therapy strategy during lipotoxicity in T2D.

Materials and methods

Chemicals and antibodies

Glibenclamide (Cat# PHR1287), glimepiride (Cat# PHR1617), sodium palmitate (PA) (Cat# P9767), and sodium oleate (OA) (Cat# O7501) were all purchased from Sigma-Aldrich Corporation. Fatty acid-free bovine serum albumin (BSA) solution was purchased from Mipbo Corporation (CA, USA). Antibodies against Kir6.2 (Cat# ab79171), SUR1 (Cat# ab32844), and Na^+/K^+ pump (Cat# ab7671) were purchased from Abcam, antibodies against α-AMPK (Cat# 2603) and phospho-AMPK (Cat# 2531) were purchased from Cell Signaling Technology Inc., and β-actin antibody was purchased from Nanjing Sunbio Technology Co., Ltd. (Hangzhou, China) Alexa Fluor 488 (donkey anti-rabbit IgG (H+L), Cat# 34206ES60) and Alexa Fluor 594 (donkey anti-mouse IgG (H+L), Cat# 34112ES60) were purchased from Yeasen Biotech Co (Shanghai, China).

Cell culture, FFA preparation, and cell treatment

MIN6 cells (passage 20–45) were routinely maintained in DMEM containing 25 mM glucose, 15% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 50 μmol/L β-mercaptoethanol at 37°C in a humidified incubator containing 5% CO_2. The fatty acid was dissolved in 50% ethanol to a concentration of 100 mM. This stock solution was diluted in culture medium to the required concentration and then incubated with fatty acid-free BSA for 30 min at 37°C to allow complex formation with a final ratio of 5 mM FFA:5% BSA. Final ethanol concentration (0.25%) was added to vehicle control cells. The cells were cultured to 80–85% confluency and stimulated with PA and OA for 0, 24, or 48 h. The cells stimulated for 48 h were washed out for another 24 h with vehicle control.

Cell counting kit-8 (CCK-8) assay

After treatments, cells were washed three times with PBS and seeded into 96-well plates at a density of 1.5×10^4 cells/well in 100 μL culture medium. Cell viability was monitored

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using WST-8 reagent according to manufacturer's instructions (CCK-8, DOJINDO).

RNA extraction and quantitative real-time PCR

Total RNA was extracted using Trizol reagent (Invitrogen) and RNA was converted into cDNA using the PrimeScript™ RT Master Mix (TAKARA). Real-time PCR was performed to assess gene expression using SYBR Green Realtime PCR Master Mix (Roche) in an Eppendorf Realplex real-time PCR system. The following forward (f) and reverse (r) primer pairs were used: SUR1 (f, 5'-AGTGGGAAGTCCTCCTTCTCTCTC-3' and r, 5'-CAGTACGAAACACTAGGCAAGCA-3'), Kir6.2 (f, 5'-TTGGAAGGCGTGGTAGAAAC-3' and r, 5'-GGACAAGGAATCTGGAGAGAT-3'), and β-actin (f, 5'-CGGGGACCTGACTGACTACC-3' and r, 5'-AGGAAGGCTGGAAGAGTGC-3'). Actin was used as the internal standard.

Total and plasma membrane protein extraction and Western blot analysis

To extract total protein, cells were lysed in RIPA lysis buffer and centrifuged at 10,600 ***g*** for 15 min at 4°C. Plasma membrane protein was extracted using a cytoplasmic and membrane protein extraction kit (BB-31161, Bestbio, Shanghai, China). Proteins were separated by SDS-PAGE gel (8–10%) electrophoresis and transferred onto 0.2 μM PVDF membranes (Millipore EMD). After blocking for 2 h in 5% skimmed milk at room temperature, membranes were incubated overnight at 4°C with the following primary antibodies at the respective dilutions: SUR1 (1:3000), Kir6.2 (1:1000), Na^+/K^+-pump (1:1500), β-actin (1:2000), α-AMPK (1:1000), and phospho-AMPK (1:1000). After three 10 min washes with TBST, immunoblots were developed using an ECL detection kit (Yeasen Biotech Co., Shanghai, China) and then visualized with a ChemiDoc™ imaging system (Bio-Rad). Protein bands were quantified using ImageJ (NIH) and normalized to the corresponding controls. The Na^+/K^+ pump was used as the membrane portion control.

Confocal laser-scanning microscopy

Cells were grown on coverslips and treated with chemicals as before and then were fixed with 4% paraformaldehyde in PBS and permeabilized with 0.25% Triton X-100 in PBS, before blocking with 2% donkey serum which was homologous with the secondary antibody. The fixed cells were incubated with anti-SUR1 and anti-Kir6.2 antibody (1:100) overnight at 4°C, and the subcellular localization of SUR1 and Kir6.2 was visualized using Alexa-488-conjugated donkey anti-rabbit immunoglobulin G (IgG) antibody (1:200; Yeasen Biotech Co.) and Alexa-594-conjugated donkey anti-mouse IgG antibody (1:200; Yeasen Biotech Co.). After washing three times in PBS, the slides were treated with DAPI Fluoromount-GTM, and images were acquired on a confocal laser-scanning microscope (Zeiss) using a 60× oil immersion lens. All experiments were repeated at least three times.

Insulin secretion assay

For the glucose-stimulated insulin secretion (GSIS) and sulfonylurea-stimulated insulin secretion (SU-SIS) assays, MIN6 cells were plated in 48-well dishes (1.5×10^4 cells/well) and exposed to PA and OA for 0, 24, or 48 h and washed out for another 24 h. After washing with Krebs–Ringer bicarbonate HEPES buffer (KRBH 135 mmol/L, NaCl 3.6 mmol/L, KCl 0.5 mmol/L, $MgSO_4$ 0.5 mmol/L, NaH_2PO_4 1.5 mmol/L, $CaCl_2$ 2 mmol/L, $NaHCO_3$ 10 mmol/L, and HEPES and 0.2% BSA adjusted to pH7.4), cells were preincubated for 1 h in glucose-free KRBH without test agent for batch incubation. Cells were then stimulated with basal (2 mmol/L) and high glucose levels (20 mmol/L) and 100 μmol/L glibenclamide and glimepiride in 0.2 mL KRBH buffer for 2 h. The insulin concentrations of the supernatants and extracts were determined using an Insulin ELISA Kit (EZassay MS300, Shenzhen, China). Insulin secretion and content were normalized to total protein content.

Statistical analysis

Results are presented as means ± S.E.M. and are from at least three independent experiments. Statistical analysis was performed using SPSS 21.0 (SPSS Inc.). Comparisons between two groups were performed using the Student's *t*-test and between multiple groups using ANOVA and Bonferroni's multiple comparisons test. A *P*-value less than 0.05 meant that differences were statistically significant.

Results

Effects of PA and OA on insulin production and secretion in MIN6 cells

We treated MIN6 cells with PA or OA (0.25/0.5/1.0 mM) for 24 or 48 h and then washed them out for 24 h (48 + 24 h) with BSA treatment (vehicle). The cell viability was assayed by CCK-8 (Supplementary Fig. 1, see section on supplementary materials given at the end of this article).

Thus, a concentration of 0.5 mM PA and OA was selected for subsequent studies.

Concentrations of glimepiride and glibenclamide we used were 100 μmol/L (16). MIN6 cells treated with FFAs exhibited impaired GSIS and SU-SIS, and after the washout step, GSIS remained inhibited while the inhibition of SUs-SIS was reversed (Fig. 1A, B, C and D). Insulin content in both GSIS and SU-SIS was lower than that in vehicle alone (Fig. 1E, F, and G).

Effects of chronic exposure to PA and OA on K_{ATP} channel expression

Total RNA and total protein were extracted from MIN6 cells after treatment with 0.5 mM PA or OA over 48 h without or with a subsequent 24 h washout treatment period. Following PA or OA treatment, Kir6.2 transcription decreased, while SUR1 remained almost the same as the control. Following lipid removal, both SUR1 and Kir6.2 expression was

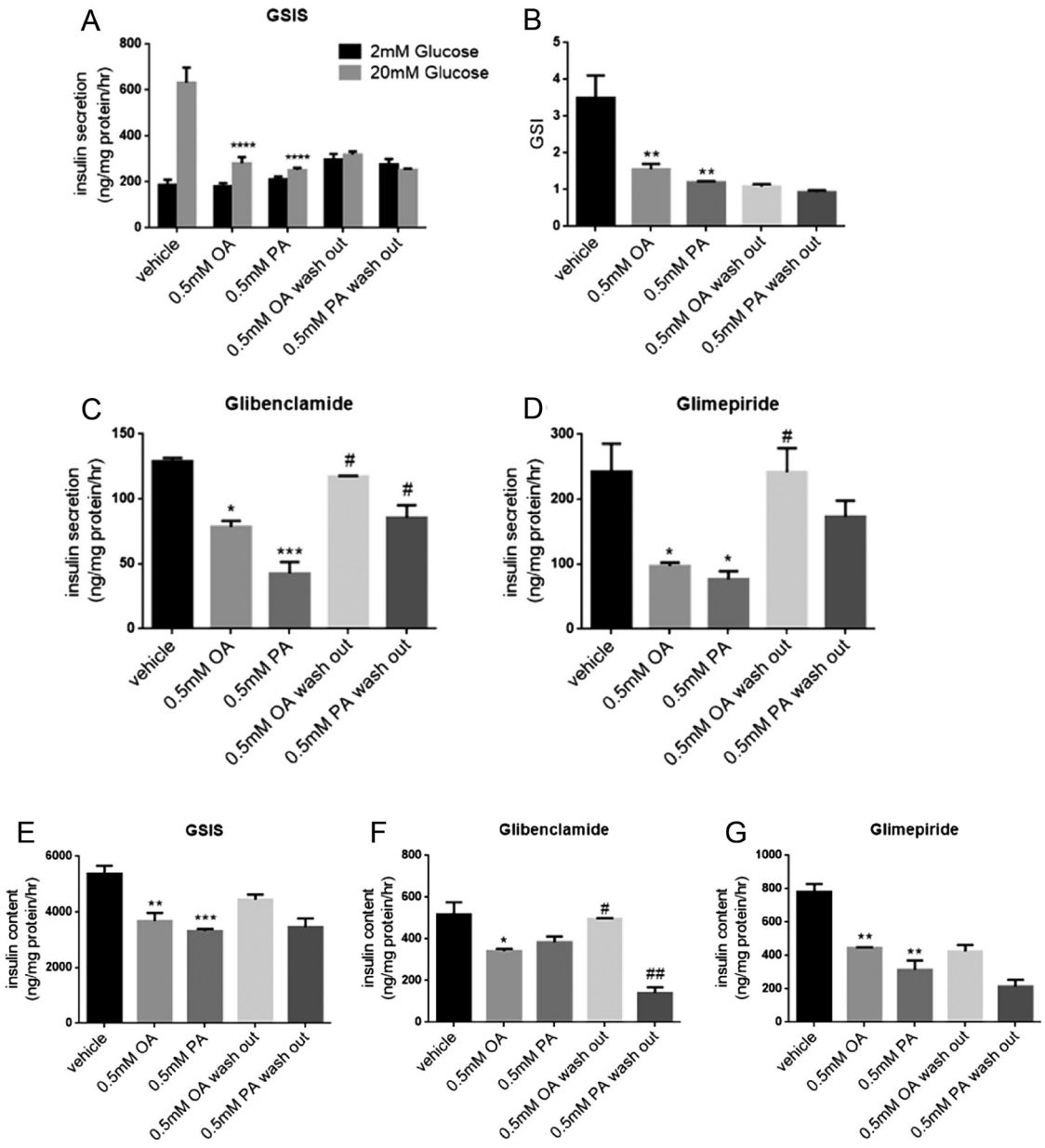

Figure 1
Effects of palmitate and oleate on insulin synthesis and secretion of MIN6 cells. Cells were exposed to 0.5 mM PA or OA for 48 h and then washed out for 24 h with vehicle. Insulin secretion (A, black bar: 2 mM glucose, gray bar: 20 mM glucose; C, 100 μM glibenclamide; D, 100 μM glimepiride) and insulin content (E–G) of MIN6 cells were analyzed by GSIS and SU-SIS assay. B: GSI: ratio of insulin secretion under high glucose (20 mM glucose) to low glucose (2 mM glucose). *$P < 0.05$ or **$P < 0.01$ or ***$P < 0.001$ or ****$P < 0.0001$ vs vehicle ($n = 3$). $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ vs 0.5 mM PA or OA ($n = 3$).

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upregulated (Fig. 2A and B). The same trends were observed in protein expression levels (Fig. 2C, D and E).

Effects of chronic PA and OA exposure on K_{ATP} channel distribution and trafficking

To provide a quantitative analysis of the FFA-induced changes in surface channel density of K_{ATP} channels, plasma membrane proteins and total proteins were both extracted, and the ratio of surface/total protein was used to represent the proportion of plasma membrane protein in total protein. To exclude the possibility that FFAs nonspecifically induced internalization of surface proteins, we examined the surface levels of the Na+/K+ pump and found that it was unaltered by FFAs. The ratios of s-SUR1/t-SUR1 and s-Kir6.2/t-Kir6.2 decreased significantly after 48 h of 0.5 mM PA or OA treatment, and these decreases appeared to be reversed with a 24 h washout period (Fig. 3A, B, and C). These results indicated that an increase in FFAs could induce the internalization of SUR1 and Kir6.2 in MIN6 cells, which could be reversed by removal of the FFAs. To dynamically observe SUR1 and Kir6.2 internalization, we labeled Kir6.2 with Alexa Flour 488 and SUR1 with Alexa Flour 594. As seen in Fig. 3D, in untreated

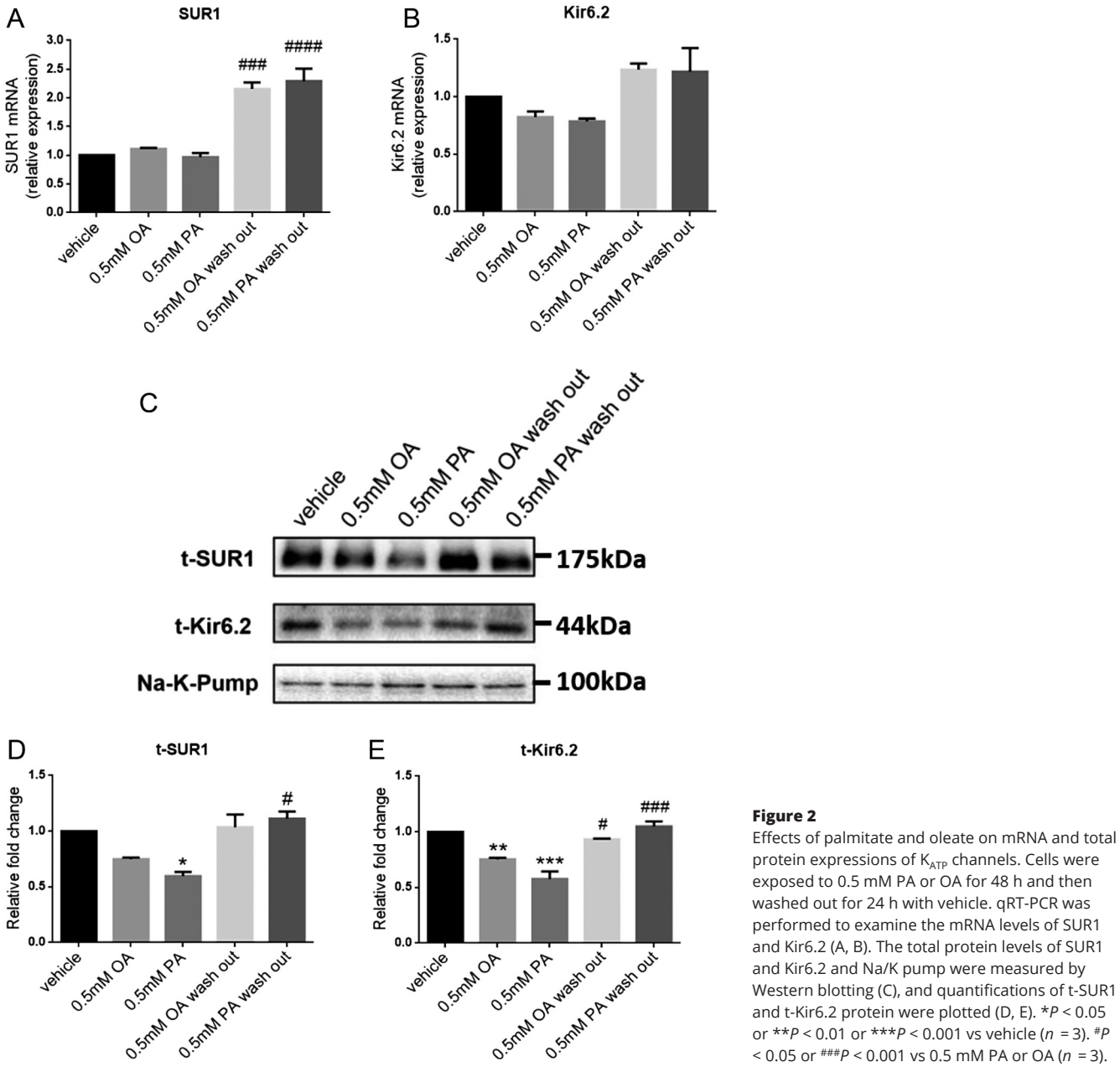

Figure 2
Effects of palmitate and oleate on mRNA and total protein expressions of K_{ATP} channels. Cells were exposed to 0.5 mM PA or OA for 48 h and then washed out for 24 h with vehicle. qRT-PCR was performed to examine the mRNA levels of SUR1 and Kir6.2 (A, B). The total protein levels of SUR1 and Kir6.2 and Na/K pump were measured by Western blotting (C), and quantifications of t-SUR1 and t-Kir6.2 protein were plotted (D, E). $^{*}P < 0.05$ or $^{**}P < 0.01$ or $^{***}P < 0.001$ vs vehicle ($n = 3$). $^{\#}P < 0.05$ or $^{\#\#\#}P < 0.001$ vs 0.5 mM PA or OA ($n = 3$).

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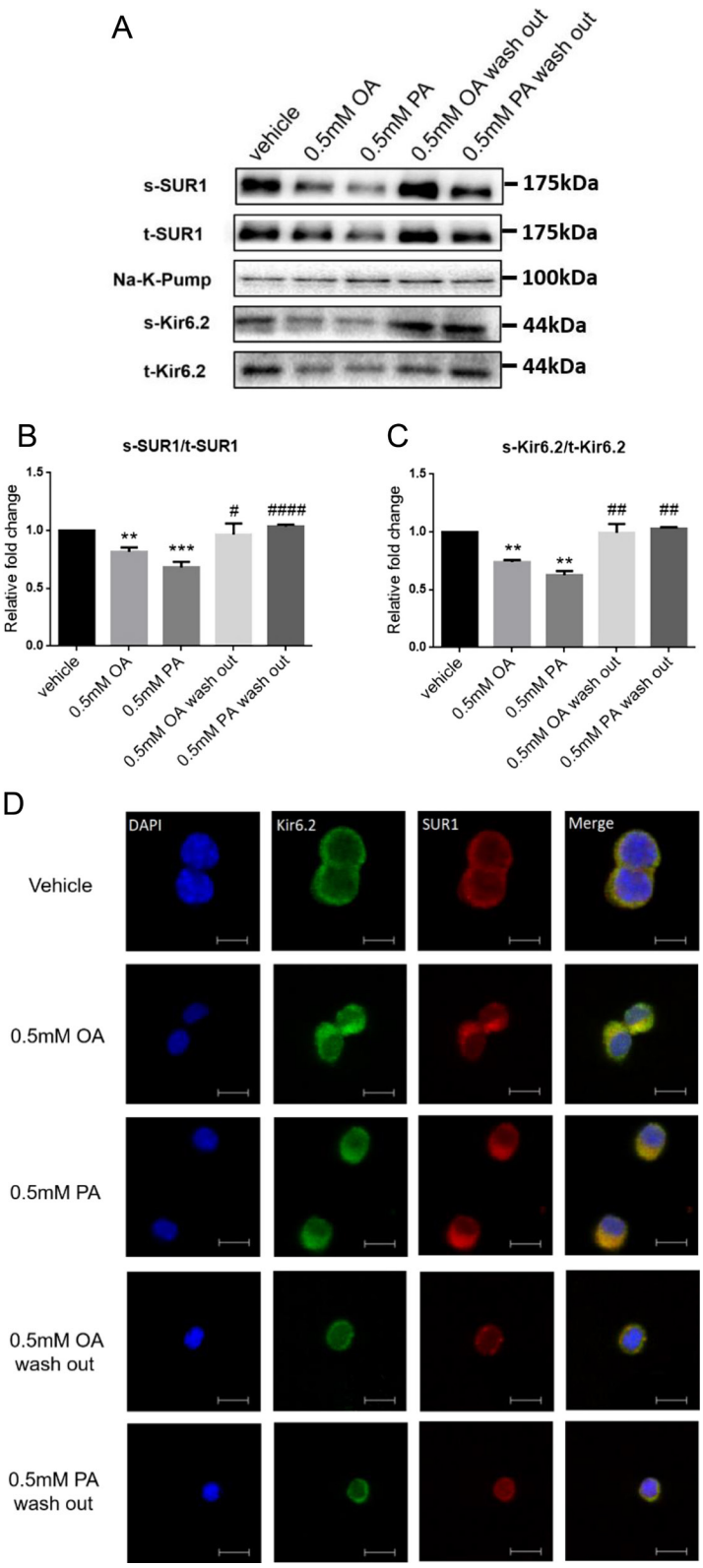

Figure 3

Effects of palmitate and oleate on the distribution and traffic of K_{ATP} channels. Cells were exposed to 0.5 mM PA or OA for 48 h and then washed out for 24 h with vehicle. Total protein and plasma membrane protein levels of SUR1 and Kir6.2 were measured by Western blot analysis. Antibodies to Na^+/K^+ pump were used as loading control for surface proteins (A). The relative ratios of surface to total SUR1 (B) and that of Kir6.2 (C) were obtained based on quantitative densitometric analysis. Confocal images of MIN6 cells stained with Alexa-488-conjugated donkey anti-rabbit IgG antibody after incubation with anti-Kir6.2 antibodies and Alexa-594-conjugated donkey anti-mouse IgG antibody after incubation with anti-Kir6.2 antibodies (D). The scale bar represents 10 μm. $^{**}P < 0.01$ or $^{***}P < 0.001$ vs vehicle ($n = 3$). $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ or $^{\#\#\#}P < 0.001$ vs 0.5 mM PA or OA ($n = 3$).

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cells, SUR1 and Kir6.2 were mostly expressed on the cell membrane, but after PA or OA treatment, the distribution of SUR1 and Kir6.2 changed gradually from cell membrane to cytoplasm, as cell membrane protein internalization occurs. Proteins translocated to the cytoplasm could be returned to the cell membrane, a process known as protein internalization reversal. In MIN6 cells, fluorescence analysis showed that SUR1 and Kir6.2 were colocalized.

PA- and OA-induced K_{ATP} channel internalization is due to increased endocytosis

FFA-induced K_{ATP} channel internalization may be due either to a decrease in channel translocation from the cytoplasm to the surface or to an increase in endocytosis. Previous studies have confirmed that AMPK signaling is involved in K_{ATP} channel translocation (14). Therefore, we

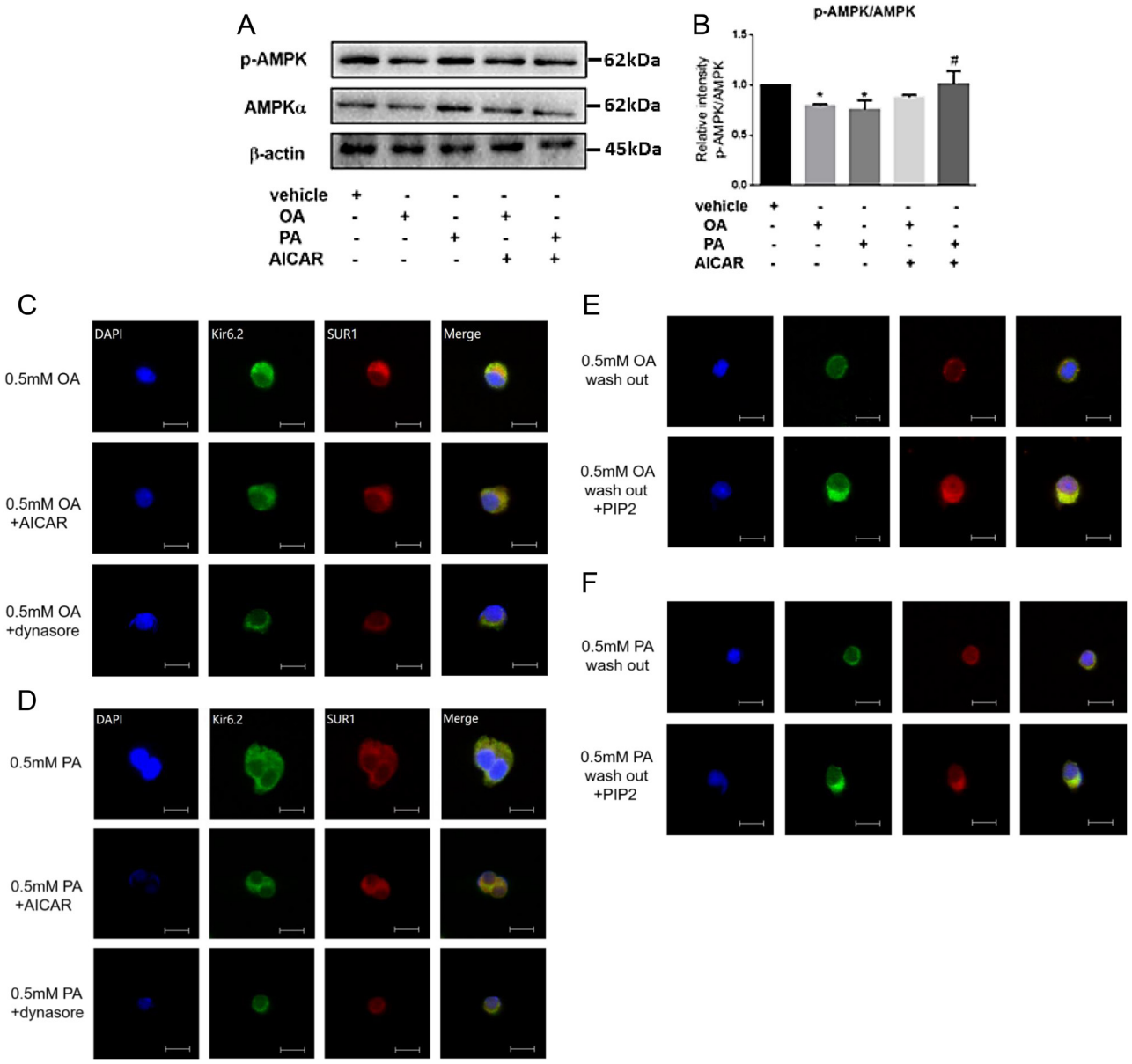

Figure 4

Palmitate- and oleate-induced internalization of K_{ATP} channels is attributable to increase in endocytosis. Western blot analysis shows the effect of 0.5 mM PA or OA on AMPK activation (A). The relative ratios of p-AMPK to total AMPKα were plotted based on quantitative densitometric analysis (B). Cells were exposed to 0.5 mM PA or OA for 48 h with AICAR or dynasore; confocal images of MIN6 cells stained with Alexa-488-conjugated donkey anti-rabbit IgG antibody after incubation with anti-Kir6.2 antibodies and Alexa-594-conjugated donkey anti-mouse IgG antibody after incubation with anti-Kir6.2 antibodies are shown (C, D). Then MIN6 cells were washed out for 24 h with PIP2, and immunofluorescence experiments were performed (E, F). The scale bar represents 10 μm. *$P < 0.05$ vs vehicle ($n = 3$). #$P < 0.05$ vs with AICAR ($n = 3$).

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used 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an AMPK activator, to test the former possibility. We showed that after FFA treatment, AMPK activity (p-AMPK/AMPKα) decreased (Fig. 4A and B), suggesting that FFAs inhibit AMPK-dependent translocation of K_{ATP} channels. However, when AICAR was added to inhibit this decrease in AMPK activity, FFA-induced internalization of K_{ATP} channels was almost unchanged (Fig. 4C and D). These results indicate that inhibiting K_{ATP} channel translocation did not have a significant effect on their internalization induced by FFAs.

Dynamin is a key molecule involved in multiple endocytic pathways. Thus, we used an inhibitor (dynasore) to test the possibility that FFAs induce internalization of K_{ATP} channels by stimulating endocytosis. We found that dynasore (25 μmol/L) almost completely blocked FFA-induced internalization of K_{ATP} channels (Fig. 4C and D). These results suggested that chronically high FFAs could promote the dynamin-mediated endocytosis of K_{ATP} channels, thereby reducing their density on the cell surface. To further verify that FFA-induced internalization of K_{ATP} channels is reversible, PIP2, a dynamin agonist, was administered after FFA removal. The results showed that PIP2 (10 μmol/L) treatment after FFA removal could reverse K_{ATP} channel exocytosis induced by high FFAs (Fig. 4E and F).

PA and OA affect SU-SIS via induced endocytosis of K_{ATP} channels

To assess whether the reversibility of FFA-induced endocytosis of K_{ATP} channels is directly related to the reversibility of SU-SIS, the effects on GSIS and SU-SIS with PA or OA treatment (± 25 μmol/L dynasore) over 48 h and after FFA washout, or vehicle treatment for additional 24 h (± 10 μmol/L PIP2), were compared in MIN6 cells. As expected, no significant effects were seen on dynamin-dependent endocytosis of K_{ATP} channels on GSIS induced by FFAs (Fig. 5A and B), but SUsSIS reversibility after FFA removal was inhibited by PIP2. We also saw that while PA-induced impairment of SU-SIS could be relieved by dynasore, it was not significant and that OA-induced impairment of SU-SIS was hardly alleviated (Fig. 5A and B).

Discussion

A lot of studies have suggested that lipotoxicity induces β cell dysfunction, mass reduction, and apoptosis, which ultimately contribute to T2D (17, 18, 19). Higher concentrations of total plasma non-esterified fatty acids (NEFA) were related to an elevated risk of T2D (20, 21). A Prospective Metabolism and Islet Cell Evaluation (PROMISE) cohort study showed that total NEFA concentration was a strong predictor of β cell dysfunction (22). However, the underlying mechanism by which this occurs remains unclear.

The regulation mechanism of K_{ATP} channels has been extensively explored, with most studies focusing on direct regulation, such as control of K_{ATP} channel sensitivity by membrane phospholipids (23, 24), but few studies have examined K_{ATP} channel density on the cell membrane. Han *et al.* showed that high glucose induces K_{ATP} channel internalization, leading to a surface density reduction. They show that K_{ATP} channel endocytosis plays a greater role in regulating β cell excitability than ATP-dependent gating (10). Beta-cell excitability is a key factor in regulation of insulin secretion, so there is a significant association between insulin secretion and density of K_{ATP} channels. Furthermore, K_{ATP} channels on β cell membranes, such as sulfonylurea-specific receptors, may affect sulfonylurea-promoted insulin secretion though surface density changes, but there are few studies on this, and the mechanism and pathway are unclear. K_{ATP} channel function is enhanced or decreased by its gating properties and expression levels. Kir6.2 or SUR1 mutations have been shown to affect K_{ATP} channel function by modifying gating properties and expression levels, leading to diseases such as permanent neonatal diabetes mellitus (25, 26) and congenital hyperinsulinism of infancy (27, 28). K_{ATP} channel density requires the correct trafficking motif of channel proteins from the ER to the cell membrane. Neither Kir6.2 nor SUR1 reaches the cell membrane when expressed alone because both contain ER retention signals, a three-amino acid motif (RKR), which are masked only in the presence of their partner subunit. When formed, the fully assembled octameric channels are then translocated to the cell surface (29). K_{ATP} channel surface density is also regulated by internalization, recycling, and/or protein degradation. In this study, we focused on the synthesis and internalization of SUR1 and Kir6.2 and did not examine recycling and degradation.

We showed that high FFAs induced endocytosis and decreased the expression of SUR1 and Kir6.2, resulting in a reduction of the K_{ATP} channel density on the cell surface, which may, in turn, decrease the level of insulin secretion following high glucose or sulfonylurea stimulation. We saw that FFA deprivation could reverse the effect of chronic FFA levels on K_{ATP} channel expression and endocytosis and that while sulfonylurea-stimulated insulin secretion was partly regained, glucose-stimulated release was not.

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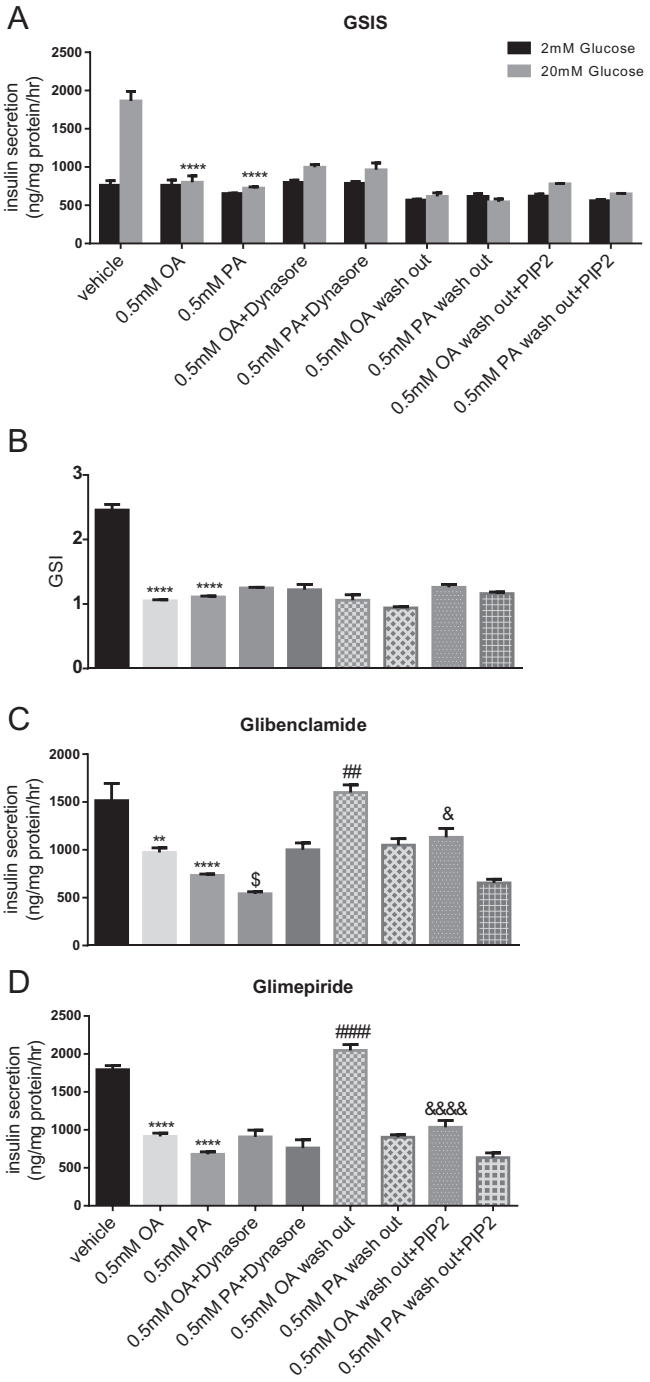

Figure 5

Palmitate and oleate affect sulfonylurea-stimulated insulin secretion (SU-SIS) via induced endocytosis of K_{ATP} channels. MIN6 cells were exposed to 0.5 mM PA or OA for 48 h with dynasore and then washed out for 24 h with PIP2. Insulin secretion (A, black bar: 2 mM glucose, gray bar: 20 mM glucose; C, 100 μM glibenclamide; D, 100 μM glimepiride) of MIN6 cells was analyzed by GSIS and SU-SIS assay. B: GSI: ratio of insulin secretion under high glucose (20 mM glucose) to low glucose (2 mM glucose). $^{**}P < 0.01$ or $^{****}P < 0.0001$ vs vehicle ($n = 3$). $^{\#\#}P < 0.01$ or $^{\#\#\#\#}P < 0.0001$ vs 0.5 mM PA or OA ($n = 3$). $^{\$}P < 0.05$ vs with dynasore ($n = 3$). $^{\&}P < 0.05$ or $^{\&\&\&\&}P < 0.0001$ vs with PIP2 ($n = 3$).

K_{ATP} channel surface levels recovered after FFA deprivation, suggesting the presence of other pathways controlling the sensitivity of the islets' glucose response. Cruz-Cruz *et al.* reported that the unitary conductance and ATP sensitivity of K_{ATP} channels in pancreatic β cells in a high-sucrose diet-induced metabolic syndrome (MS) model and controls were similar. In contrast, MS produced variability in the sensitivity to glibenclamide of K_{ATP} channels (30). Our study showed that OA and PA had the same effects on SUR1 and Kir6.2 expression and endocytosis together with changes with insulin secretion. But the partial reversibility was only seen in the OA group, indicating the difference of different FFAs. This may partially be related to the PA's capacity to induce islet cell apoptosis (31, 32).

The large GTPase dynamin is the first protein involved in membrane fission and plays a central role in many cell functions, from endocytosis to organelle division and fusion, but precisely how it works has not yet be uncovered (33). We found that the reversibility of FFA-induced endocytosis of K_{ATP} channels was mediated by dynamin. When examining insulin secretion, we found that after the OA or PA washout step, immediately adding PIP2 recovered SU-SIS but adding dynasore did not. As we all know, K_{ATP} channel function depends on channel gating properties and the number of channels expressed at the cell surface (10, 11). The surface K_{ATP} channel density has relation with both channel expression and subcellular distribution. The above results indicate that K_{ATP} channel total protein levels after FFA treatment were reduced, so although K_{ATP} channel endocytosis is inhibited by dynasore, membrane density could not significantly increase and so is FFA-induced SU-SIS. Electrophysiology of K_{ATP} channels is the best demonstration to explain it, but it is a pity that we failed to continue with this part due to technical limitations.

While we did see any significant effect on AMPK-mediated endocytosis induced by high FFAs in terms of Kir6.2 and SUR1 transport to the cell membrane, we cannot rule out that this could be due to insufficient excitatory activity and high specificity of AICAR (an AMPK agonist). An agonist with stronger affinity and specificity will need to be selected so that its action and downstream mechanism can be studied further.

Previous studies have shown that high FFAs induce ER stress in islet β cells, resulting in dysregulation of protein folding, production, and trafficking and in an overbalance of calcium ion homeostasis (34, 35). Chronic exposure to high FFA levels resulting in calcium persistent efflux will induce mitochondrial dysfunction

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and increase nitric oxide levels (36). Organelles injured by high FFA levels, especially the ER, play important roles in synthesis and transport of Kir6.2 and SUR1. Clearly, further studies are required to unravel whether FFAs can directly regulate the synthesis and transport of SUR1 and Kir6.2.

In brief, we present the first investigation into the effects of high FFAs on K_{ATP} channel expression and endocytosis in β cells and related insulin secretion. We have preliminarily explored the molecular mechanism of this and revealed the role of endocytosis of Kir6.2 and SUR1, but we did not examine recycling and degradation of K_{ATP} channels. This study provides a new perspective for uncovering the mechanisms of lipotoxicity-induced β cell dysfunction in T2D and helps improve clinical understanding of the conditions and timing of sulfonylurea treatment. Future work using animal models will be needed to understand the mechanism of this recovery and its clinical importance.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-22-0221.

Declaration of interest

The authors declare that they have no conflict of interest.

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