Rapid Publication

Insulin Activates ATP-Sensitive K$^+$ Channels in Pancreatic β-Cells Through a Phosphatidylinositol 3-Kinase–Dependent Pathway

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Insulin is known to regulate pancreatic β-cell function through the activation of cell surface insulin receptors, phosphorylation of insulin receptor substrate (IRS)-1 and -2, and activation of phosphatidylinositol (PI) 3-kinase. However, an acute effect of insulin in modulating β-cell electrical activity and its underlying ionic currents has not been reported. Using the perforated patch clamp technique, we found that insulin (1–600 nmol/l) but not IGF-1 (100 nmol/l) reversibly hyperpolarized single mouse β-cells and inhibited their electrical activity. The dose-response relationship for insulin yielded a maximal change (mean ± SE) in membrane potential of −13.6 ± 2.0 mV ($P < 0.001$) and a 50% effective dose of 25.9 ± 0.1 nmol/l ($n = 63$). Exposing patched β-cells within intact islets to 200 nmol/l insulin produced similar results, hyperpolarizing islets from −47.7 ± 3.3 to −65.6 ± 3.7 mV ($P < 0.0001$, $n = 11$). In single cells, insulin-induced hyperpolarization was associated with a threefold increase in whole-cell conductance from 0.6 ± 0.1 to 1.7 ± 0.2 nS ($P < 0.001$, $n = 10$) and a shift in the current reversal potential from −25.7 ± 2.5 to −63.7 ± 1.0 mV ($P < 0.001$ vs. control, $n = 9$; calculated K$^+$ equilibrium potential = −90 mV). The effects of insulin were reversed by tolbutamide, which decreased cell conductance to 0.5 ± 0.1 nS and shifted the current reversal potential to −25.2 ± 2.3 mV. Insulin-induced β-cell hyperpolarization was sufficient to abolish intracellular calcium concentration ([Ca^{2+}]$_i$) oscillations measured in pancreatic islets exposed to 10 mmol/l glucose. The application of 100 nmol/l wortmannin to inactivate PI 3-kinase, a key enzyme in insulin signaling, was found to reverse the effects of 100 nmol/l insulin. In cell-attached patches, single ATP-sensitive K$^+$ (K$_{ATP}$) channels were activated by bath-applied insulin and subsequently inhibited by wortmannin. Our data thus demonstrate that insulin activates the K$_{ATP}$ channels of single mouse pancreatic β-cells and islets, resulting in membrane hyperpolarization, an inhibition of electrical activity, and the abolition of [Ca^{2+}]$_i$ oscillations. We thus propose that locally released insulin might serve as a negative feedback signal within the islet under physiological conditions. *Diabetes* 50: 2192–2198, 2001

ATP-sensitive K$^+$ (K$_{ATP}$) channels of the pancreatic β-cell play a pivotal role in stimulus-secretion coupling, setting the resting membrane potential below the activation threshold of voltage-gated Ca^{2+} channels (1). Cellular metabolism of fuel secretagogues raises the ATP/ADP ratio, resulting in the closure of K$_{ATP}$ channels (1–3) and concomitant β-cell depolarization (3,4). This depolarization in turn activates voltage-gated Ca^{2+} channels, resulting in Ca^{2+} influx and Ca^{2+}-dependent insulin exocytosis (1,2,4,5). Locally released insulin may bind to β-cell insulin receptors (6–8), triggering receptor autophosphorylation (8), receptor tyrosine kinase activation (8), insulin receptor substrate (IRS)-1 phosphorylation (7,8), and phosphatidylinositol (PI) 3-kinase activation (8,9). This autocrine action of insulin has been proposed to regulate a number of functions in β-cells, including insulin gene transcription (10,11). The importance of insulin signaling for normal β-cell function has been demonstrated by recent studies of insulin receptor knockout mice (12). These animals exhibit long-term insulin secretory defects and resemble human type 2 diabetic patients. However, changes caused by islet insulin receptor knockout could reflect the long-term trophic effects of insulin rather than acute effects of locally (and newly) secreted insulin on β-cells. Although the hypothesis that secreted insulin provides negative feedback to the islet to limit further insulin secretion has been studied for a number of years, the data in the literature are conflicting. Thus, whereas some in vivo (13) and in vitro (14,15) studies support this mechanism, other data support the converse hypothesis that insulin facilitates its own secretion, possibly by releasing Ca^{2+} from endoplasmic reticulum (ER) Ca^{2+} stores in an IRS-1– and PI 3-kinase–dependent manner (16,17). However, this action, which was demonstrated using β-cells bathed in 3

mmol/l glucose, apparently does not involve changes in β-cell membrane potential. In contrast, other studies show that inhibiting PI 3-kinase augments insulin secretion, in support of the negative feedback hypothesis (18,19).

The present study was prompted by an intriguing recent report showing that insulin activates the K_{ATP} channels of rat hypothalamic neurons (20). We hypothesized that if insulin had a similar action on the pancreatic β-cell K_{ATP} channels, the acute release of insulin from β-cells within the islet might be capable of uncoupling fuel metabolism from β-cell electrical activity. Because metabolism-induced electrical activity is an important component of stimulus-secretion coupling in islet β-cells, K_{ATP} channels opened by insulin could thus constitute a novel autocrine negative feedback mechanism to locally regulate islet insulin secretion. This mechanism might work in tandem with the more global negative feedback regulation of β-cell insulin secretion that occurs when secreted insulin binds to its peripheral target tissues, lowering the plasma glucose concentration. In this model, locally released insulin as well as decreased plasma glucose turn off islet secretion by opening β-cell K_{ATP} channels, leading to a cessation of β-cell electrical activity, Ca^{2+}-influx, and Ca^{2+}-induced insulin granule exocytosis.

We now report for the first time that exogenous insulin hyperpolarized isolated as well as in situ mouse pancreatic β-cells in a dose-dependent manner, inhibited their electrical activity and abolished their intracellular calcium concentration ($[Ca^{2+}]_i$) oscillations. These effects were mediated by insulin-induced activation of K_{ATP} channels, because insulin increased whole-cell K_{ATP} conductance in a tolbutamide-blockable manner and directly activated single K_{ATP} channels in cell-attached patches. Because wortmannin reversed insulin-induced β-cell hyperpolarization and closed insulin-activated K_{ATP} channels, this action of insulin is likely mediated by PI 3-kinase, a key signaling enzyme in insulin signaling.

RESEARCH DESIGN AND METHODS

Tissue culture. Mouse pancreases were isolated from 25- to 30-g male Swiss-Webster mice. After cannulation of the bile duct, 2 mg/ml collagenase was injected into the pancreas, which was then removed and incubated for 5–10 min at 37°C to isolate individual islets. Single-cell suspensions were obtained by gently shaking islets in a low-calcium medium. Cell suspensions were plated onto glass coverslips in 35-mm petri dishes containing RPMI-1640 medium supplemented with fetal bovine serum, L-glutamate, and penicillin-streptomycin and were kept in a 95/5% air/CO_2 incubator at 37°C. Single cells were fed every other day and were used for up to 1 week after isolation. Whole islets were cultured in a similar fashion and used within 48 h in culture. To record electrical activity from in situ β-cells, islets were held in place by a wide-tipped glass suction pipette, which was mounted on a micromanipulator (21) (Fig. 2D).
Electrophysiology. A small-volume recording chamber containing β-cells or islets was mounted on an inverted microscope (Olympus IM-T2; Tokyo), which in turn was placed on a TMC air isolation table (TMC, Peabody, MA). Recording solutions were maintained at 35°C using a TC-1 temperature controller, a preheater, and a second transparent heater attached to the bottom of the recording chamber (Cell Micro Controls, Virginia Beach, VA). The electrophysiological recording solution contained (in millimoles per liter) 140 NaCl, 3.6 KCl, 2 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $MgSO_4$, 5 HEPES, and 10 glucose, pH 7.4, with NaOH. Extracellular solution was superfused at a rate of 1–2 ml/min. Recording pipettes had resistances ranging from 5 to 10 MΩ and were pulled from borosilicate glass capillaries using a Sutter micropuller (P-97; Sutter Instrument, Novato, CA). The pipette tips were fire-polished and then filled with internal solution containing (in millimoles per liter) 76 K_2SO_4, 10 NaCl, 10 KCl, 1 $MgCl_2$, and 5 HEPES, pH 7.35, with KOH. The pipettes were then back-filled with the same solution containing 0.1 mg/ml amphotericin-B. The pipette solution for cell-attached single K_{ATP} channel recordings con-

tained (in millimoles per liter) 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, pH 7.3, with KOH. Seal resistances were >1 GΩ. Wortmannin was obtained from Calbiochem (Calbiochem-Novabiochem, La Jolla, CA), whereas all other chemicals, including recombinant human insulin, were obtained from Sigma (Sigma Chemical, St. Louis, MO). Insulin solutions were prepared fresh daily from 1 mmol/l stock solutions. Cytochrome-C, at a concentration of 0.1 mg/ml, was added to insulin containing solutions to prevent insulin from adhering to the plastic tubing or the experimental chamber. Control solutions contained an identical concentration of cytochrome-C.

Using the perforated patch clamp technique (22), isolated β-cells were identified by their size and characteristic patterns of electrical activity in 10 mmol/l glucose (23), whereas in situ β-cells were identified by their medium bursting in 10 mmol/l glucose (21,24). Integrity of the gigaseals was checked periodically. Data were acquired using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA) and a Macintosh Quadra G3 computer (Apple Computer, Cupertino, CA) using an ITC-16 computer interface (Instrutech, Elmont, NY) and Igor Pro software (Wavemetrics, Lake Oswego, OR). Data were also stored on videotapes for off-line analysis. Linear voltage ramps from −90 to +50 mV lasting 6.2 s were applied from a holding potential of −65 mV after switching the amplifier from current to voltage clamp mode. Whole-cell conductance was measured by averaging currents obtained using five voltage pulses of ±10 mV from a holding potential of −65 mV.

Single K_{ATP} channels were recorded in the cell-attached patch configuration at a pipette potential of 0 mV using electrodes containing 140 mmol/l KCl. Nifedipine (10 μmol/l) was added to the bath solution in this configuration to prevent capacitatively coupled action potentials. Data were acquired at 5 kHz after filtering at 2 kHz. Single K_{ATP} channel openings were analyzed using TAC software to generate an event list (Bruxton, Seattle, WA). Single-channel conductance was determined by stepping the pipette potential from −20 to +20 mV. The change in mean single channel current amplitude for the applied step voltage was used to calculate the single-channel conductance, using the equation $g = \Delta I/\Delta V$ (g = single-channel conductance, ΔV = step voltage, and ΔI = change in mean single channel current amplitude). Mean channel activity (NP_o) was calculated using 10-s sample traces, using the equation $NP_o = I_{avg}/i$ (I_{avg} = the average current of the analyzed trace and i = the mean single channel current amplitude). The fact that liquid junction potentials were not compensated and the resting membrane potential of the cell was unclamped may have influenced the amplitude of the unitary currents. K_{ATP} channels were identified in cell-attached patch recordings by their characteristic slope conductance and their sensitivity to changes in bath glucose concentration. To further establish that these events correspond to the opening of single K_{ATP} channels, we confirmed that they were strongly activated by the addition of 2 mmol/l sodium azide to bath solutions containing 10 mmol/l glucose and were promptly blocked by the addition of 1 μmol/l glyburide (data not shown).
**$[Ca^{2+}]_i$ measurement using Fura-2 fluorescence.** Islets were loaded with the Ca^{2+}-sensitive probe Fura-2/acetoxymethyl. This probe (2 μmol/l) and 2 μl of 2.5% pluronic acid solution were added to culture dishes containing 1 ml of medium and were incubated for 30 min in an air/CO^2 incubator at 37°C. After loading, islets were washed and incubated in recording solution for 20 min. $[Ca^{2+}]_i$ was measured using an IX-50 inverted fluorescence microscope (Olympus, Tokyo) in conjunction with a xenon arc excitation source, a photomultiplier, and a photon counter (Ionoptix, Milton, MA). Data were acquired and analyzed with Ionwizard software provided by the manufacturer. Epifluorescence was measured from single islets, and the values in $[Ca^{2+}]_i$ were determined as the fluorescence ratio (R) of Ca^{2+}-bound Fura-2 (340 nm) to unbound Fura-2 (380 nm), with emission collected at 510 nm. R was converted to free Ca^{2+} concentration using an in vitro standard curve generated with a calibration kit (Molecular Probes, Eugene, OR).
Statistics. Statistical analysis was done using paired t test or nonparametric one-way analysis of variance with Newman-Keuls Multiple Comparison Test to compare more than two values. P values <0.05 were considered statistically significant.

RESULTS

Insulin, but not IGF-1, hyperpolarized isolated single β-cells and islets and abolished regular $[Ca^{2+}]_i$ oscillations. The electrical activity of single dispersed β-cells in 10 mmol/l glucose consists of repeated large spikes, fast (e.g., <5-s) bursts, or plateau depolarizations (23), whereas medium bursting having a duration of 10–20 s only occurs in intact islets (21,24).
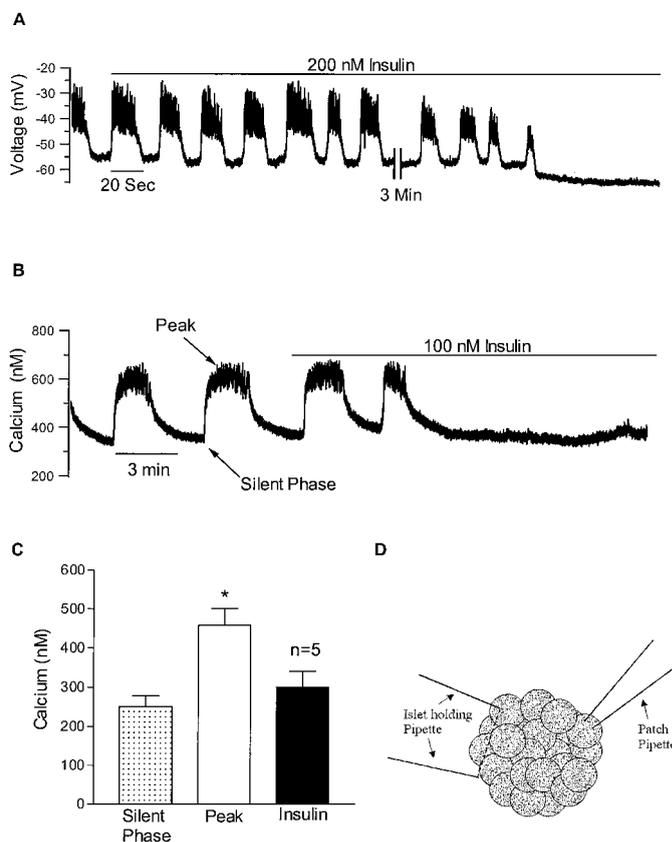
Using the perforated patch clamp technique (22) to record from single mouse β-cells bathed in 10 mmol/l glucose, we found that the application of insulin (1–600
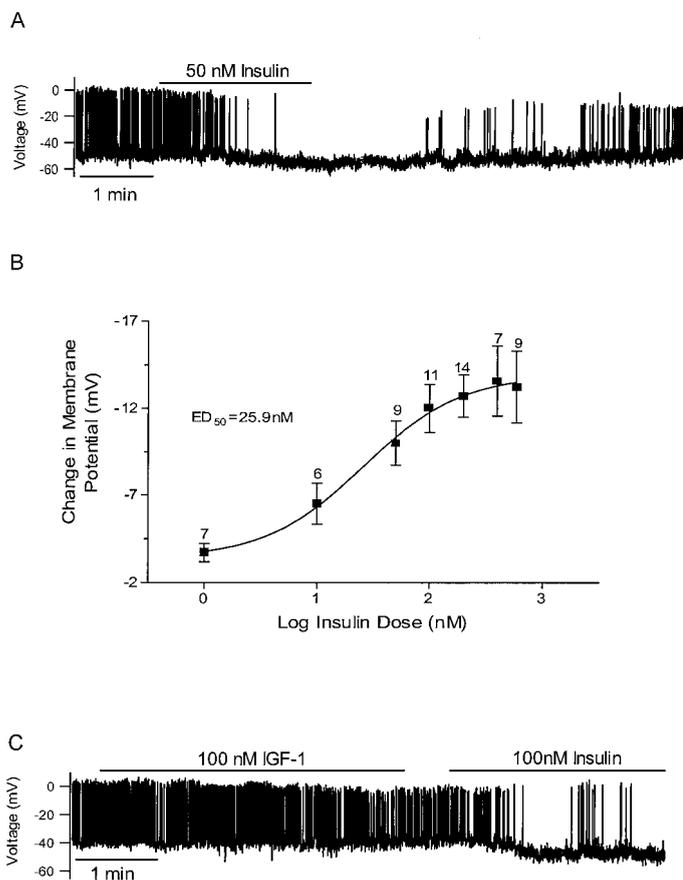
FIG. 1. Effect of exogenous insulin and IGF-1 on the membrane potential of single mouse pancreatic β-cells. *A*: The application of insulin reversibly hyperpolarized single mouse β-cells bathed in 10 mmol/l glucose at 35°C. The trace shown is representative of 63 of 86 cells tested with 1–600 nmol/l insulin. *B*: The changes in membrane potential caused by insulin were dose-responsive in single β-cells over the range of 1–600 nmol/l. The numbers listed above the error bars indicate the number of cells tested in each group. The maximal change in membrane potential was -13.6 ± 2.0 mV, and the calculated ED_{50} from these data were 25.9 ± 0.1 nmol/l. *C*: The application of 100 nmol/l IGF-1 had little or no effect on β-cell electrical activity, whereas subsequent exposure to 100 nmol/l insulin hyperpolarized the cells. The mean membrane potential in control solutions and in solutions containing 100 nmol/l IGF-1 and 100 nmol/l insulin were -45.0 ± 2.6, -45.0 ± 3.2, and -56.3 ± 4.5 mV ($P < 0.001$, $n = 6$), respectively.

FIG. 2. Insulin-induced hyperpolarization of in situ β-cells and inhibition of regular $[Ca^{2+}]_i$ oscillations. *A*: Insulin (200 nmol/l) hyperpolarized β-cells within intact islets. The application of 200 nmol/l insulin hyperpolarized islets from -47.7 ± 3.3 to -65.6 ± 3.7 mV ($n = 11$). Insulin took significantly longer to act in intact islets compared with single β-cells (9.4 ± 1.7 vs. 3.7 ± 0.5 min, $n = 11$ and 14, respectively). *B*: Regular $[Ca^{2+}]_i$ oscillations observed in 10 mmol/l glucose were inhibited by insulin addition within ~9 min. *C*: $[Ca^{2+}]_i$ levels corresponding to the silent and peak phases of glucose-induced $[Ca^{2+}]_i$ oscillations were 251.0 ± 26.8 and 457.6 ± 42.9 nmol/l, respectively ($P < 0.05$, $n = 5$). The application of insulin (100 nmol/l) inhibited these oscillations, producing a new steady-state $[Ca^{2+}]_i$ value of 300.4 ± 40.0 nmol/l. *D*: A graphic representation of the technique used to gather data from in situ β-cells.

nmol/l) resulted in a reversible inhibition of β-cell electrical activity in 73% (63 of 86) of the cells tested (Fig. 1*A*), with insulin doses >1 nmol/l producing statistically significant changes in β-cell membrane potential. The dose-response relationship for exogenous insulin yielded a maximal change (mean ± SE) in membrane potential of -13.6 ± 2.0 mV and a 50% effective dose (ED_{50}) of 25.9 ± 0.1 nmol/l, with an R^2 value of 0.9959, $n = 63$ (Fig. 1*B*). Although insulin-induced hyperpolarization was reversible, the recovery time appeared to be proportional to the total duration of insulin application.

Insulin is known to bind to and activate IGF-1 and insulin receptors (8,25). IGF-1 receptors are structurally and functionally homologous to insulin receptors and belong to the same family of receptors having tyrosine kinase activity (25). To rule out the possibility that insulin-induced β-cell hyperpolarization resulted from binding to IGF-1 receptors, equimolar concentrations of IGF-1 and insulin were tested on the same β-cells. As shown in Fig. 1*C*, 100 nmol/l IGF-1 applied alone for up to 5 min failed to

suppress β-cell electrical activity in 10 mmol/l glucose ($n = 6$). However, the subsequent addition of 100 nmol/l insulin resulted in β-cell hyperpolarization within 2 min. Thus, the mean membrane potential after IGF-1 application was -45.0 ± 2.6 mV, which was not significantly different from control (-45.0 ± 3.2 mV), whereas the subsequent addition of 100 nmol/l insulin to the bath hyperpolarized these β-cells to -56.3 ± 4.5 mV ($P < 0.001$, $n = 4$).

Recordings from β-cells within intact islets exposed to 200 nmol/l insulin produced similar results (Fig. 2*A*). Thus, insulin hyperpolarized islets from -47.7 ± 3.3 to -65.6 ± 3.7 mV ($P < 0.0001$, $n = 11$). The mean change in membrane potential for islets versus single dispersed β-cells was -17.9 ± 2.8 mV ($n = 11$) vs. -12.7 ± 1.2 mV ($n = 14$), respectively ($P > 0.05$). However, the mean time to maximum response was 9.4 ± 1.7 min for islets ($n = 11$) and 3.7 ± 0.5 min for isolated β-cells ($n = 14$, $P < 0.05$), indicating that insulin took a significantly longer time to produce its effect on in situ β-cells. This difference likely reflects the longer diffusion time required for insulin to

activate a sufficient number of β-cells within the intact islet (R. Bertram and L. Satin, unpublished observations).

We next tested whether insulin was capable of abolishing the regular $[Ca^{2+}]_i$ oscillations of intact mouse islets bathed in 10 mmol/l glucose. Using the ratiometric Ca^{2+}-sensing dye Fura-2 (see RESEARCH DESIGN AND METHODS), regular $[Ca^{2+}]_i$ oscillations were observed (Fig. 2B) between 251.0 ± 26.8 and 457.6 ± 42.9 nmol/l ($P < 0.05$, $n = 5$) (Fig. 2C), corresponding to the silent and peak phases of the $[Ca^{2+}]_i$ oscillations. As shown in Fig. 2B, the $[Ca^{2+}]_i$ oscillations were completely abolished within ~9 min of exposure to 100 nmol/l insulin, resulting in a new steady-state $[Ca^{2+}]_i$ level of 300.4 ± 40.0 nmol/l ($n = 5$) (Fig. 2B and C). These data clearly demonstrate that insulin not only hyperpolarizes islets but abolishes the regular $[Ca^{2+}]_i$ oscillations, which are known be important for pulsatile insulin secretion.

Insulin-induced β-cell hyperpolarization is caused by an increase in whole-cell K^+ conductance. Insulin has been shown to hyperpolarize hypothalamic neurons by activating their K_{ATP} channels (20). We therefore hypothesized that a similar mechanism might account for the insulin-induced hyperpolarization of β-cells. In support of this hypothesis, we found that tolbutamide, a specific blocker of K_{ATP} channels (26), completely reversed insulin-induced β-cell hyperpolarization (Fig. 3A). Thus, whereas 100 nmol/l insulin hyperpolarized β-cells from −46.7 ± 2.4 to −62.1 ± 1.9 mV ($P < 0.001$, $n = 7$), the subsequent addition of 100 μmol/l tolbutamide depolarized membrane potential back to control levels (−44.1 ± 2.1 mV). Whole-cell conductance measured using voltage pulses in voltage clamp was 0.6 ± 0.1, 1.7 ± 0.2 ($P < 0.001$), and 0.5 ± 0.1 nS for control, insulin, and insulin plus tolbutamide-containing solutions ($n = 10$), respectively. Thus, insulin-induced hyperpolarization was associated with a threefold increase in cell membrane conductance, which was completely reversed by tolbutamide (Fig. 3B). Whole-cell conductance corresponding to the period of insulin-induced hyperpolarization was within the reported range (e.g., 0.27–7.6 nS) for the calculated whole-cell K_{ATP} conductance of single rodent β-cells (1,27). Figure 3B shows cell conductance after normalization for cell capacitance under various conditions.

Application of voltage ramps to single β-cells voltage-clamped to −65 mV in 10 mmol/l glucose revealed an outward-rectifying whole-cell current that reversed at −25.7 ± 2.5 mV ($n = 9$). Insulin (100 nmol/l) increased the slope of the linear part of the current-voltage relationship between −90 to −40 mV, which is known to reflect the contribution of K_{ATP} channels to β-cell whole-cell current, and shifted the reversal potential to −63.7 ± 1.0 mV ($P < 0.001$ vs. control, $n = 9$), toward the calculated K^+ equilibrium potential of −90 mV (Fig. 3C). The subsequent application of tolbutamide inhibited the insulin-activated current and shifted its reversal potential to near control values (−25.2 ± 2.3 mV). These data thus strongly suggest that insulin hyperpolarizes β-cells by activating K_{ATP} channels and increasing β-cell K^+ conductance.

Wortmannin reversed insulin-induced activation of K_{ATP} channels and hyperpolarization of β-cells. PI 3-kinase is a pivotal enzyme in insulin signal transduction both in classical insulin target tissues (28) and insulin-
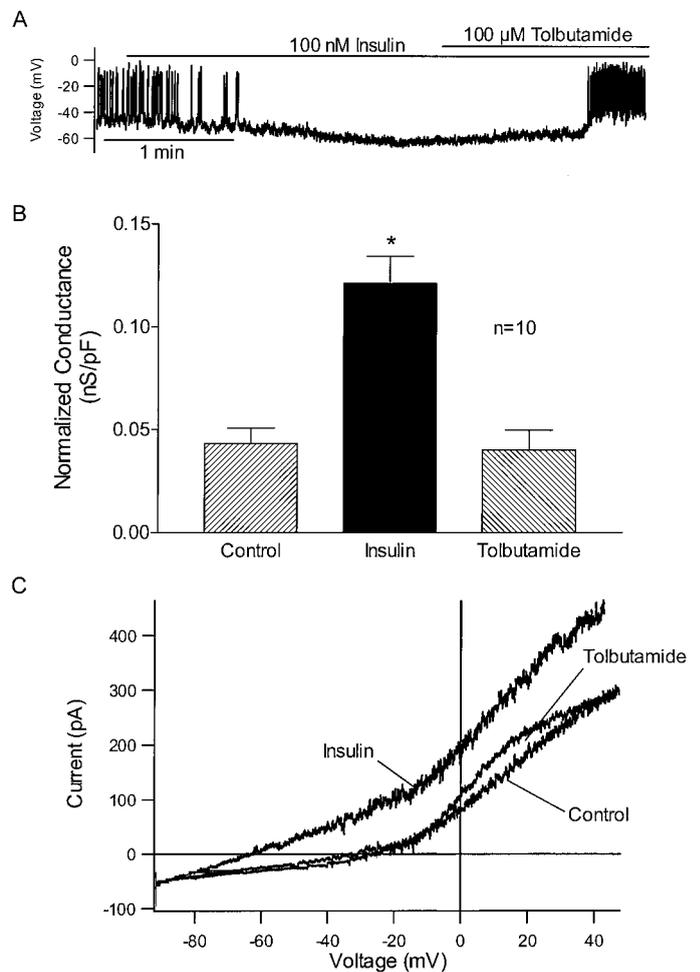
FIG. 3. Tolbutamide reversed insulin-induced hyperpolarization and increased K_{ATP} conductance in single pancreatic β-cells. *A*: Insulin-induced (100 nmol/l) hyperpolarization was reversed by the application of 100 μmol/l tolbutamide. The membrane potentials measured in control, 100 nmol/l insulin, or insulin + 100 μmol/l tolbutamide solutions were −46.7 ± 2.4, −62.1 ± 1.9 ($P < 0.001$), and −44.1 ± 2.1 mV ($n = 7$), respectively. *B*: Whole-cell conductance, adjusted for cell capacitance, is shown for control, 100 nmol/l insulin, or insulin + 100 μmol/l tolbutamide solutions. Insulin addition increased normalized cell conductance, which could be reversed to control levels by tolbutamide. *C*: Currents recorded from cells in control, 100 nmol/l insulin, or insulin + 100 μmol/l tolbutamide solutions are plotted against cell voltage. The reversal potentials corresponding to β-cells exposed to control, insulin, or insulin + tolbutamide solutions were −25.7 ± 2.5, −63.7 ± 1.0 ($P < 0.001$), and −25.2 ± 2.3 mV, respectively ($n = 9$). Insulin shifted the reversal potential toward K_{ATP} equilibrium potential. This shift was reversed by the application of tolbutamide.

secreting β-cells (8,9). A PI 3-kinase–dependent pathway has also been implicated in leptin activation of K_{ATP} channels in the insulin-secreting CRI-G1 cell line (29) and in insulin activation of neuronal K_{ATP} channels (20). We thus tested whether wortmannin, a PI 3-kinase inhibitor, could reverse insulin-induced β-cell hyperpolarization. As expected, the application of 100 nmol/l insulin hyperpolarized β-cells from −53.6 ± 1.6 to −69.8 ± 2.2 mV ($P < 0.001$, $n = 5$) and terminated their electrical activity (Fig. 4A). The subsequent application of 100 nmol/l wortmannin to inhibit PI 3-kinase slowly depolarized these cells to −53.2 ± 2.1 mV ($P < 0.05$) over a mean period of 4.5 ± 1.1 min ($n = 5$). In separate experiments, we found that pretreating β-cells with wortmannin fully blocked the inhibitory effects of insulin (data not shown).
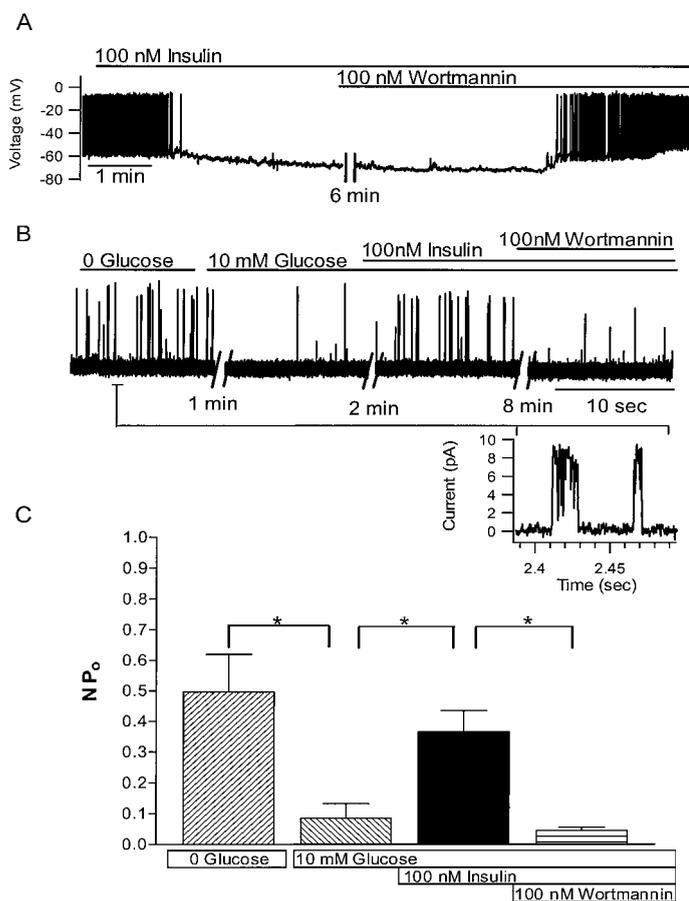
FIG. 4. Wortmannin reversed insulin-induced β-cell hyperpolarization. *A*: **Insulin-induced (100 nmol/l) β-cell hyperpolarization (from −53.6 ± 1.6 to −69.8 ± 2.2 mV, $P < 0.001$) was reversed by the subsequent application of 100 nmol/l wortmannin to −53.2 ± 2.1 mV ($n = 5$). *B*: Representative traces from cell-attached patch recordings of single K_{ATP} channels, where the bath solution contained 0 mmol/l glucose, 10 mmol/l glucose, 10 mmol/l glucose + 100 nmol/l insulin, or insulin + 100 nmol/l wortmannin. *C*: NP_o (N = the number of functional channels, P_o = the channel open probability) was calculated using 10 s–long recordings of K_{ATP} channel activity obtained in solutions containing 0 mmol/l glucose (0.49 ± 0.1), 10 mmol/l glucose (0.08 ± 0.04), 10 mmol/l glucose + 100 nmol/l insulin (0.37 ± 0.07), or in insulin + 100 nmol/l wortmannin (0.04 ± 0.01).**

We next tested the effects of insulin and wortmannin on unitary K_{ATP} channel activity measured in cell-attached patch recordings from single mouse β-cells. As shown in Fig. 4*B*, in the absence of glucose, single channels having a slope conductance of 44.8 ± 4.8 pS ($n = 5$) were observed. This conductance corresponds to the reported unitary conductance for β-cell K_{ATP} channels (~50 pS) (1–3). NP_o in glucose-free solution was 0.49 ± 0.1 ($n = 4$) (Fig. 4*C*), whereas increasing the bath glucose concentration to 10 mmol/l decreased channel activity to 0.08 ± 0.04 ($P < 0.005$). The subsequent application of 100 nmol/l insulin reversed the action of glucose, resulting in an increase in mean K_{ATP} channel activity to 0.37 ± 0.07 ($P < 0.005$), supporting the hypothesis that glucose-inhibited K_{ATP} channels are activated by insulin. Because single K_{ATP} channels in this recording configuration were not directly exposed to insulin, the efficacy of bath-applied insulin suggests that insulin opens K_{ATP} channels through an intracellular second messenger. The subsequent addition of 100 nmol/l wortmannin resulted in the suppression of NP_o to 0.04 ± 0.01 ($P < 0.005$), which was not

significantly different from that observed in solutions containing 10 mmol/l glucose ($P > 0.05$) (Fig. 4*C*).

DISCUSSION

We thus report that insulin activates the K_{ATP} channels of mouse pancreatic β-cells through a PI 3-kinase–dependent pathway, resulting in β-cell hyperpolarization, a cessation of glucose-induced electrical activity, and inhibition of islet $[Ca^{2+}]_i$ oscillations. The peptide hormones leptin (29–31), somatostatin (32), and galanin (33) and "K_{ATP}-opener" drugs, such as diazoxide (34) and minoxidil (35), hyperpolarize β-cells and inhibit insulin secretion by activating K_{ATP} channels, as we found for insulin. In the case of leptin, which activates K_{ATP} channels in *ob/ob* mouse β-cells (31) and rat CRI-G1 insulinoma cells, a PI 3-kinase-dependent pathway also appears to be involved (30), as we found for insulin activation of β-cell K_{ATP} channels.

The actions of insulin that we describe are likely to be mediated by insulin receptors because IGF-1 failed to hyperpolarize β-cells, and the EC_{50} we obtained for insulin compared favorably with the reported EC_{50} (~4 nmol/l) of β-cell insulin receptors (8,16,17). Although we and others (8,11,16,17) have exposed β-cells to higher concentrations of insulin to observe acute effects (e.g. 100 nmol/l), even low nanomolar doses of insulin significantly activated β-cell K_{ATP} channels. Circulating insulin levels in this range have been reported in the plasma of insulin-resistant human subjects after a glucose load (36–39) and in the portal circulation (40). Additionally, we would expect that still higher insulin concentrations occur within islets because of the local accumulation of newly secreted insulin into their tight interstitial spaces. Thus, the mechanism that we describe is likely to functionally alter β-cell electrophysiology and modify insulin secretion.

β-cells began to hyperpolarize within tens of seconds of adding exogenous insulin, whereas maximal hyperpolarization occurred after several minutes. These kinetics are consistent with a large number of studies of insulin action in its classic target tissues, where insulin-induced insulin receptor phosphorylation, phosphorylation of IRS proteins, and activation of PI 3-kinase and Akt/protein kinase B occur tens of seconds to minutes after exposure to insulin (41–45), whereas the translocation of glucose transporters and glucose transport takes many minutes (46). In βTC-3 cells, the autocrine action of insulin to stimulate insulin receptor phosphorylation occurs within ~2 minutes (8). It thus seems reasonable to conclude that the time lag we observed for insulin activation of β-cell K_{ATP} channels reflects the time course of the insulin receptor signal transduction cascade, as in muscle cells and adipocytes. We attribute the longer delays we observed using in situ versus isolated β-cells to the time required for insulin to diffuse within the intact islet. Although we do not yet know how insulin activates the K_{ATP} channels of β-cells, PI 3-kinase could conceivably activate K_{ATP} channels by altering membrane phosphoinositide levels, because these have been shown to reduce the ATP sensitivity of K_{ATP} channels (47).

Although this is the first report showing that insulin activates β-cell K_{ATP} channels, insulin activates the K_{ATP} channels of rat hypothalamic neurons through a similar mechanism involving PI 3-kinase (20). Thus, these authors

found that 5–300 nmol/l insulin hyperpolarized a subpopulation of the hypothalamic neurons of lean Zucker rats and decreased their input resistance. Voltage clamp studies established that insulin activated an ionic current under these conditions that reversed at the K^+ equilibrium potential and was blocked by 200 μmol/l tolbutamide. The authors extended these studies to the single-channel level and showed that insulin activated single K_{ATP} channels in cell-attached patches. As we found in β-cells, insulin-induced hyperpolarization of hypothalamic neurons required PI 3-kinase activation, as it was blocked by wortmannin or LY294002. Based on these findings, Spanswick et al. (20) proposed that insulin serves as a negative feedback signal in the hypothalamus to reduce food intake through the inhibition of glucose-responsive neurons.

However, in contrast to our findings, Aspinwall et al. (16,17) reported that 1–100 nmol/l insulin applied to single mouse β-cells increased rather than decreased insulin exocytosis, as assayed using 5–hydroxytryptamine amperometry, and did so independently of changes in β-cell membrane potential. These authors proposed that insulin stimulates insulin secretion by releasing Ca^{2+} from ER Ca^{2+} stores via a mechanism involving IRS-1 and PI 3-kinase, because the insulin-induced changes they observed were wortmannin-sensitive. However, because Aspinwall et al. (16,17) carried out their studies in 3 mmol/l glucose, it is reasonable to expect that most of the β-cell's K_{ATP} channels were already open and were thus relatively insensitive to further modulation (1). Under basal conditions such as these, minimal amounts of insulin would be secreted, which further questions the relevance of these findings for glucose-stimulated insulin secretion. In another recent study, Harvey and Ashford (29) reported that whereas leptin activated CRI-G1 cell K_{ATP} channels, insulin did not. Although we do not know why insulin failed to open K_{ATP} channels in this preparation, CRI-G1 cells differ in several respects from the adult mouse β-cells we used and are glucose insensitive. Thus, it may be that metabolic differences between the two preparations might account for this discrepancy.

Insulin activation of β-cell K_{ATP} channels has a number of implications for understanding islet function. First, in general, this negative feedback mechanism would be expected to restrain excessive insulin exocytosis when intra-islet insulin is sufficiently high, preventing hyperinsulinemia. Second, in terms of islet electrophysiology, the mechanism may help explain why intact islets have regular 10- to 60-s electrical oscillations (21,24), compared with the fast bursting or spiking of isolated β-cells (23). Thus, it may be that insulin released within islets during glucose-stimulated electrical activity in turn inhibits islet electrical activity by activating β-cell K_{ATP} channels. Secretion would then decrease, as $[Ca^{2+}]_i$ decreases, and the cycle would be repeated as secreted insulin diffuses away from β-cell insulin receptors and K_{ATP} channels close. However, in isolated β-cells lacking tight intercellular spaces, insulin once secreted would be likely to dissipate quickly, precluding autocrine or possibly paracrine effects. Thus, a loss of intra-islet signaling might account for the lack of islet-like electrical activity displayed by isolated β-cells (21,23,24).

We have quantitatively tested the feasibility of this proposed mechanism using a recent paradigm of bursting, the phantom burster model (48). In this model, islet bursting results from the interaction of two slow processes, one (S1) with a time constant of a few seconds, and another (S2) with a time constant of a few minutes. The slow kinetics of activation of K_{ATP} current by insulin makes this mechanism an attractive candidate for the slower S2 process. In support of this hypothesis, we found that a variation of the phantom burster model incorporating insulin activation of K_{ATP} channels as the slow S2 process was able to simulate the medium bursting of intact islets (A. Sherman, L. Satin, R. Bertram, unpublished observations). Furthermore, removing the slow process, which might be akin to dispersing islets into single β-cells and thus eliminating the intercellular spaces needed for insulin to accumulate in, resulted in faster, S1-dominated bursting resembling that of dispersed β-cells (23). These computations thus support the general feasibility of this proposed mechanism of bursting, although the hypothesis remains to be tested by experiment.

Dysregulated insulin signaling in this novel negative feedback system, perhaps resulting from biochemical or molecular defects in the β-cell insulin receptor signaling cascade, could be significant for diabetes, because it may result in abnormal insulin secretion including defective pulsatility if insulin activation of K_{ATP} channels is required for periodic islet electrical activity.

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