Activation of ATP-sensitive potassium channels in rat pancreatic β -cells by linoleic acid through both intracellular metabolites and membrane receptor signalling pathway

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Abstract

ATP-sensitive potassium channels (K_{ATP} channels) determine the excitability of pancreatic β -cells and importantly regulate glucose-stimulated insulin secretion (GSIS). Long-chain free fatty acids (FFAs) decrease GSIS after long-term exposure to β -cells, but the effects of exogenous FFAs on K_{ATP} channels are not yet well clarified. In this study, the effects of linoleic acid (LA) on membrane potential (MP) and K_{ATP} channels were observed in primary cultured rat pancreatic β -cells. LA (20 μ M) induced hyperpolarization of MP and opening of K_{ATP} channels, which was totally reversed and inhibited by tolbutamide, a K_{ATP} channel blocker. Inhibition of LA metabolism by acyl-CoA synthetase inhibitor, triacsin C (10 μ M), partially inhibited LA-induced opening of K_{ATP} channels by 64%. The non-FFA G protein-coupled receptor

(GPR) 40 agonist, GW9508 (40 μ M), induced an opening of K_{ATP} channels, which was similar to that induced by LA under triacsin C treatment. Blockade of protein kinases A and C did not influence the opening of K_{ATP} channels induced by LA and GW9508, indicating that these two protein kinase pathways are not involved in the action of LA on K_{ATP} channels. The present study demonstrates that LA induces hyperpolarization of MP by activating K_{ATP} channels via both intracellular metabolites and activation of GPR40. It indicates that not only intracellular metabolites of FFAs but also GPR40-mediated pathways take part in the inhibition of GSIS and β -cell dysfunction induced by FFAs.

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Introduction

Type 2 diabetes is linked to obesity and high levels of free fatty acids (FFAs) (Unger 1995, Kahn & Flier 2000, Wyne 2003, Moller & Kaufman 2005). One of the pathways by which FFAs contribute to the occurrence of type 2 diabetes is to deteriorate pancreatic β -cell function (Yaney & Corkey 2003, Haber *et al.* 2006). FFAs have multiple effects on pancreatic β -cells, including inducing apoptosis and stimulating insulin secretion. Although exogenous FFAs stimulate insulin secretion, FFAs substantially decreases glucose-stimulated insulin secretion (GSIS) under a long-term treatment condition (Zraika *et al.* 2002). The discovery that FFAs activate the G-protein-coupled membrane receptor, GPR40, gives an explanation of how FFAs acutely stimulate insulin secretion (Briscoe *et al.* 2003, Itoh *et al.* 2003, Shapiro *et al.* 2005). GPR40 is abundantly expressed in β -cells, and activation of GPR40 induces an increase in intracellular calcium concentration and insulin secretion (Briscoe *et al.* 2003, Fujiwara *et al.* 2005). On the other hand, intracellular metabolism and the generation of lipid-derived signal

molecules from FFAs are generally accepted to mediate the effects of FFAs on insulin secretion. This hypothesis postulates that FFAs cross the β -cells plasma membrane via flip-flop to activate long-chain acyl-CoA esters, which in turn modulate intracellular targets that regulate insulin secretion (Kamp *et al.* 1995, Faergeman & Knudsen 1997, Corkey *et al.* 2000).

The pancreatic β -cell is excitatory and exhibits depolarization of membrane potential (MP) and bursting of action potentials when stimulated by glucose (Rorsman 1997). MP is very important to insulin secretion because it determines the opening and closure of voltage-dependent calcium channels and other voltage-dependent channels. There is evidence that linoleic acid (LA) influences the action of voltage-dependent potassium channels on the membrane of rat pancreatic β -cells via GPR40 and the cAMP and protein kinase A (PKA) intracellular signalling system (Feng *et al.* 2006). In addition, it is found that LA inhibits voltage-dependent calcium channels via calcium-dependent inactivation pathway in rat β -cells (Feng *et al.* 2008). However, the effects of LA on MP and K_{ATP} channel activity are not clarified. In this study, we first observed the effects of LA on MP. Surprisingly, we found that

LA strongly hyperpolarizes MP of rat pancreatic β -cells by activating K_{ATP} channels. Both the intracellular metabolic pathway and the GPR40 receptor pathway are involved in the effects of LA on K_{ATP} channels.

Materials and Methods

Chemicals

LA was purchased from Sigma. GW9508 was obtained from GlaxoSmithKline. Histopaque-1077, dispase, collagenase (type V), DNase I, BSA, Roswell Park Memorial Institute (RPMI) 1640 medium, tolbutamide and all reagents for bath solution and pipette solutions were purchased from Sigma. H89 and chelerythrine was from Calbiochem (San Diego, CA, USA). Fetal calf serum, HEPES and penicillin/streptomycin were obtained from Thermo Electron (Melbourne, Australia).

Preparation and culture of rat pancreatic β -cells

Pancreatic islets were isolated from 10- to 12-week-old male Sprague–Dawley rats as described previously (Zhao *et al.* 2003). The rats were obtained from the Monash University and killed by CO₂ inhalation as approved by the Animal Ethics Committee of Monash University. The pancreas was inflated by injecting 10 ml collagenase solution into it through the bile duct. The collagenase solution was composed of 0.5 mg/ml collagenase, 0.1 mg/ml DNase I and 1 mg/ml BSA in Hank's balanced salt solution (HBSS). The pancreas was collected and digested at 37 °C for 30 min in a stationary state, and then dispersed by shaking. The islets were separated from the pancreas by Histopaque-1077 density gradient centrifugation and collected under microscope. The islets were dispersed into single cells by digestion with dispase solution (1 mg/ml dispase, 0.1 mg/ml DNase I and 1 mg/ml BSA in Ca²⁺-free HBSS). The islet cells were plated onto 35 mm Petri dishes and cultured in RPMI 1640 medium at 8 mM glucose supplemented with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin and 100 g/ml streptomycin in a humidified atmosphere of 95% air and 5% CO₂. The culture medium was changed every 2 days. The experiments were performed during days 3–6 in culture.

Electrophysiological recording

Cells were washed and kept in bath solution before recording. The recordings were made under perforated whole-cell patch-clamp configuration. Electrodes were pulled by a Sutter P-87 microelectrode puller from borosilicate micropipettes and had an initial input resistance of 3–5 M Ω . All recordings were performed using the Axopatch 200A amplifier (Axon Instrument, Union City, CA, USA). The bath solution for MP and K_{ATP} current recordings was composed of (mM): 140 NaCl, 4.7 KCl, 2.6 CaCl₂, 1.2 MgSO₄, 1 NaHCO₃, 1.2 Na₂HPO₄, 5 glucose and 5 HEPES (pH 7.4 with NaOH).

The pipette solution for the recording was composed of (mM): 76 K₂SO₄, 10 KCl, 10 NaCl, 8 MgSO₄, 20 HEPES (pH 7.3 with NaOH). After the formation of a high-resistance seal, the voltage in the pipette was held at -80 mV. Membrane perforation was achieved by 0.24 mg/ml nystatin in the pipette solution, and a series resistance decreasing to lower than 30 M Ω was considered indicative of sufficient access to the cell interior. The whole-cell capacitance and series resistance were well compensated. MP or K_{ATP} current was recorded using different protocols. MP was recorded under current-clamp mode using AxoScope 8 program, as the current was held at 0 pA. K_{ATP} current was recorded under voltage-clamp mode using Clampex 8 program. The cells were held at -80 mV, and a trial of sweeps was obtained by clamping from -110 to -50 mV at intervals of 1 s, with 20 mV increments and 300 ms duration of each step. Drugs were given through a perfusion system at 3 ml/min. β -Cells were identified by cell size and cell membrane capacitance. The mean capacitances of β - and α -cells are 5.5 ± 0.3 and 2.8 ± 0.1 pF respectively (Gopel *et al.* 2000, Leung *et al.* 2005). For the current experiment, β -cell measurements were limited to cells whose whole-cell capacitance, after clamping, equalled to or exceeded 6 pF. Experiments were performed at room temperature.

Statistical analysis

Data are presented as mean $\pm$ S.E.M. for each group. One-way ANOVA was used to analyse the statistical significance between different groups. $P < 0.05$ was taken as the minimum level of significance.

Results

Effects of LA on MP of rat pancreatic β -cells

Rat pancreatic β -cells had a MP between -60 and -40 mV at 5 mM glucose condition. MP remained stable during 20 min recording in control. LA induced a quick hyperpolarization of MP. The MP of the control was -48.33 ± 3.964 mV and became hyperpolarized to -76.25 ± 1.962 mV when 20 μ M LA was added ($P < 0.01$, $n = 12$). LA instantly induced hyperpolarization and the time to reach the stable level was 4.199 ± 0.562 min ($n = 12$). After washout of LA, the MP had a slow partial recovery, and 8.547 ± 2.041 min elapsed before 50% recovery was reached (Fig. 1A and B). Glucose (15 mM) stimulated depolarization of β -cells, and in the presence of glucose, LA induced hyperpolarization of β -cells (-40.69 ± 5.062 mV at 15 mM glucose and -73.58 ± 4.531 mV with LA, $P < 0.01$, $n = 6$; Fig. 1C and D).

Tolbutamide (0.1 mM), a K_{ATP} channel blocker, totally reversed the LA-induced hyperpolarization at 5 mM glucose (Fig. 2A). In 2 mM glucose, rat β -cells had a lower MP of -60.50 ± 5.439 mV. 0.1 mM tolbutamide depolarized the

Figure 1 Effects of LA on membrane potential of rat β -cells. (A) LA-induced hyperpolarization of rat β -cells. (B) The statistical results of membrane potential (** $P < 0.01$ versus control, $n = 12$). (C) LA-induced hyperpolarization at high glucose levels. (D) The statistical results of membrane potential (** $P < 0.01$ versus control, $n = 6$). Data are presented as mean $\pm$ S.E.M.

Figure 2 Effects of tolbutamide on LA-induced hyperpolarization of rat β -cells. (A) LA-induced hyperpolarization was reversed by 0.1 mM tolbutamide at 5 mM glucose (** $P < 0.01$ versus LA, $n = 6$). (B) Tolbutamide-induced depolarization of rat β -cells at 2 mM glucose. LA could not induce hyperpolarization of rat β -cells with the addition of tolbutamide (** $P < 0.01$ versus control; NS means no significant difference, $n = 4$). Data are presented as mean $\pm$ S.E.M.

MP to -29.50 ± 2.255 mV ($P < 0.01$ versus control, $n = 4$). With the addition of tolbutamide, LA could not induce hyperpolarization of rat β -cells (Fig. 2B).

Effects of LA on K_{ATP} channels in rat pancreatic β -cells

The blockade of LA-induced hyperpolarization by tolbutamide, a specific blocker of K_{ATP} channels, indicated that activation of K_{ATP} channels is responsible for the LA-induced hyperpolarization. In 5 mM glucose, there was a small K_{ATP} current. LA induced an increased opening of K_{ATP} channels. Ten minutes after LA incubation, the K_{ATP} currents reached maximal levels. The I-V curve shows that K_{ATP} currents were significantly increased at each clamping step. K_{ATP} currents increased from -2.052 ± 0.273 pA/pF to -24.621 ± 3.818 pA/pF at -110 mV ($P < 0.01$, $n = 12$; Fig. 3A). With the addition of 0.1 mM tolbutamide, LA could not induce the opening of K_{ATP} channels (Fig. 3B).

Figure 3 Effects of LA on K_{ATP} channels of rat β -cells. (A) I-V curve of K_{ATP} currents. Cells were held at -80 mV and clamped from -110 to -50 mV in 20 mV step. K_{ATP} currents were significantly increased at each clamping voltage from -110 to -50 mV. (** $P < 0.01$ versus control at same clamping voltage, $n = 12$). (B) Tolbutamide deterred LA-induced opening of K_{ATP} channels. Data are presented as mean $\pm$ S.E.M.

Effects of triacsin C on LA-induced opening of K_{ATP} channels in rat pancreatic β -cells

LA can get into β -cells by flip-flop across the plasma membrane and metabolized to generate acyl-CoA, and it was found that acyl-CoA can induce opening of K_{ATP} channels (Larsson *et al.* 1996, Branstrom *et al.* 2004). We next tested the effects of blockade of acyl-CoA formation by inhibiting acyl-CoA synthetase using triacsin C. Triacsin C (10 μ M) itself did not influence K_{ATP} currents after 10-min incubation. In the presence of triacsin C, LA could induce opening of K_{ATP} channels (Fig. 4A). However, the maximal levels of K_{ATP} currents were significantly reduced by triacsin C treatment. The change in the currents from -110 to -50 mV was significantly reduced in the group with triacsin C treatment (19.09 ± 4.403 in control versus 6.950 ± 2.240 in triacsin C treatment, $P < 0.05$, $n = 6$; Fig. 4B).

Figure 4 Effects of triacsin C on LA-induced opening of K_{ATP} channels in rat β -cells. (A) With addition of triacsin C, LA induced opening of K_{ATP} channels, but the maximal amplitude was partly inhibited. (B) The changes in the currents from -110 to -50 mV showed that triacsin C treatment partly but significantly inhibited the opening of K_{ATP} channels (* $P < 0.05$ versus LA, $n = 6$). Data are presented as mean $\pm$ S.E.M.

Effects of GW9508 on MP and K_{ATP} channels in rat pancreatic β -cells

The partial inhibition of LA-induced activation of K_{ATP} channels by triacsin C indicates that non-metabolic pathways such as the GPR40 receptor pathway may take part in the action of LA on K_{ATP} channels. Since use of LA could not exclude the involvement of metabolic pathways, we used GW9508, a small-molecule non-metabolic agonist of GPR40, to test whether GPR40 activation contributes to LA-induced opening of K_{ATP} channels. GW9508 induced hyperpolarization and opening of K_{ATP} channels in rat β -cells. As shown in Fig. 5A, GW9508 at 40 μ M hyperpolarized the MP of rat β -cells from -48.25 ± 2.477 to -66.50 ± 1.812 mV ($P < 0.01$, $n = 8$). Tolbutamide totally blocked the hyperpolarization induced by GW9508 (-28.14 ± 3.203 mV versus -30.01 ± 1.543 mV, $P > 0.5$, $n = 7$). In contrast to LA-induced hyperpolarization, the

Figure 5 Effects of GW9508 on membrane potential and K_{ATP} channels of rat β -cells. (A) GW9508 at 40 μ M induced hyperpolarization of β -cells, and the effect of GW9508 on membrane potential was totally blocked by 0.1 mM tolbutamide (** $P < 0.01$, $n = 8$; NS means no significant difference, $n = 7$). (B) GW9508 induced opening of K_{ATP} channels, which was totally blocked by 0.1 mM tolbutamide ($P < 0.01$, $n = 8$). Data are presented as mean $\pm$ S.E.M.

hyperpolarization induced by GW9508 recovered fully and quickly in 5 min after washout of GW9508. According to the hyperpolarization, GW9508 induced opening of K_{ATP} channels. It increased the K_{ATP} currents from -2.691 ± 0.478 to -11.056 ± 1.599 pA/pF at -110 mV ($P < 0.01$, $n = 8$). However, the K_{ATP} currents induced by GW9508 were significantly smaller than that induced by LA (-11.056 ± 1.599 pA/pF at -110 mV for GW9508, $n = 8$; -24.621 ± 3.818 pA/pF at -110 mV for LA, $n = 12$, $P < 0.01$). The opening of K_{ATP} channels induced by GW9508 was totally reversed by tolbutamide in 5 min (Fig. 5B). To exclude the possibility of non-receptor-mediated effects of GW9508 on K_{ATP} channels, we observed the effects of GW9508 on K_{ATP} channels in GH3 cells, a growth hormone (GH)-secreting cell line that does not express GPR40 as identified by reverse transcription PCR (Yang *et al.* 2005, Feng *et al.* 2006). GW9508 at 40 μ M did not increase the K_{ATP} channels in GH3 cells (-1.587 ± 0.208 pA/pF in control and -1.641 ± 0.249 pA/pF after GW9508, no significant difference, $n = 5$).

Effects of protein kinase inhibitors on LA-induced opening of K_{ATP} channels in rat pancreatic β -cells

We next tested whether protein kinases A and C (PKA and PKC) signalling pathways mediate the effects of LA on K_{ATP} channels. PKA inhibitor, 1 μ M H89, did not influence K_{ATP} channels. After treatment with H89 for 10 min, LA induced opening of K_{ATP} channels, and we observed no difference in the amplitude at each clamping step. PKC inhibitor, 10 μ M chelerythrine, neither influenced K_{ATP} channels by itself nor influenced LA-induced opening of K_{ATP} channels. In addition, H89 and chelerythrine did not influence GW9508-induced opening of K_{ATP} channels. The amplitude of K_{ATP} currents at -110 mV is shown in Fig. 6.

Discussion

In this report, we demonstrated that LA induces hyperpolarization of MP in rat β -cells by activating K_{ATP} channels via

Figure 6 Effects of inhibitors of protein kinases A and C on K_{ATP} channels induced by LA and GW9508 in rat β -cells. (A) Neither protein kinase A inhibitor, 10 μ M H89, nor protein kinase C inhibitor, 10 μ M chelerythrine, influenced LA-induced opening of K_{ATP} channels. There was no significant difference in the maximal amplitude of K_{ATP} currents at -110 mV ($n = 9$). (B) H89 and chelerythrine did not influence GW9508-induced opening of K_{ATP} channels ($n = 6$). Data are presented as mean $\pm$ S.E.M.

two distinct pathways: the intracellular metabolite-mediated signalling and membrane receptor GPR40-mediated signalling pathways.

First, we showed that activation of K_{ATP} channels is responsible for LA-induced hyperpolarization in rat β-cells. Pancreatic K_{ATP} channels are formed by two different types of subunits, the Kir6.2 inwards rectifying potassium channels and sulphonylurea receptor subunit SUR1 (Mikhailov & Ashcroft 2000, Mikhailov *et al.* 2000). Pancreatic K_{ATP} channels can be specifically blocked by sulphonylurea such as tolbutamide (Ashfield *et al.* 1999, Gribble & Ashcroft 2000). LA-induced hyperpolarization was reversed by tolbutamide and was deterred by tolbutamide pretreatment, indicating that opening of K_{ATP} channels is responsible for the hyperpolarization. This was confirmed by the current recording. LA induced strong non-voltage-dependent currents that were totally blocked by tolbutamide, confirming that LA opened K_{ATP} channels. K_{ATP} channels play an important role in the process of GSIS. Glucose goes to aerobic oxidation to produce ATP from ADP in β-cells. The resulting increase in the ATP/ADP ratio closes K_{ATP} channels and leads to depolarization of MP and subsequent activation of voltage-gated Ca²⁺ channels (Ca²⁺_v channels). The influx of Ca²⁺ results in an increase in intracellular Ca²⁺ concentration ([Ca²⁺]_i), which triggers exocytosis of insulin granules (Rorsman 1997). Activation of K_{ATP} channels results in a decrease in the excitability of β-cells and inhibits insulin secretion. Although FFAs stimulate insulin secretion, FFAs substantially inhibits GSIS in a long-term condition (Zraika *et al.* 2002, Haber *et al.* 2003). LA-induced activation of K_{ATP} channels may play a role in decreasing β-cell glucose sensitivity and be the reason for the inhibition of GSIS by FFAs. The stimulatory effects of FFAs on insulin secretion should be due to the K_{ATP} channel-independent pathway. For example, FFAs stimulate calcium release from intracellular calcium stores via GPR40 membrane receptor and then trigger insulin secretion. Moreover, FFAs metabolites such as long-chain acyl-CoA activate PKC that facilitates insulin secretion in the distal step of exocytosis. Long-chain acyl-CoA may involve acylation of exocytosis-related proteins and facilitate the exocytosis of secretory granules of insulin (Hess *et al.* 1992, Braun & Scheller 1995).

The mechanism of activation of K_{ATP} channels was not previously fully understood. It was suggested that long-chain acyl-CoA activates K_{ATP} channels (Larsson *et al.* 1996, Branstrom *et al.* 2004, Manning Fox *et al.* 2004). Single-channel recording has shown that long-chain acyl-CoA esters induce a rapid, strong and slowly reversible opening of K_{ATP} channels in mouse β-cells (Larsson *et al.* 1996). Long-chain acyl-CoA is the metabolically active form of FFAs: an important signalling molecule in β-cells (Corkey *et al.* 2000). Extracellular FFAs enter β-cells and are first acylated by acyl-CoA synthetase to form long-chain acyl-CoA before moving to other pathways. Triacsin C is the inhibitor of acyl-CoA synthetase and inhibits the formation of long-chain acyl-CoA (Omura *et al.* 1986). It partly inhibited LA-induced activation of K_{ATP} channels, suggesting the involvement of LA

metabolism in the activation of K_{ATP} channels. The slow partial recovery of hyperpolarization after washout of LA also indicates that LA metabolism is involved in the opening of K_{ATP} channels.

On the other hand, blockade of LA metabolism only partially inhibited the effects of LA on K_{ATP} channels, indicating another signalling pathway is involved in the action of LA on K_{ATP} channels. The GPR40 signalling pathway is a newly discovered pathway for FFAs in mediating β-cell function (Briscoe *et al.* 2003, Itoh *et al.* 2003). FFAs not only bind to GPR40 as ligands but also cross the β-cells plasma membrane to be metabolized. The metabolic effects of FFAs cannot be fully excluded when FFAs are used to activate GPR40. The discovery of a new molecule, GW9508, sheds light on the research in GPR40. GW9508 is a small-molecule agonist of FFA's membrane receptor GPR40 (Briscoe *et al.* 2006, Sum *et al.* 2007). GW9508 induced opening of K_{ATP} channels in a manner similar to that of LA under triacsin C treatment. This supports the view that GPR40 activation also takes part in the activation of K_{ATP} channels. Moreover, compared with the effects of LA on K_{ATP} channels, GW9508-induced opening of K_{ATP} channels was recovered fully and quickly, suggesting that its effect is mediated by the membrane receptor. In addition, GH3 cells with K_{ATP} channels but without GPR40 expression did not show K_{ATP} current change to the action of GW9508. This suggests that the effects of GW9508 on K_{ATP} channels are mediated by GPR40.

The involvement of GPR40 in the activation of K_{ATP} channels describes a new mechanism for FFAs to regulate K_{ATP} channels and GSIS. There are two apparently conflicting hypotheses regarding the regulation of β-cell function by FFAs. First, considerable evidence supports the notion that intracellular metabolism of FFAs is essential to their effects on β-cells (Haber *et al.* 2006). On the other hand, the role of GPR40 receptor in FFA-induced insulin secretion is reported (Itoh *et al.* 2003). The results of our experiments demonstrate that both signalling pathways mediate the effects of FFAs on K_{ATP} channels. This result may suggest a complimentary action of the two pathways in the functional regulation of β-cells. GPR40 knockout mice do not express dysfunction in GSIS (Steneberg *et al.* 2005, Latour *et al.* 2007). However, GPR40-overexpressing mice have impaired GSIS (Steneberg *et al.* 2005), the mechanism of which was not understood. Our study demonstrates that activation of K_{ATP} channels due to overexpression of GPR40 may be responsible for the impaired GSIS in GPR40-overexpressing mice.

We attempted to clarify the molecular mechanism of LA-induced activation of K_{ATP} channels. Both activation of GPR40 and FFA metabolites can activate PKC (Poitout 2003) and GPR40 may also activate PKA (Feng *et al.* 2006). H89 is a cell-permeable and potent inhibitor of PKA with IC₅₀ of 50 nM (Chijiwa *et al.* 1990). Chelerythrine is a potent and selective PKC inhibitor with IC₅₀ of 0.66 μM (Herbert *et al.* 1990). By inhibiting these protein kinases by H89 and chelerythrine, we found that neither PKA nor PKC signalling pathways take part in the effects of LA on K_{ATP}

channels. Further research is required to clarify the molecular mechanisms of intracellular metabolites of FFAs and GR40-mediated activation of K_{ATP} channels.

In conclusion, our study demonstrates that both intracellular metabolites and GPR40 membrane receptors take part in LA-induced opening of K_{ATP} channels. This describes a new mechanism for FFA-induced impairment of GSIS. The present study provides new data to reevaluate the role of GPR40 in pancreatic β -cells.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Ashfield R, Gribble FM, Ashcroft SJ & Ashcroft FM 1999 Identification of the high-affinity tolbutamide site on the SUR1 subunit of the $K(ATP)$ channel. *Diabetes* **48** 1341–1347.
- Branstrom R, Aspinwall CA, Valimaki S, Ostensson CG, Tibell A, Eckhard M, Brandhorst H, Corkey BE, Berggren PO & Larsson O 2004 Long-chain CoA esters activate human pancreatic β -cell $KATP$ channels: potential role in type 2 diabetes. *Diabetologia* **47** 277–283.
- Braun JE & Scheller RH 1995 Cysteine string protein, a DnaJ family member, is present on diverse secretory vesicles. *Neuropharmacology* **34** 1361–1369.
- Briscoe CP, Tadayon M, Andrews JL, Benson WG, Chambers JK, Eilert MM, Ellis C, Elshourbagy NA, Goetz AS, Minnick DT *et al.* 2003 The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *Journal of Biological Chemistry* **278** 11303–11311.
- Briscoe CP, Peat AJ, McKeown SC, Corbett DE, Goetz AS, Littleton TR, McCoy DC, Kenakin TP, Andrews JL, Ammala C *et al.* 2006 Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. *British Journal of Pharmacology* **148** 619–628.
- Chijiwa T, Mishima A, Hagiwara M, Sano M, Hayashi K, Inoue T, Naito K, Toshioka T & Hidaka H 1990 Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. *Journal of Biological Chemistry* **265** 5267–5272.
- Corkey BE, Deeney JT, Yaney GC, Tornheim K & Prentki M 2000 The role of long-chain fatty acyl-CoA esters in β -cell signal transduction. *Journal of Nutrition* **130** 299S–304S.
- Faergeman NJ & Knudsen J 1997 Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling. *Biochemical Journal* **323** 1–12.
- Feng DD, Luo Z, Roh SG, Hernandez M, Tawadros N, Keating DJ & Chen C 2006 Reduction in voltage-gated K^+ currents in primary cultured rat pancreatic β -cells by linoleic acids. *Endocrinology* **147** 674–682.
- Feng DD, Zhao YF, Luo ZQ, Keating DJ & Chen C 2008 Linoleic acid induces Ca^{2+} -induced inactivation of voltage-dependent Ca^{2+} currents in rat pancreatic β -cells. *Journal of Endocrinology* **196** 377–384.
- Fujiwara K, Maekawa F & Yada T 2005 Oleic acid interacts with GPR40 to induce Ca^{2+} signaling in rat islet β -cells: mediation by PLC and L-type Ca^{2+} channel and link to insulin release. *American Journal of Physiology. Endocrinology and Metabolism* **289** E670–E677.
- Gopel SO, Kanno T, Barg S, Weng XG, Gromada J & Rorsman P 2000 Regulation of glucagon release in mouse α -cells by $KATP$ channels and inactivation of TTX-sensitive Na^+ channels. *Journal of Physiology* **528** 509–520.
- Gribble FM & Ashcroft FM 2000 Sulfonylurea sensitivity of adenosine triphosphate-sensitive potassium channels from β cells and extrapancreatic tissues. *Metabolism* **49** 3–6.
- Haber EP, Ximenes HM, Procopio J, Carvalho CR, Curi R & Carpinelli AR 2003 Pleiotropic effects of fatty acids on pancreatic β -cells. *Journal of Cellular Physiology* **194** 1–12.
- Haber EP, Procopio J, Carvalho CR, Carpinelli AR, Newsholme P & Curi R 2006 New insights into fatty acid modulation of pancreatic β -cell function. *International Review of Cytology* **248** 1–41.
- Herbert JM, Augereau JM, Gleye J & Maffrand JP 1990 Chelerythrine is a potent and specific inhibitor of protein kinase C. *Biochemical and Biophysical Research Communications* **172** 993–999.
- Hess DT, Slater TM, Wilson MC & Skene JH 1992 The 25 kDa synaptosomal-associated protein SNAP-25 is the major methionine-rich polypeptide in rapid axonal transport and a major substrate for palmitoylation in adult CNS. *Journal of Neuroscience* **12** 4634–4641.
- Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H *et al.* 2003 Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40. *Nature* **422** 173–176.
- Kahn BB & Flier JS 2000 Obesity and insulin resistance. *Journal of Clinical Investigation* **106** 473–481.
- Kamp F, Zakim D, Zhang F, Noy N & Hamilton JA 1995 Fatty acid flip-flop in phospholipid bilayers is extremely fast. *Biochemistry* **34** 11928–11937.
- Larsson O, Deeney JT, Branstrom R, Berggren PO & Corkey BE 1996 Activation of the ATP-sensitive K^+ channel by long chain acyl-CoA. A role in modulation of pancreatic β -cell glucose sensitivity. *Journal of Biological Chemistry* **271** 10623–10626.
- Latour MG, Alquier T, Oseid E, Tremblay C, Jetton TL, Luo J, Lin DC & Poutout V 2007 GPR40 is necessary but not sufficient for fatty acid stimulation of insulin secretion *in vivo*. *Diabetes* **56** 1087–1094.
- Leung YM, Ahmed I, Sheu L, Tushima RG, Diamant NE, Hara M & Gaisano HY 2005 Electrophysiological characterization of pancreatic islet cells in the mouse insulin promoter-green fluorescent protein mouse. *Endocrinology* **146** 4766–4775.
- Manning Fox JE, Nichols CG & Light PE 2004 Activation of adenosine triphosphate-sensitive potassium channels by acyl coenzyme A esters involves multiple phosphatidylinositol 4,5-bisphosphate-interacting residues. *Molecular Endocrinology* **18** 679–686.
- Mikhailov MV & Ashcroft SJ 2000 Interactions of the sulfonylurea receptor 1 subunit in the molecular assembly of β -cell $K(ATP)$ channels. *Journal of Biological Chemistry* **275** 3360–3364.
- Mikhailov MV, Mikhailova EA & Ashcroft SJ 2000 Investigation of the molecular assembly of β -cell $K(ATP)$ channels. *FEBS Letters* **482** 59–64.
- Moller DE & Kaufman KD 2005 Metabolic syndrome: a clinical and molecular perspective. *Annual Review of Medicine* **56** 45–62.
- Omura S, Tomoda H, Xu QM, Takahashi Y & Iwai Y 1986 Triacins, new inhibitors of acyl-CoA synthetase produced by *Streptomyces* sp. *Journal of Antibiotics* **39** 1211–1218.
- Poutout V 2003 The ins and outs of fatty acids on the pancreatic β cell. *Trends in Endocrinology and Metabolism* **14** 201–203.

- Rorsman P 1997 The pancreatic β -cell as a fuel sensor: an electrophysiologist's viewpoint. *Diabetologia* **40** 487–495.
- Shapiro H, Shachar S, Sekler I, Hershfinkel M & Walker MD 2005 Role of GPR40 in fatty acid action on the β cell line INS-1E. *Biochemical and Biophysical Research Communications* **335** 97–104.
- Steneberg P, Rubins N, Bartoov-Shifman R, Walker MD & Edlund H 2005 The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metabolism* **1** 245–258.
- Sum CS, Tikhonova IG, Neumann S, Engel S, Raaka BM, Costanzi S & Gershengorn MC 2007 Identification of residues important for agonist recognition and activation in GPR40. *Journal of Biological Chemistry* **282** 29248–29255.
- Unger RH 1995 Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* **44** 863–870.
- Wyne KL 2003 Free fatty acids and type 2 diabetes mellitus. *American Journal of Medicine* **115** 29S–36S.
- Yaney GC & Corkey BE 2003 Fatty acid metabolism and insulin secretion in pancreatic β cells. *Diabetologia* **46** 1297–1312.
- Yang SK, Parkington HC, Blake AD, Keating DJ & Chen C 2005 Somatostatin increases voltage-gated K^+ currents in GH3 cells through activation of multiple somatostatin receptors. *Endocrinology* **146** 4975–4984.
- Zhao YF, Xu R, Hernandez M, Zhu Y & Chen C 2003 Distinct intracellular Ca^{2+} response to extracellular adenosine triphosphate in pancreatic β -cells in rats and mice. *Endocrine* **22** 185–192.
- Zraika S, Dunlop M, Proietto J & Andrikopoulos S 2002 Effects of free fatty acids on insulin secretion in obesity. *Obesity Reviews* **3** 103–112.

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