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\textsubscript{ATP} channels) determine the excitability of pancreatic \(\beta\)-cells and importantly regulate glucose-stimulated insulin secretion (GSIS). Long-chain free fatty acids (FFAs) decrease GSIS after long term exposure to \(\beta\)-cells, but the effects of exogenous FFAs on K\textsubscript{ATP} channels are not yet well clarified. In this study, the effects of linoleic acid (LA) on membrane potential (MP) and K\textsubscript{ATP} channels were observed in primary cultured rat pancreatic \(\beta\)-cells. LA (20 \(\mu\)M) induced hyperpolarization of MP and opening of K\textsubscript{ATP} channels, which was totally reversed and inhibited by tolbutamide, a K\textsubscript{ATP} channel blocker. Inhibition of LA metabolism by acyl-CoA synthetase inhibitor, triacsin C (10 \(\mu\)M), partially inhibited LA-induced opening of K\textsubscript{ATP} channels by 64\%. The non-FFA GPR40 agonist, GW9508 (40 \(\mu\)M), induced an opening of K\textsubscript{ATP} channels, which was similar to that induced by LA under triacsin C treatment. Blockade of protein kinase A and protein kinase C did not influence the opening of K\textsubscript{ATP} channels induced by LA and GW9508, indicating that these two protein kinase pathways are not involved in the action of LA on K\textsubscript{ATP} channels. The present study demonstrates that LA induces hyperpolarization of MP by activating K\textsubscript{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 \(\beta\)-cell dysfunction induced by FFAs.
INTRODUCTION

Type 2 diabetes is linked to obesity and high levels of free fatty acids (FFAs) (Kahn and Flier 2000; Moller and Kaufman 2005; Unger 1995; Wyne 2003). One of the pathways by which FFAs contribute to the occurrence of type 2 diabetes is to deteriorate pancreatic β-cell function (Haber, et al. 2006; Yaney and Corkey 2003). 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-cell’s plasma membrane via flip-flop to activate long-chain acyl-CoA esters, which in turn modulate intracellular targets that regulate insulin secretion (Corkey, et al. 2000; Faergeman and Knudsen 1997; Kamp, et al. 1995).

The pancreatic β-cell is excitatory and exhibits depolarization of membrane potential and bursting of action potentials when stimulated by glucose (Rorsman 1997). Membrane potential 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 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 membrane potential and K\textsubscript{ATP} channel activity are not clarified. In this study, we firstly observed the effects of LA on membrane potential. Surprisingly we found that LA strongly hyperpolarizes membrane potential of rat pancreatic β-cells by...
activating $K_{\text{ATP}}$ channels. Both the intracellular metabolic pathway and the GPR40 receptor pathway are involved in the effects of LA on $K_{\text{ATP}}$ channels.

MATERIALS AND METHODS

1. **Chemicals:** Linoleic acid was purchased from Sigma (St. Louis, USA). GW9508 was obtained from GlaxoSmithKline (Hertfordshire, United Kingdom). Histopaque-1077, dispase, collagenase (type V), deoxyribonuclease I (DNase I), bovine serum albumin (BSA), RPMI-1640, tolbutamide and all reagents for bath solution and pipette solutions were purchased from Sigma. H89 and chelerythrine was from Calbiochem (San Diego, USA). Fetal calf serum, HEPES and penicillin/streptomycin were obtained from Thermo Electron (Melbourne, Australia).

2. **Preparation and culture of rat pancreatic β-cells:** Pancreatic islets were isolated from 10-12 week-old male Sprague-Dawley rats as previously described (Zhao, et al. 2003). Rats were obtained from Monash University and killed by CO$_2$ inhalation as approved by 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 0.5mg/ml collagenase, 0.1mg/ml DNase I and 1mg/ml BSA in Hank’s balanced salt solution (HBSS). The pancreas was collected and digested at 37°C for 30 minutes 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. Islets were dispersed into single cells by digestion with dispase solution (1mg/ml dispase, 0.1mg/ml DNase I and 1mg/ml BSA in Ca$^{2+}$-free HBSS). The islet cells were plated into 35 mm petri dishes and cultured in RPMI-1640 medium at 8mM glucose supplemented with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO$_2$. The culture medium was changed every 2 days. The experiments were performed during days 3-6 in culture.

3. **Electrophysiological recording:** Cells were washed and kept in bath solution before recording. 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 3-5MΩ. All
recordings were performed using the Axopatch 200A amplifier (Axon Instrument, CA, USA). The bath solution for membrane potential recording and $K_{ATP}$ current
recording was composed of (mM): 140 NaCl, 4.7 KCl, 2.6 CaCl$_2$, 1.2 MgSO$_4$, 1 NaHCO$_3$, 1.2 Na$_2$HPO$_4$, 5 glucose and 5 HEPES (pH=7.4 with NaOH). The pipette
solution for recording was composed of (mM): 76 K$_2$SO$_4$, 10 KCl, 10 NaCl, 8 MgSO$_4$, 20 HEPES (pH=7.3 with NaOH). After formation of a high-resistance seal, the voltage in the pipette was held at –80mV. Membrane perforation was achieved by
0.24mg/ml nystatin in pipette solution, and a series resistance decreasing to lower
than 30 MΩ was considered indicative of sufficient access to the cell interior. The
whole-cell capacitance and series resistance were well compensated. Membrane
potential or $K_{ATP}$ 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. $K_{ATP}$ current was recorded under voltage-clamp mode using
Clampex 8 program. The cells were held at –80mV, and a trial of sweeps was
obtained by clamping from -110mV to -50mV at intervals of 1 second, with 20mV
increments and 300 ms duration of each step. Drugs were given through a perfusion
system at 3ml/min. β-cells were identified by cell size and cell membrane
capacitance. The mean capacitances of β-cells and α-cells are 5.5±0.3 pF 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.

**4. Statistics:** Data are presented as Mean ± SEM 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**

1. **Effects of linoleic acid on membrane potential of rat pancreatic β-cells.** Rat
pancreatic β-cells had a membrane potential (MP) between -60mV and -40mV at 5
mM glucose condition. MP remained stable during 20 minutes 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 20µM 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 minutes (n=12). After washout of LA, the MP had a slow partial recovery, and 8.547±2.041 minutes elapsed before 50% recovery was reached (Fig 1A and B). Glucose (15mM) stimulated depolarization of β-cells, and in the presence of glucose, LA induced hyperpolarization of β-cells (-40.69±5.062 mV at 15mM glucose and -73.58±4.531 mV with LA, P<0.01, n=6, Fig 1 C and D).

Tolbutamide (0.1mM), a K<sub>ATP</sub> channel blocker, totally reversed the LA-induced hyperpolarization at 5mM glucose (Fig 2A). In 2mM glucose, rat β-cells had a lower MP of -60.50±5.439 mV. 0.1mM tolbutamide depolarized the MP to -29.50±2.255 mV (P<0.01 vs control, n=4). With the addition of tolbutamide, LA could not induce hyperpolarization of rat β-cells (Fig 2B).

2. Effects of linoleic acid on K<sub>ATP</sub> channels in rat pancreatic β-cells. The blockade of LA-induced hyperpolarization by tolbutamide, a specific blocker of K<sub>ATP</sub> channels, indicated that activation of K<sub>ATP</sub> channels is responsible for the LA-induced hyperpolarization. In 5mM glucose, there was a small K<sub>ATP</sub> current. LA induced an increased opening of K<sub>ATP</sub> channels. 10 minutes after LA incubation, the K<sub>ATP</sub> currents reached maximal levels. The I-V curve shows that K<sub>ATP</sub> currents were significantly increased at each clamping step. K<sub>ATP</sub> currents increased from -2.052 ± 0.273 pA/pF to -24.621 ± 3.818 pA/pF at -110mV (P<0.01, n=12, Fig 3A). With the addition of 0.1mM tolbutamide, LA could not induce the opening of K<sub>ATP</sub> channels (Fig 3B).

3. Effects of triacsin C on linoleic acid-induced opening of K<sub>ATP</sub> 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<sub>ATP</sub> channels (Branstrom, et al. 2004; Larsson, et al. 1996). We next tested the effects of blockade of acyl-CoA formation by inhibiting acyl-CoA synthetase using triacsin C. Triacsin C (10µM) itself did not influence K<sub>ATP</sub> currents after 10 minutes incubation. In the presence of triacsin C, LA could induce opening of K<sub>ATP</sub> channels (Fig 4A). However, the maximal levels of K<sub>ATP</sub> currents were
significantly reduced by triacsin C treatment. The change of the currents from -110mV to -50mV was significantly reduced in the group with Triacsin C treatment (19.09±4.403 in control vs 6.950±2.240 in Triacsin C treatment, P<0.05, n=6, Fig 4B).

4. Effects of GW9508 on membrane potential and \( K_{ATP} \) channels in rat pancreatic \( \beta \)-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 \( \beta \)-cells. As shown in Fig 5A, GW9508 at 40\( \mu \)M hyperpolarized the MP of rat \( \beta \)-cells from -48.25±2.477 mV to -66.50±1.812 mV (P<0.01, n=8). Tolbutamide totally blocked the hyperpolarization induced by GW9508 (-28.14±3.203 mV vs -30.01±1.543 mV, P>0.5, n=7). In contrast to LA-induced hyperpolarization, the hyperpolarization induced by GW9508 recovered fully and quickly in 5 minutes after washout of GW9508. Accordant to the hyperpolarization, GW9508 induced opening of \( K_{ATP} \) channels. It increased the \( K_{ATP} \) currents from -2.691 ± 0.478 pA/pF to -11.056 ± 1.599 pA/pF at -110mV (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 -110mV for GW9508, n=8; -24.621 ± 3.818 pA/pF at -110mV for LA, n=12, P<0.01). The opening of \( K_{ATP} \) channels induced by GW9508 was totally reversed by tolbutamide in 5 minutes (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-secreting cell line which does not express GPR40 as identified by reverse transcriptional PCR (Feng, et al. 2006)(Yang, et al. 2005). GW9508 at 40\( \mu \)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).

5. Effects of Protein Kinase inhibitors on linoleic acid-induced opening of \( K_{ATP} \) channels in rat pancreatic \( \beta \)-cells. We next tested whether protein kinase A and
protein kinase C signalling pathways mediate the effects of LA on $K_{\text{ATP}}$ channels. Protein kinase A inhibitor, 1 µM H89, did not influence $K_{\text{ATP}}$ channels. After treatment with H89 for 10 minutes, LA induced opening of $K_{\text{ATP}}$ channels, and we observed no difference in the amplitude at each clamping step. Protein kinase C inhibitor, 10µM chelerythrine, neither influenced $K_{\text{ATP}}$ channels by itself nor influenced LA-induced opening of $K_{\text{ATP}}$ channels. In addition, H89 and chelerythrine did not influence GW9508-induced opening of $K_{\text{ATP}}$ channels. The amplitude of $K_{\text{ATP}}$ currents at -110mV is shown in Fig 6.

**DISCUSSION**

In this report, we demonstrate that linoleic acid induces hyperpolarization of membrane potential in rat β-cells by activating $K_{\text{ATP}}$ channels via two distinct pathways, the intracellular metabolite-mediated signaling pathway and membrane receptor GPR40-mediated signaling pathway.

Firstly, we show that activation of $K_{\text{ATP}}$ channels is responsible for LA-induced hyperpolarization in rat β-cells. Pancreatic $K_{\text{ATP}}$ channels are formed by two different types of subunits, the Kir6.2 inward rectifying potassium channels and sulphonylurea receptor subunit SUR1 (Mikhailov and Ashcroft 2000; Mikhailov, et al. 2000). Pancreatic $K_{\text{ATP}}$ channels can be specifically blocked by sulphonylurea such as tolbutamide (Ashfield, et al. 1999; Gribble and Ashcroft 2000). LA-induced hyperpolarization was reversed by tolbutamide and was deterred by tolbutamide pretreatment, indicating that opening of $K_{\text{ATP}}$ channels is responsible for the hyperpolarization. This was confirmed by the current recording. LA induced strong non-voltage dependent currents which were totally blocked by tolbutamide, confirming that LA opened $K_{\text{ATP}}$ channels. $K_{\text{ATP}}$ channels play an important role in the process of GSIS. Glucose goes to aerobic oxidation to produce adenosine triphosphaste (ATP) from adenosine diphosphaste (ADP) in β-cells. The resulting increase in the ATP/ADP ratio closes $K_{\text{ATP}}$ channels and leads to depolarization of membrane potential and subsequent activation of voltage-gated Ca$^{2+}$ channels ($Ca^{2+}_{(v)}$ channels). The influx of Ca$^{2+}$ results in an increase in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]i), which triggers exocytosis of insulin granules (Rorsman 1997). Activation of
K\textsubscript{ATP} channels results in a decrease in excitability of \(\beta\)-cells and inhibits insulin secretion. Although FFAs stimulate insulin secretion, FFAs substantially inhibits GSIS in a long term condition (Haber, et al. 2003; Zraika et al. 2002). LA-induced activation of K\textsubscript{ATP} channels may play a role in decreasing \(\beta\)-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\textsubscript{ATP} channel-independent pathway. For example, FFAs stimulates calcium release from intracellular calcium stores via GPR40 membrane receptor and then triggers insulin secretion. Moreover, FFAs metabolites such as long-chain Acyl-CoA activate protein kinase C 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 (Braun and Scheller 1995; Hess, et al. 1992).

The mechanism of activation of K\textsubscript{ATP} channels was not previously fully understood. It was suggested that long-chain acyl-CoA activates K\textsubscript{ATP} channels (Branstrom et al. 2004; Larsson et al. 1996; 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\textsubscript{ATP} channels in mouse \(\beta\)-cells (Larsson et al. 1996). Long-chain acyl-CoA is the metabolically active form of FFAs an important signalling molecule in \(\beta\)-cells (Corkey et al. 2000). Extracellular FFAs enter \(\beta\)-cells and are firstly 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\textsubscript{ATP} channels, suggesting the involvement of LA metabolism in activation of K\textsubscript{ATP} channels. The slow partial recovery of hyperpolarization after washout of LA also indicates that LA metabolism is involved in the opening of K\textsubscript{ATP} channels.

On the other hand, blockade of LA metabolism only partially inhibited the effects of LA on K\textsubscript{ATP} channels, indicating another signalling pathway is involved in the action of LA on K\textsubscript{ATP} channels. The GPR40 signalling pathway is a newly discovered pathway for FFAs in mediating \(\beta\)-cell function (Briscoe et al. 2003; Itoh et al. 2003). FFAs not only bind to GPR40 as ligands but also enter cross the \(\beta\)-cells plasma membrane to be metabolized. The metabolic effects of FFAs can not 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 FFAs membrane receptor GPR40 (Briscoe, et al. 2006; Sum, et al. 2007). GW9508 induced opening of K\textsubscript{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\textsubscript{ATP} channels. Moreover, compared to the effects of LA on K\textsubscript{ATP} channels, GW9508-induced opening of K\textsubscript{ATP} channels was recovered fully and quickly, suggesting its effect is mediated by the membrane receptor. In addition, GH3 cells with K\textsubscript{ATP} channels but without GPR40 expression did not show K\textsubscript{ATP} current change to the action of GW9508. This suggests that the effects of GW9508 on K\textsubscript{ATP} channels are mediated by GPR40.

The involvement of GPR40 in the activation of K\textsubscript{ATP} channels describes a new mechanism for FFAs to regulate K\textsubscript{ATP} channels and GSIS. There are two apparently conflicting hypotheses regarding the regulation of β-cell function by FFAs. Firstly, 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 FFAs-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\textsubscript{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 (Latour, et al. 2007; Steneberg, et al. 2005). 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\textsubscript{ATP} channels due to over expression of GPR40 may be responsible for the impaired GSIS in GPR40-over expressing mice.

We attempted to clarify the molecular mechanism of LA-induced activation of K\textsubscript{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 50nM (Chijiwa, et al. 1990). Chelerythrine is a potent and selective PKC inhibitor with IC50 of 0.66µM (Herbert, et al. 1990). By inhibiting these protein kinases by H89 and chelerythrine, we found that neither
protein kinase A nor protein kinase C signalling pathways take part in the effects of LA on \( K_{\text{ATP}} \) channels. Further research is required to clarify the molecular mechanisms of intracellular metabolites of FFAs and GR40-mediated activation of \( K_{\text{ATP}} \) channels.

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

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Legend

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 (** means P<0.01 vs control, n=12). C: LA induced hyperpolarization at high glucose levels. D: The statistical results of membrane potential (** means P<0.01 vs control, n=6). Data are presented as Mean±SEM.

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

Figure 3: Effects of LA on K\textsubscript{ATP} channels of rat β-cells. A: I-V curve of K\textsubscript{ATP} currents. Cells were held at -80mV and clamped from -110mV to -50mV in 20mV step. K\textsubscript{ATP} currents were significantly increased at each clamping voltage from -110mV to -50mV. (** means P<0.01 vs control at same clamping voltage, n=12). B: tolbutamide deterred LA-induced opening of K\textsubscript{ATP} channels. Data are presented as Mean±SEM.

Figure 4: Effects of Triacsin C on LA-induced opening of K\textsubscript{ATP} channels in rat β-cells. A: With addition of Triacsin C, LA induced opening of K\textsubscript{ATP} channels, but the maximal amplitude was partly inhibited. B: The changes of the currents from -110mV to -50mV showed that Triacsin C treatment partly but significantly inhibited the opening of K\textsubscript{ATP} channels (* means P<0.05 vs LA, n=6). Data are presented as Mean±SEM.

Figure 5: Effects of GW9508 on membrane potential and K\textsubscript{ATP} channels of rat β-cells. A: GW9508 at 40µM induced hyperpolarization of β-cells, and the effect of GW9508 on membrane potential was totally blocked by 0.1 mM tolbutamide (** means 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 0.1mM tolbutamide ($P<0.01$, n=8).

Data are presented as Mean±SEM.

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

![Graph A]

**Membrane Potential (mV)**

- Control
- Linoleic acid
- Linoleic acid + Tolbutamide

B

![Graph B]

**Membrane Potential (mV)**

- Control
- Tolbutamide
- Tolbutamide + linoleic acid

**NS**

**Statistical Significance**:

- **Control** vs. **Linoleic acid**: Significant difference
- **Tolbutamide** vs. **Tolbutamide + linoleic acid**: Significant difference

**Notes**: NS indicates no significant difference.
In Figure A, the plot shows the relationship between membrane voltage (mV) and the ratio of pA/pF. The lines represent three different conditions: Control, Triacsin C, and Triacsin C + linoleic acid. The asterisks indicate significant differences.

In Figure B, the bar graph compares the ratio of current (K<sub>ATP</sub>) to basal level under two conditions: Linoleic acid and Triacsin C + Linoleic acid. The control group is not shown in this graph.