Supporting Information

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SI Materials and Methods

Islets and β-Cell Isolations. Mice (2- to 5-mo-old, both sexes) were deeply anesthetized with inhaled isoflurane and killed by cervical dislocation. The mouse abdominal wall was then cut open. The pancreas was distended by injection of 1 mL of ice-cold Krebs Ringer bicarbonate (KRB) buffer containing 2.2 mg/mL collagenase type IV (Worthington) and 0.5 μL/mL DNase I (Boehringer-Mannheim) through a cannula inserted into the common bile duct. The pancreas was then removed, minced into tiny tissue blocks, and transferred to a 50-mL Falcon tube containing the above enzyme solution and incubated in a water bath at 37 °C for 18–30 min. The digested pancreas was washed with ice-cold KRB buffer four times, and islets of Langerhans were hand-picked under a dissection microscope. Islets were either used directly for perifusion experiments or dispersed into single cells for electrophysiological recordings by vigorous shaking in Ca^{2+}/Mg^{2+}-free HBSS supplemented with 0.05% trypsin/EDTA (wt/vol) (Invitrogen). The dispersed islet cells were cultured on small glass coverslips (10 mm in diameter) precoated by poly-L-lysine in RPMI 1640 media supplemented with 10% (vol/vol) FBS and 1% P/S/G (penicillin and streptomycin) (vol/vol) in a 37-°C humidified chamber containing 5% (vol/vol) CO_2. Short-term cultured (1–2 d) islet cells were used in the patch-clamp experiments. Individual coverslips were transferred to the test chamber and placed on an inverted microscope for electrophysiological experiments. In electrophysiological recordings, β cells are distinguished from other islet cells by their large size (usually a capacitance ≥7 pF). There was no detectable Na^+ current when holding at −70 mV, and characteristic oscillatory or bursting electrical activity when exposed to 10 mM glucose (1, 2).

Islet Perifusion Assay. Islets from $G_o\alpha$ (G_o2, G_o1), $G_i\alpha$ (G_i1/G_i3 double, G_i2) knockouts, and wild-type controls were loaded into perifusion chambers (Millipore) and perifused with KRB buffer at 37 °C at a flow rate of 0.25 mL/min in the presence of 1.8 mM glucose to establish basal and stable insulin secretory rates. The buffer contained (in mmol/L) $CaCl_2$ 2.5, Hepes 10.0, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, NaCl 129.0, $NaHCO_3$ 5.0, and 0.2% BSA. After a 1-h stabilization period, islets were perifused with KRB supplemented with 16.2 mmol/L glucose for 120 min, and galanin (100 nM) was present during the 60–90 min period. The perifusion solution was then switched back to 1.8 mM (low) glucose for 30 min. Finally, islets were perifused with KCl (30 mM) for an additional 30 min. Eluent was collected at 2-min intervals with a fraction collector. Insulin content was determined by ELISA.

Electrophysiological Recordings. The patch-clamp technique was used to record whole-cell Ca^{2+} and ATP-sensitive potassium (K_{ATP}) currents in the voltage-clamp mode. Glass coverslips attached to primary-cultured pancreatic β cells were placed into the perifusion chamber, which was mounted on the stage of an inverted phase-contrast microscope (IX 71; Olympus). Cells were washed with bath solution in the chamber for 3–5 min before starting an experiment. Patch pipettes were pulled from borosilicate glass capillaries with filament, with a tip resistance of 2–5 MΩ when filled with pipette solution. Various whole-cell ionic currents were recorded with an EPC-10 patch-clamp amplifier (HEKA Elektronic) and controlled by an MP-225 interface (Sutter Instrument) and Patchmaster software (version 2.0; HEKA Elektronic). Digitized signals were filtered at 2.9 kHz and sampled at 15.6 kHz, and were stored for analysis by an online computer using Patchmaster software. At the beginning of each experiment, the junctional potential between pipette solution and bath solution was electronically adjusted to zero. Capacitive transients were automatically compensated by C-fast and C-slow. No leakage subtraction was performed for the original recordings, and all cells with visible changes in leakage currents during the course of the study were excluded from further analysis. I–V (current-voltage) curves were constructed using the current amplitude measured during the whole period of 500-ms test pulses. Test pulses were made at 10-mV increments from −150 to +50 mV. The holding potential was set at −20 mV, at which the outward voltage-dependent K^+ currents were largely inactivated. The pipette solution for K_{ATP} current recording included (in mmol/L) NaCl 5, KCl 125, $MgCl_2$ 4, $CaCl_2$ 2, Hepes 5, EGTA 10, MgATP 0.3, and Na_2GTP 0.3 (pH 7.2), whereas bath solution for K_{ATP} currents contained NaCl 138, KCl 5.6, $MaCl_2$ 1.2, $CaCl_2$ 2.6, Hepes 10, and glucose 0, 2.8, or 16.2, as stated in the text (pH = 7.4). L-type Ca^{2+} currents were recorded by a 200-ms step pulse ranging from −80 to +40 mV with a holding potential of −70 mV. The pipette solution for Ca^{2+} currents contained (mM) CsCl 120, TEACl 20, $MgCl_2$ 1, Hepes 10, EGTA 5, MgATP 5, and Na_2GTP 0.3 (pH = 7.2), whereas bath solution for Ca^{2+} currents included NaCl 100, TEACl 20, $BaCl_2$ 20, $MgCl_2$ 1, Hepes 10, and glucose 10 (pH = 7.4). For Ca^{2+} current recording, Giga-ohm seal was made in normal extracellular high-Na^+ solution. After formation of whole-cell mode, Ba^{2+}-carried Ca^{2+} currents were recorded in a Ba^{2+}-containing bath solution. The osmolarity of the recording solutions was adjusted to 290 mOsmol/L and pH to 7.2–7.4. Cells were continuously perifused with the bath solution containing the tested chemicals at the desired final concentrations. All experiments were performed at room temperature.

Effects of Galanin on Glucose Levels in Vivo. Mice (8- to 12-wk-old) were fasted overnight and 2 μg/kg bodyweight galanin or saline was given via retroorbital vein at time point zero. Blood glucose levels were monitored at the time points indicated in the figures using a One-Touch Ultra blood glucose meter (LifeScan).

Chemicals and Data Analysis. Galanin (1-16) (porcine, rat) was purchased from AnaSpec. Diazoxide (Alexis Biochemicals), nifedipine (Tokyo Kasei Kogyo), and Bay K-8644 (Alexis Biochemicals) were purchased. Stock solutions of diazoxide and nifedipine were made in DMSO and diluted to the desired concentrations immediately before use. DMSO alone was without effect at the concentration used (up to 0.3% vol/vol). MgATP and Na_2GDP were directly dissolved in the pipette solution to achieve the desired concentrations on the day of experiment. Anti-G_o2 antibody (Rb9576; 0.46 mg/mL) was custom-made in our laboratory. All data are expressed as means ± SEM from at least three independent experiments performed in duplicate unless otherwise stated. Statistical analyses were done using paired and unpaired Student's *t* tests and analyses of variance in conjunction with Newman–Keuls tests where appropriate. Group differences at the level of $P < 0.05$ were considered statistically significant.

1. Göpel SO, Kanno T, Barg S, Rorsman P (2000) Patch-clamp characterisation of somatostatin-secreting δ-cells in intact mouse pancreatic islets. *J Physiol* 528: 497–507.

2. Mears D, Zimliki CL (2004) Muscarinic agonists activate Ca^{2+} store-operated and -independent ionic currents in insulin-secreting HIT-T15 cells and mouse pancreatic β-cells. *J Membr Biol* 197(1):59–70.

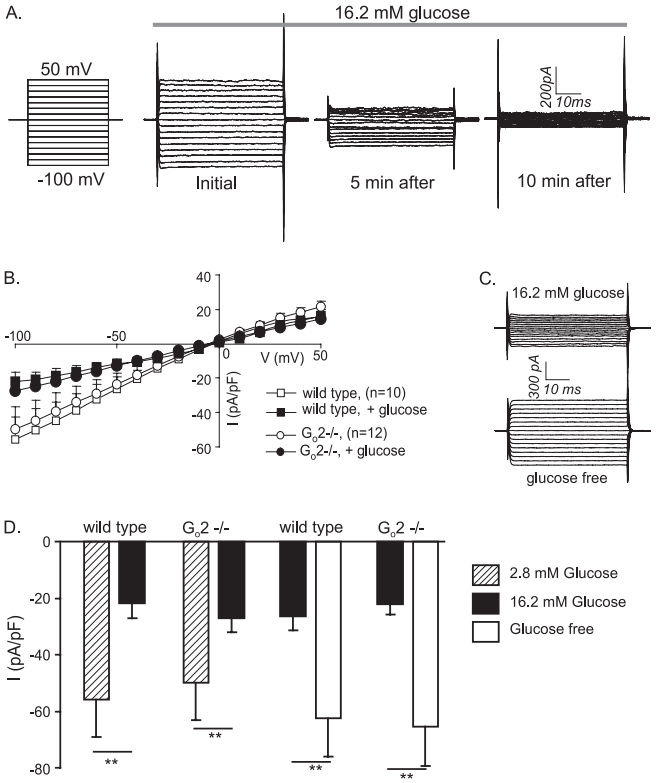

Fig. S1. $G_o2^{-/-}$ mice exhibit normal sensitivity of the K_{ATP} channel to glucose. (*A*) Representative K_{ATP} current traces recorded from β cells initially and 5 and 10 min after application of 16.2 mM glucose. Testing potential (TP) = −100 to +50 mV, holding potential (HP) = −20 mV. (*B*) The mean I–V (current-voltage) relationship curves show that a high concentration of glucose (16.2 mM) inhibited K_{ATP} currents in pancreatic β cells of $G_o2^{-/-}$ and wild-type mice (n = 10–12, TP = −100 to +50 mV, HP = −20 mV). (*C*) Representative K_{ATP} current traces before and after the removal of 16.2 mM glucose from the bath solution. (*D*) Summary of K_{ATP} currents inhibited by 16.2 mM glucose and stimulated by removal of glucose in $G_o2^{-/-}$ and wild-type β cells (n = 9–14, TP = −100 mV to +50 mV, HP = −20 mV, $^{**}P < 0.01$).