GW9508 inhibits insulin secretion by activating ATP-sensitive potassium channels in rat pancreatic β-cells

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Abstract

GW9508 is an agonist of G protein-coupled receptor 40 (GPR40) that is expressed in pancreatic β-cells and is reported to regulate insulin secretion. However, the effects of GW9508 on pancreatic β-cells in primary culture have not been well investigated. This study measured the acute effects of GW9508 on insulin secretion from rat pancreatic islets in primary culture, and the insulin secretion-related events such as the changes in membrane potential, ATP-sensitive potassium currents (KATP currents), and intracellular Ca2+ concentrations ([Ca2+]i) of rat islet β-cells were also recorded. GW9508 (10–40 μM) did not influence basal insulin levels at 2 mM glucose, but it (above 20 μM) significantly inhibited 5 and 15 mM glucose-stimulated insulin secretion (GSIS). GW9508 did not inhibit insulin secretion stimulated by tolbutamide, the closer of KATP channels. GW9508 activated KATP channels and blocked the membrane depolarization and the increase in [Ca2+]i that were stimulated by glucose. GW9508 itself stimulated a transient increase in [Ca2+]i, which was fully blocked by depletion of intracellular Ca2+ stores with thapsigargin or by inhibition of phospholipase C (PLC) activity with U73122. GW9508-induced activation of KATP channels was only partly inhibited by U73122 treatment. In conclusion, although it stimulates a transient release of Ca2+ from intracellular Ca2+ stores via activation of PLC, GW9508 inhibits GSIS by activating KATP channels probably in a distal step to GPR40 activation in rat β-cells.

Key Words

► GW9508
► insulin
► KATP channels
► β-cells

Introduction

GW9508 (3-(4-(((3-(phenyloxoy)phenyl)methyl)amino)phenyl)propanoic acid) was first identified as an agonist for free fatty acid receptors (FFARs) such as G protein-coupled receptor 40 (GPR40) and GPR120 from a high-throughput screen of the GlaxoSmithKline chemical collection in 2006 (Briscoe et al. 2006). In previous reports about FFARs actions on pancreatic β-cells, FFAs were commonly used as agonists of FFARs, but the effects of GW9508 on β-cell function were seldom reported. There are only reports showing that GW9508 potentiated glucose-stimulated insulin secretion (GSIS) in insulinoma cell lines such as MIN6 cells and INS-1E cells (Briscoe et al. 2006, Yang et al. 2010). And it was shown that GW9508 does not influence insulin secretion in rat
and mouse islets in primary culture (Briscoe et al. 2006). The detailed effects of GW9508 on the function of β-cells in primary culture are not well known.

In our previous study, we found that GW9508 activates ATP-sensitive potassium channels (K_{ATP} channels) on rat β-cells in primary culture (Zhao et al. 2008). The K_{ATP} channel is a key controller to β-cell excitability and GSIS. It is known that pancreatic β-cells metabolize glucose to generate ATP from ADP, and the increased ATP:ADP ratio closes K_{ATP} channels and leads to membrane depolarization and a concomitant rise in the levels of intracellular Ca^{2+} concentration ([Ca^{2+}]_i) and insulin secretion (Ashcroft et al. 1994, Rorsman 1997, Drews et al. 2010, Rorsman et al. 2011). In the present study, we further investigated the effects of GW9508 on insulin secretion, K_{ATP} channels, and [Ca^{2+}]_i levels in primary rat islets and β-cells. It was found that GW9508 activates K_{ATP} channels and inhibits GSIS.

Materials and methods

Materials

GW9508 was obtained from GlaxoSmithKline and Sigma. Histopaque-1077, Dispase, collagenase (type V), DNase I, BSA, RPMI-1640, thapsigargin, tolbutamide, and all reagents for bath solution and pipette solutions were purchased from Sigma. U73122 was obtained from Calbiochem (San Diego, CA, USA). Fura-2/AM was purchased from Invitrogen. FCS, HEPES, and penicillin/streptomycin were obtained from Gibco. QIAamp DNA Investigator kits were obtained from Qiagen and the Qubit dsDNA HS assay kits were purchased from Invitrogen.

Preparation and culture of rat pancreatic β-cells

Sprague Dawley rats (8–10 weeks old) were purchased from the Animal House of The University of Queensland (UQ). The animal experiment was reviewed and approved by the Animal Care and Use Committee of UQ. Pancreatic islets were isolated from 10- to 12-week-old male Sprague Dawley rats as described previously (Zhao et al. 2008). Briefly, rats were killed and 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 were collected and digested at 37°C for 30 min in stationary state and then they were dispersed by shaking. The islets were separated by Histopaque-1077 density gradient centrifugation and collected under microscope.

Insulin secretion assay

After culture overnight, the islets were washed twice with cold incubating solution and then separated into 48-well plates with 20 islets per well in 0.5 ml incubating solution. The samples were randomly separated into different groups with eight samples per group. To observe the dose effects of GW9508, the incubating solution was collected after incubating with different treatments for 1 h at stationary state. To observe the effects of GW9508 on the first and second phases of GSIS, 200 islets per sample were put into perfusion chamber and perfused at a speed of 0.1 ml/min under the control of a peristaltic pump. Each experiment began after a 20-min equilibrium period of perfusion with perfusion solution containing 2 mM glucose. Then the perfusion solution was collected every 5 min. After being perfused at 2 mM glucose to get the basal insulin secretion, the islets were exposed to 15 mM glucose for 60 min and then washed with 2 mM glucose. GW9508 was put into the perfusion solution 10 min before the exposure to 15 mM glucose. The perfusion and incubating solution was composed of the following (mM): 140 NaCl, 4.7 KCl, 2.6 CaCl_2, 1.2 MgSO_4, 1 NaHCO_3, 1.2 Na_2HPO_4, 2 glucose, and 5 HEPES (pH = 7.4 with NaOH). Insulin levels in the incubating solution were assayed by insulin ELISA kit as indicated by the kit instructions (DSL Laboratory, Webster, TX, USA). In brief, samples were pipetted into an ELISA 96-well plates in duplicate. The anti-insulin conjugate antibodies were then added, and the plates were incubated on a plate shaker at 700 r.p.m. for 2 h at room temperature. Then the plates were washed thoroughly and 3,3',5,5'-tetramethylbenzidine substrate solution was added and incubated for 15 min at room temperature in the dark. Stop buffer was added and then the absorbance was measured at 450 nM.
were well compensated and then the membrane potential.

The whole-cell capacitance and series resistance lower than 30 MΩ.

10 KCl, 10 NaCl, 8 MgSO4, and 20 HEPES (pH 7.3 with KOH). Membrane perforation was achieved by nystatin with a MultiSkran ELISA plate reader. At the end of the collection of perfusion solution and the incubating solution, the islets in each sample were centrifuged and the suspension solution was removed. Total DNA from the samples was extracted using QIAamp DNA Investigator kits and was quantified using Qubit dsDNA HS assay kits and was quantified using Qubit dsDNA HS assay kits. The insulin level was rectified by the total DNA quantity for each sample.

**Effects of GW9508 on glucose-stimulated insulin secretion.** Glucose at 2 mM glucose. GW9508 at 20 and 40 µM but not at 10 µM significantly inhibited insulin secretion stimulated by 5 and 15 mM glucose. GW9508 did not influence insulin secretion under 2 mM glucose (*P<0.05 and **P<0.01 vs control at same glucose level, n=8).

**Measurement of [Ca2+]i in single rat β-cells.** Islet cells were loaded with 1 µM Fura-2/AM in RPMI-1640 medium for 30 min at 37 °C. Cells were subsequently rinsed with bath solution and kept for 20 min in this solution to allow full de-esterification of the dye and tranquillization of the cells. Fura-2 in the cell was alternately excited by 340 and 380 nm light (100 ms exposure and 2-s cycle) under the control of Dual-Wavelength Photometry Controller (SDR, NSW, Australia). Emission at 510 nm was detected by a photomultiplier tube (PMT model 77348, Oriel, Bozeman, MT, USA). The signal was transmitted into DigiData and recorded by or KATP current was recorded using different protocols. Membrane potential was recorded under current-clamp mode using Axoscope 8 program, as the current was held at 0 pA. KATP 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 −130 to −30 mV at intervals of 1 s with 20 mV increments and a duration of 300 ms each step. Pancreatic β-cells were identified by cell size and cell membrane capacitance. The mean capacitance 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 this study, the cells whose whole-cell capacitances were above 6 pF were recorded and the data were used for analysis. Experiments were performed at room temperature.
Axoscope 8.2 (Axon Instrument). \([Ca^{2+}]_{i}\) was calculated according to the formula described by Gryniewicz et al. (1985). \([Ca^{2+}]_{i}\) \((nM)=K_d \times (F_0/F_s)\times (R−R_{min})/(R_{max}−R)\), where \(K_d\) the Fura-2 dissociation constant (225 nM); \(F_0\), the 380 nm fluorescence in the absence of \(Ca^{2+}\); \(F_s\), the 380 nm fluorescence with saturating \(Ca^{2+}\); \(R\), the 340:380 nm fluorescence ratio; \(R_{max}\), the 340:380 nm ratio with saturating \(Ca^{2+}\); and \(R_{min}\), the 340:380 nm ratio in the absence of \(Ca^{2+}\). \(F_0/F_s\), \(R_{max}\), and \(R_{min}\) were determined in the recorded cells. Briefly, the cells were permeabilized by 20 \(\mu\)M ionomycin for 10 min to allow sufficient extracellular \(Ca^{2+}\) entry and the resulting 340:380 nm ratio is \(R_{max}\). After a steady value of \(R_{max}\) had been obtained, the \(R_{min}\) value was determined by chelating \(Ca^{2+}\) with 8 mM EGTA. Cells were constantly perfused at a rate of 3 ml/min. Experimental reagents were dissolved in the bath solution just before the recordings and delivered through the perfusion. The bath solution used for \([Ca^{2+}]_{i}\) measurements was the same as that used for the insulin assay.

**Statistical analysis**

The data are represented 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 statistical significance.

**Results**

**GW9508 dose dependently inhibited GSIS from rat islets**

Glucose significantly stimulated insulin secretion from rat pancreatic islets at 5 and 15 mM concentrations during 1-h incubation compared with the basal level of 2 mM glucose. GW9508 did not significantly influence insulin secretion at 2 mM glucose. However, it dose dependently inhibited GSIS during 1-h incubation. GW9508 at 10 \(\mu\)M did not influence insulin secretion at either 5 or 15 mM glucose levels. At 5 mM glucose, GW9508 at 20 and 40 \(\mu\)M significantly inhibited insulin secretion 33 and 64% respectively (4.77 \(\pm\) 0.65 ng/\(\mu\)g islets DNA in control; 3.2 \(\pm\) 0.39 ng/\(\mu\)g islets DNA at 20 \(\mu\)M GW9508, \(P<0.05\) vs control, \(n=8\); 1.71 \(\pm\) 0.31 ng/\(\mu\)g islets DNA at 40 \(\mu\)M GW9508, \(P<0.01\) vs control, \(n=8\)). At 15 mM glucose, GW9508 at 20 and 40 \(\mu\)M significantly inhibited insulin secretion 19 and 51% respectively (13.66 \(\pm\) 0.74 ng/\(\mu\)g islets DNA in control; 11.11 \(\pm\) 0.49 ng/\(\mu\)g islets DNA at 20 \(\mu\)M GW9508, \(P<0.05\) vs control, \(n=8\); 6.67 \(\pm\) 0.76 ng/\(\mu\)g islets DNA at 40 \(\mu\)M GW9508, \(P<0.01\) vs control, \(n=8\); Fig. 1). Glucose stimulated two phases of insulin secretion in rat islets as shown by the perfusion experiment. When GW9508 was given 10 min before the exposure to 15 mM glucose, GW9508 (40 \(\mu\)M) completely inhibited the first phase of GSIS. The second phase of GSIS was also significantly inhibited by GW9508, and the inhibitory ratio was about 51% (Fig. 2). GW9508 at 20 and 40 \(\mu\)M could not inhibit insulin secretion that was stimulated by 0.1 mM tolbutamide at 15 mM glucose during 1-h incubation (Fig. 3).

**GW9508 induced hyperpolarization and activated \(K_{ATP}\) channels in rat \(\beta\)-cells**

The resting membrane potential of rat \(\beta\)-cells was \(-48 \pm 2\) mV at 5 mM glucose and was significantly hyperpolarized to \(-66 \pm 2\) mV by 40 \(\mu\)M GW9508 \((P<0.01, n=8)\). The cells recovered from the hyperpolarization fully in 5 min after washout of GW9508 (Fig. 4A and B). GW9508 (40 \(\mu\)M) immediately increased \(K_{ATP}\) currents from \(-4 \pm 0.7\) to \(-16 \pm 2\) pA/pF at \(-130\) mV \((P<0.01, n=8)\). The current recovered completely to \(-4 \pm 0.5\) pA/pF, 5 min after washout of GW9508 (Fig. 4C). Tolbutamide decreased \(K_{ATP}\) current from \(-4 \pm 0.5\) to \(-2 \pm 0.3\) pA/pF at \(-130\) mV \((P<0.01, n=5)\). GW9508 (40 \(\mu\)M), up to 10 min, did not affect this current in the presence of tolbutamide (Fig. 4D). The dose-responses of GW9508 on membrane potential and \(K_{ATP}\) currents were shown in Fig. 4E and F. At 5 mM glucose, GW9508 at 10 \(\mu\)M did not affect the membrane potential and \(K_{ATP}\) currents, and GW9508 at 20 and 40 \(\mu\)M significantly induced hyperpolarization of membrane potential and increase in \(K_{ATP}\) currents dose dependently.

![Figure 3](http://jme.endocrinology-journals.org/C209/DOI: 10.1530/JME-13-0019) Effects of GW9508 on tolbutamide-stimulated insulin secretion. 0.1 mM tolbutamide stimulated high levels of insulin secretion, and GW9508 at 20 and 40 \(\mu\)M could not inhibit tolbutamide-stimulated insulin secretion.
GW9508 inhibited glucose-stimulated increase in \([\text{Ca}^{2+}]\), in rat \(\beta\)-cells

The resting \([\text{Ca}^{2+}]\) level in \(\beta\)-cells was low at 3 mM glucose, but it significantly increased after glucose stimulation. Glucose repetitively induced increase in \([\text{Ca}^{2+}]\) in rat \(\beta\)-cells (Fig. 6A). The second increase in \([\text{Ca}^{2+}]\) was analysed and the \([\text{Ca}^{2+}]\) levels were shown in Fig. 6B (15 ± 2 vs 115 ± 13 nM, \(P < 0.01\), \(n = 7\)). GW9508 (40 \(\mu\)M) treatment completely blocked 15 mM glucose-stimulated increase in \([\text{Ca}^{2+}]\) but not 0.1 mM tolbutamide-stimulated increase in \([\text{Ca}^{2+}]\), in rat \(\beta\)-cells (Fig. 6C). The \([\text{Ca}^{2+}]\) levels in the second stimulation were analysed and shown in Fig. 6D (18 ± 3 nM at basal level, 20 ± 2 nM after 15 mM glucose, \(P > 0.5\) vs basal level; 126 ± 17 nM after tolbutamide, \(P < 0.01\) vs basal level, \(n = 7\)).

GW9508 stimulated increase in \([\text{Ca}^{2+}]\), by mobilizing calcium release in rat \(\beta\)-cells

GW9508 itself stimulated a transient increase in \([\text{Ca}^{2+}]\), in rat \(\beta\)-cells (Fig. 7A). Removal of extracellular \(\text{Ca}^{2+}\) did not eliminate the GW9508-stimulated increase (Fig. 7B). Pretreatment of the cells with thapsigargin (1 \(\mu\)M for 30 min) totally eliminated the GW9508-stimulated \([\text{Ca}^{2+}]\) increase (Fig. 7C). The mean \([\text{Ca}^{2+}]\) changes (\(\Delta[\text{Ca}^{2+}]\)), the mean \([\text{Ca}^{2+}]\) levels in 3 min after GW9508 stimulation subtracting basal levels of \([\text{Ca}^{2+}]\) are shown in Fig. 7D (\(P < 0.01\) vs control in thapsigargin group, \(n = 8\)).
GW9508 inhibits insulin secretion

GW9508 was first identified as FFAR agonist to activate GPR40 and GPR120 (Briscoe et al. 2006, Sum et al. 2007, Tikhonova et al. 2007). In the present study, we found that GW9508 inhibits GSIS by activating K\textsubscript{ATP} channels in rat pancreatic \(\beta\)-cells, which is supported by the results of insulin secretion assay, electrophysiological recording, and \([\text{Ca}^{2+}]\text{i}\) recording.

Glucose stimulates insulin secretion in a biphasic manner that is mediated by two different signalling pathways, the K\textsubscript{ATP} channel-dependent pathway and K\textsubscript{ATP} channel-independent pathway (Gembal et al. 1992, Komatsu et al. 1997, Straub et al. 1998, Henquin 2000, Aizawa et al. 2002). ATP derived from glucose oxidation closes K\textsubscript{ATP} channels and leads to depolarization of membrane potential and subsequent activation of voltage-gated Ca\(^{2+}\) channels (Ca\(^{2+}\text{[v]}\) channels). The increase in \([\text{Ca}^{2+}]\text{i}\) following the influx of Ca\(^{2+}\) via Ca\(^{2+}\text{[v]}\) channels triggers the first phase of insulin secretion in K\textsubscript{ATP} channel-dependent manner (Rorsman 1997). Augmentation of Ca\(^{2+}\)-triggered insulin secretion by K\textsubscript{ATP} channels independent of signals that follow glucose metabolism takes part in the second phase of insulin secretion (Straub et al. 1998, Straub & Sharp 2002). GW9508 completely inhibited the first phase of GSIS, and it partially inhibited the second phase of GSIS. It supports that GW9508 mainly inhibits the K\textsubscript{ATP}-dependent pathway but not K\textsubscript{ATP}-independent pathway.

Pancreatic K\textsubscript{ATP} channels are formed by two different types of subunits, the Kir6.2 inwardly rectifying potassium channels and sulphonylurea receptor subunit SUR1. Pancreatic K\textsubscript{ATP} channels are specifically blocked by sulphonylurea such as tolbutamide. Although GW9508 inhibits GSIS by activating K\textsubscript{ATP} channels in rat pancreatic \(\beta\)-cells, which is supported by the results of insulin secretion assay, electrophysiological recording, and \([\text{Ca}^{2+}]\text{i}\) recording.

Discussion

Effects of U73122 treatment on GW9508-induced activation of K\textsubscript{ATP} channels and increase in \([\text{Ca}^{2+}]\text{i}\) in \(\beta\)-cells

Activation of phospholipase C (PLC) and the subsequent production of inositol trisphosphate (IP3) is one of the signalling pathways for ligand-stimulated calcium release from intracellular calcium stores (Briscoe et al. 2003, Fujiwara et al. 2005). We tested the effect of PLC blockade by U73122 on GW9508-induced activation of K\textsubscript{ATP} channels and increase in \([\text{Ca}^{2+}]\text{i}\) in \(\beta\)-cells. The K\textsubscript{ATP} currents were activated to 567±85% of basal levels by 40 \(\mu\)M GW9508. After U73122 treatment (10 \(\mu\)M for 10 min), the K\textsubscript{ATP} currents were activated to 250±51% of basal levels by 40 \(\mu\)M GW9508 (Fig. 8A, \(P<0.01\) vs control, \(n=8\)). The \([\text{Ca}^{2+}]\text{i}\) levels increased significantly by GW9508 stimulation, and this increase was totally blocked by U73122 treatment (10 \(\mu\)M for 10 min). The mean level of GW9508-stimulated \([\text{Ca}^{2+}]\text{i}\) was 287±65% of the control, which decreased to 108±12% of the control in U73122 group (Fig. 8B, \(P<0.01\) vs non-U73122 control group, \(n=8\)).
inhibited GSIS, it could not inhibit tolbutamide-stimulated insulin secretion. This supports that GW9508 inhibits GSIS by opening K\textsubscript{ATP} channels. The subsequent electrophysiological recording showed that GW9508 induced hyperpolarization of membrane potential and increase in K\textsubscript{ATP} currents, giving strong evidence that GW9508 activated K\textsubscript{ATP} channels. 

\[ \text{[Ca}^{2+}\text{]}_i \] recording further confirmed that GW9508 inhibited GSIS by opening K\textsubscript{ATP} channels because the increase in \([\text{Ca}^{2+}]_i\) stimulated by glucose but not by tolbutamide was inhibited GW9508. 

Although GW9508 mobilizes intracellular calcium stores, the present study suggests that the effect is not strong enough to stimulate insulin secretion and to counteract its inhibitory effects on GSIS. GW9508-stimulated increase in \([\text{Ca}^{2+}]_i\) was eliminated by depletion of intracellular endoplasmic reticulum calcium stores by thapsigargin treatment, but not by removal of extracellular calcium. It is therefore suggested that GW9508 stimulates calcium release from intracellular calcium stores. Previous reports suggested that FFAR such as GPR40 activation is linked to PLC and mobilization of intracellular calcium stores by production of IP\textsubscript{3} (Briscoe \textit{et al.} 2003, Fujiwara \textit{et al.} 2005). Moreover, it has been confirmed that GPR40 activation can stimulate phosphatidylinositol hydrolysis, further supporting that the IP\textsubscript{3} signalling pathway is employed by GPR40 (Meidute \textit{Abaraviciene et al.} 2008). The present results indicate that GW9508 may activate GPR40 and subsequently stimulate calcium release from intracellular calcium stores. This is further confirmed by the observation that inhibition of PLC activity by U73122 totally blocked GW9508-stimulated increase in \([\text{Ca}^{2+}]_i\), in rat β-cells. However, this increase in \([\text{Ca}^{2+}]_i\) is not sufficient enough to stimulate insulin secretion as there was no significant increase in insulin secretion after GW9508 stimulation at 2 mM glucose and no augmenting effect was observed of GW9508 on tolbutamide-stimulated insulin secretion. As the increase in \([\text{Ca}^{2+}]_i\), stimulated by GW9508 is transient and comes from release of intracellular Ca\textsuperscript{2+} stores, it may not be sufficient in either a temporal or spatial manner to stimulate exocytosis of insulin granules.

On the other hand, the process of exocytosis can be regulated by membrane potential, and it is indicated that depolarization can enhance exocytosis voltage dependently (Mochida \textit{et al.} 1998). Hyperpolarization of the membrane potential by GW9508 may counteract the stimulatory effects of \([\text{Ca}^{2+}]_i\) increase on exocytosis of insulin granules.

GW9508 mobilized intracellular calcium by activating PLC, but the effects of GW9508 on K\textsubscript{ATP} channels were not solely mediated by PLC. Blockade of PLC by U73122 only inhibited the activation of K\textsubscript{ATP} channels by GW9508 in part, suggesting that other signalling pathways may also be involved. It has been reported that oleic acid promotes the activation of the extracellular signal-regulated protein kinase–MAPK signalling pathway, mainly via FFAR (Zhang \textit{et al.} 2007). This indicates that multiple signalling pathways are employed by FFAR. In our previous study, we also tried to clarify the signalling molecules that are involved in the activation of K\textsubscript{ATP} channels by GW9508, but it was found that neither protein kinase A nor C is involved in the action of GW9508 on K\textsubscript{ATP} channels (Zhao \textit{et al.} 2008). Further investigation will be required to clarify the candidate signals in mediating the activation of K\textsubscript{ATP} channels by GW9508 and the involvement of FFAR.
Previous reports mostly indicate that FFAR activation by FFAs stimulates insulin secretion (Itoh et al. 2003, Fujiwara et al. 2005, Salehi et al. 2005, Shapiro et al. 2005). While it was reported that GW9508 is an agonist of FFAR, the inhibitory effects of GW9508 on GSIS are controversial to the stimulatory effects of FFAs on insulin secretion. The discrepancy may be due to differences in species and the significant physiological difference between cell lines and islets β-cell in primary culture. Briscoe et al. (2006) did not see the stimulatory effects of 10 μM GW9508 on insulin secretion in mouse or rat islets, although they observed the stimulatory effects of GW9508 on insulin secretion in MIN6 cells. On the other hand, FFAs not only act as ligands to activate FFAR but also enter cells to be metabolized. The metabolic effects of FFAs could not be fully excluded in previous reports about the stimulation of insulin secretion by FFAs through FFAR. GW9508 does not generate intracellular metabolic effects by itself upon activation of FFAR (Briscoe et al. 2006, Sum et al. 2007). The different chemical properties between FFAs and GW9508 may be one reason for the discrepancy in action on insulin secretion. Moreover, the possibility that GW9508 activates K<sub>ATP</sub> channels via a non-FFAR signalling pathway has not been ruled out. This possibility can be clarified by observing the effects of GW9508 on insulin secretion from islets of FFAR knockout animals such as GPR40 knockout mice. Owing to the limitation to obtaining the animal models, we at present are not able to exclude the possibility of non-FFAR effects of GW9508. However, our results suggest that it is worthy to observe the effects of GW9508 in GPR40 knockout mice, which would deepen our comprehension of the mechanism of GW9508-induced activation of K<sub>ATP</sub> channels.

In summary, the present study suggests that GW9508 is not a secretagogue for insulin secretion in rat islets. GW9508 above 20 μM inhibits insulin secretion via activating K<sub>ATP</sub> channels. Although PLC is involved in the action of GW9508 on K<sub>ATP</sub> channels, the detailed signalling pathways for the effects of GW9508 needs to be further investigated.

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.

Funding
This work is supported by the grant from Australian NH and MRC and the fund from National Natural Sciences Foundation of China (no. 30971078).

References


Gembal M, Gilon P & Henquin JC 1992 Evidence that glucose can control insulin release independently from its action on ATP-sensitive K<sup>+</sup> channels in mouse β-cells. Journal of Clinical Investigation 89 1288–1295. (doi:10.1172/JCI115714)


The authors are grateful to GlaxoSmithKline for providing GW9508.

Acknowledgements
The authors would like to acknowledge the contributions of the members of the research group for their valuable advice and support throughout the course of this project. This work was supported by the National Natural Science Foundation of China (grant 30971078) and the Australian National Health and Medical Research Council (grant 407744).
mediated via the synaptic protein interaction site of N-type Ca\(^{2+}\) channels. *PNAS* **95** 14523–14528. (doi:10.1073/pnas.95.24.14523)


Received in final form 23 April 2013

Accepted 29 April 2013

Accepted Preprint published online 29 April 2013