Insulin Feedback Alters Mitochondrial Activity Through an ATP-sensitive K^+ Channel–Dependent Pathway in Mouse Islets and β-Cells

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Recent work suggests that insulin may exert both positive and negative feedback directly on pancreatic β-cells. To investigate the hypothesis that insulin modulates β-cell metabolism, mouse islets and β-cell clusters were loaded with rhodamine 123 to dynamically monitor mitochondrial membrane potential ($\Delta\Psi_m$). Spontaneous oscillations in $\Delta\Psi_m$ (period: 218 ± 26 s) were observed in 17 of 30 islets exposed to 11.1 mmol/l glucose. Acute insulin application (100 nmol/l) hyperpolarized $\Delta\Psi_m$, indicating a change in mitochondrial activity. The ATP-sensitive K^+ (K_{ATP}) channel opener diazoxide or the L-type calcium channel blocker nifedipine mimicked the effect of insulin, suggesting that insulin activates K_{ATP} channels to hyperpolarize $\Delta\Psi_m$ by inhibiting calcium influx. Treatment with forskolin, which increases endogenous insulin secretion, also mimicked the effect of exogenous insulin, suggesting physiological feedback. Pretreatment with nifedipine or the K_{ATP} inhibitor glyburide prevented insulin action, further implicating a K_{ATP} channel pathway. Together, these data suggest a feedback mechanism whereby insulin receptor activation opens K_{ATP} channels to inhibit further secretion. The resulting reduction in β-cell calcium increases the energy stored in the mitochondrial gradient that drives ATP production. Insulin feedback onto mitochondria may thus help to calibrate the energy needs of the β-cell on a minute-to-minute basis.
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Electrical activity plays a prominent role in β-cell stimulus-secretion coupling in mouse islets (1,2). At low glucose concentrations (<3 mmol/l), β-cells are electrically silent and secrete low or basal levels of insulin. In response to glucose stimulation (>5–7 mmol/l), metabolism and mitochondrial energy production increase (3–5). The resulting increase in the ATP/ADP ratio closes ATP-sensitive K^+ (K_{ATP}) channels and depolarizes the β-cell to initiate electrical activity and insulin secretion (2,6). β-cell electrical activity typically follows a burst pattern consisting of slow oscillations or plateaus in membrane potential with superimposed fast calcium spikes (7). The resulting calcium influx induces insulin secretion and may activate several types of K^+ channels to assist in terminating each burst (8–11).

Recent work suggests that this process may be regulated by insulin. Exogenous insulin has been found to modulate gene transcription and translation, intracellular signaling, intracellular calcium ($[Ca^{2+}]_i$), and insulin secretion itself (12–19). We have previously shown that insulin acutely opens the K_{ATP} channel through a phosphatidylinositol (PI) 3-kinase–sensitive mechanism, leading to hyperpolarization of the β-cell plasma membrane and a reduction in calcium influx as voltage-gated L-type calcium channels close (14). Insulin thus negatively feeds back to inhibit further insulin secretion via this pathway. Other studies, however, have suggested that insulin receptor activation results in increased $[Ca^{2+}]_i$ and increased secretion, suggesting a positive feedback due to insulin receptor activation (15,16,18,19).

In the present study, we sought to determine whether insulin could also influence metabolic activity in β-cells. Previous studies using the dye rhodamine 123 (Rh123) to dynamically measure mitochondrial membrane potential ($\Delta\Psi_m$) as a marker of cell metabolic activity have established that $\Delta\Psi_m$ hyperpolarizes in response to increased glucose in β-cells (20–22), consistent with the known action of glucose metabolism to increase the proton gradient that drives mitochondrial ATP production. Oscillatory changes in Rh123 have been found to mirror oscillatory changes in $[Ca^{2+}]_i$ (21,22), suggesting that increased $[Ca^{2+}]_i$ in β-cells decreases the ATP/ADP ratio, as discussed in the Keizer-Magnus model (23,24). To investigate the feedback effects of insulin on metabolism and their possible relation to plasma membrane ion channel activity, we measured Rh123 fluorescence in β-cells while selectively blocking specific β-cell ion channels to test the hypothesis that insulin modifies mitochondrial metabolism via ion channel activity in mouse islets and β-cells.

RESEARCH DESIGN AND METHODS

Culture of islets and islet β-cells. Mouse islets were isolated by collagenase digestion from the pancreata of Swiss-Webster mice (ages 2–3 months, weight 25–35 g), as previously described (14,25). Islets were dissociated into single cells or clusters by gentle trituration in a low-calcium medium (26,27). Islets or islet cell suspensions were then plated on glass coverslips pretreated with 0.1% gelatin to facilitate attachment (Sigma-Aldrich, St. Louis, MO) and placed in 35-mm plastic Petri dishes for tissue culture. Islet tissue was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin (Gibco, Grand Island, NY) and

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$\Delta\Psi_m$, mitochondrial membrane potential; FCCP, fluoro-carbonyl cyanide phenylhydrazone; K_{ATP} channel, ATP-sensitive K^+ channel; PI, phosphatidylinositol; Rh123, rhodamine 123; ROI, region of interest.

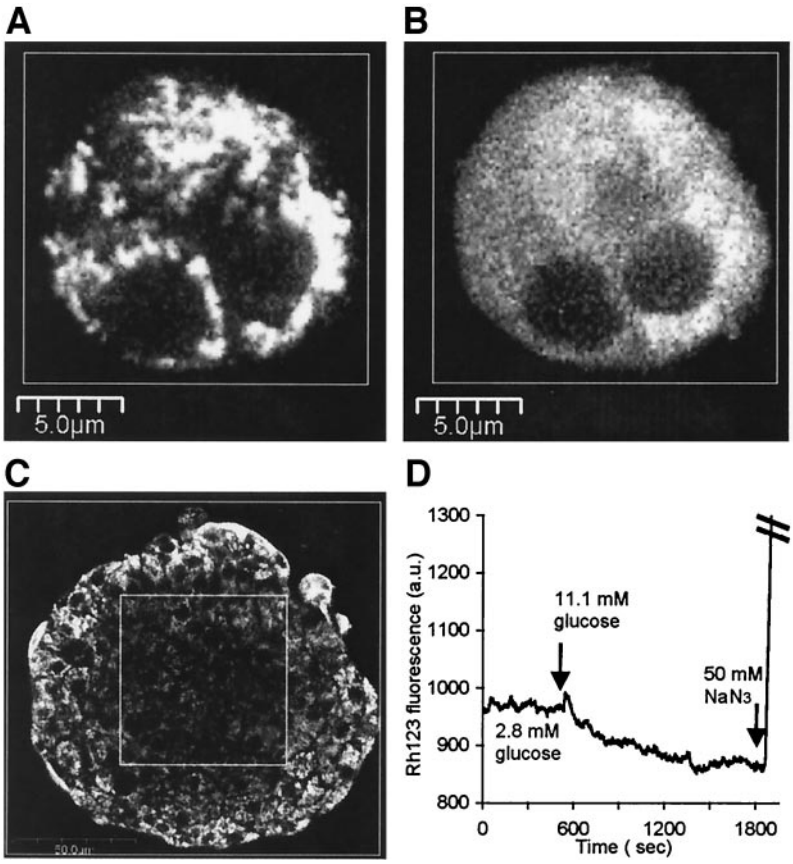

FIG. 1. Rhodamine fluorescence from islets and clusters. *A* and *B*: Images of a small cluster of cells during 11.1 mmol/l glucose control conditions (*A*) and after depolarizing $\Delta\Psi$m with NaN_3 (*B*). *C*: Image of an islet during 11.1 mmol/l glucose control conditions. Scale bar in lower left corner denotes size. Boxes (ROIs) represent the area analyzed to produce plots of fluorescence intensity. *D*: Plot of fluorescence intensity versus time for an islet incubated for 8 min in 2.8 mmol/l glucose, followed by 22 min in 11.1 mmol/l glucose and then 3 min in 50 mmol/l NaN_3 (representative of eight islets tested).

incubated at 37°C in a 95% air/5% CO_2 mixture. Cultures were fed every other day. Clusters of islet cells were studied after 2–5 days in culture, and islets were studied within 2–3 days.

Rhodamine 123 loading and fluorescence measurements. Cultures were loaded with 5 μmol/l Rh123 (Molecular Probes, Eugene, OR) in RPMI and incubated for 10–15 min at 37°C. Cultures were then washed and incubated in Rh123-free RPMI before each experiment. Shards of coverslips containing mouse islets or single cells were placed in a recording chamber and perfused with external solution containing (in mmol/l): 11.1 glucose, 130.5 NaCl, 3 $CaCl_2$, 5 KCl, 2 $MgCl_2$, 10 HEPES (pH 7.3). All chemicals were obtained from Sigma-Aldrich, unless otherwise indicated. A Minipulse II peristaltic pump (Gilson, Villiers Le Bel, France) perfused various solutions through the recording chamber at 32–35°C (±0.3°C intra-experimental range) using an in-line heater (Cell Micro Controls, Virginia Beach, VA).

Rh123 fluorescence was measured using an Olympus BX61WI upright laser scanning confocal microscope and Fluoview image acquisition and analysis software (Olympus America, Melville, NY). Rh123 has been used in numerous studies (28,29), including several using β-cells (20–22), to monitor changes in $\Delta\Psi_m$. Hyperpolarization of $\Delta\Psi_m$ leads to increased mitochondrial Rh123 uptake, Rh123 concentration, and self-quenching of fluorescence within the mitochondria. In contrast, as $\Delta\Psi_m$ diminishes, the relative depolarization of $\Delta\Psi_m$ leads to dye efflux from the mitochondria into the cytosol as oxidation decreases, causing fluorescence to increase. This explains why mitochondrial inhibitors, such as NaN_3 and fluoro-carbonyl cyanide phenylhydrazone (FCCP), consistently increase Rh123 fluorescence. Rh123 was excited at 488 nm, and its emission was collected at 535 nm. Cell-free calibration experiments carried out using different concentrations of free dye confirmed a linear relation between dye concentration and fluorescence intensity within the range monitored, and rhodamine-coated Teflon beads (6 μm diameter; Molecular Probes) confirmed the stability of the fluorescence measurements (data not shown). For each experiment, the fluorescence of single β-cells, β-cell clusters, or islets was recorded in 11.1 mmol/l glucose for 10–20 min, with images acquired at 3-s intervals to establish baseline fluorescence and detect possible oscillations. This control period was followed by a 5-min treatment period with one of several ion channel–modulating drugs (100 nmol/l glyburide, 250 μmol/l diazoxide, 50 μmol/l nifedipine) or a 10-min exposure to insulin to test for concomitant changes in mitochondrial activity. In some studies, several drugs were given in series or in combination, as detailed in the results. In studies using high KCl, extracellular NaCl was lowered accordingly to maintain osmolarity. The mitochondrial poison NaN_3 (5 or 50 mmol/l) or the mitochondrial uncoupler FCCP (5 μmol/l) was applied for 5 min at the end of each of the experiments to confirm that Rh123 acted appropriately. Clusters and islets that did not respond to NaN_3 or FCCP were not included for further analysis.

Data analysis. To determine mean Rh123 fluorescence, rectangular regions of interest (ROIs) were drawn around the images of each islet, cell, or cell cluster after each experiment (Fig. 1*A*–*C*). (We refer to clusters of cultured islet cells as β-cell clusters from here onward based on prior work demonstrating that >80% of cultured islet cells are typically β-cells [25].) In the case of islets, ROIs were drawn around the central portion of the islet for comparison (Fig. 1*C*). Rh123 fluorescence could be observed several cell layers below the surface of the islet. This allowed fluorescence measurements to be made in the interior of the islet, where endogenous changes in Rh123 fluorescence were often greater. Because the center of each optical section represented the interior of the islet, this region was expected to have a higher proportion of β-cells (30). Treatment-induced changes in Rh123 fluorescence were typically greater in whole-islet versus central ROIs (likely due to the somewhat slow diffusion of drugs to the interior), so whole-islet ROIs were preferred for analysis. The mean fluorescence of all pixels within each ROI was calculated for each image taken at 3-s intervals and then plotted versus time using arbitrary units of fluorescence. To prevent photobleaching of Rh123, laser intensity was set to 3% of the maximum power. Decreases in mean fluorescence of <1% could be established between two consecutive control periods of 5 min each using this approach (n = 30 islets). In the core of the islets, a mean decrease of <0.05% could even be observed. Changes in fluorescence attributed to endogenous oscillations in mitochondrial activity were assessed by collecting images during control periods of 10–20 min in 11.1 mmol/l glucose. The periodicity of Rh123 oscillations was determined visually by identifying two or more peaks in fluorescence having amplitudes >5% of the baseline signal, and then calculating the time interval between the peaks observed for each recording. Comparisons between successive treatment phases were made by averaging the fluorescence of each ROI during the last 2–3 min of drug exposure. Comparisons between control and drug treatment phases were carried out using a two-tailed t test of paired mean fluorescence, with significance at $P < 0.05$. One-way ANOVA was used to compare multiple treatments. Data are presented as the percent of control fluorescence. Note that although some results were presented as "responders" (>5% change) and "nonresponders" (<5% change) to provide more detailed

information, all statistical comparisons were carried out using means for the entire treatment group.

RESULTS

Confirmation of the relation between Rh123 fluorescence and $\Delta\Psi_m$. Before investigating the effects of insulin treatment on mitochondrial activity, we first confirmed that Rh123 reported changes in $\Delta\Psi_m$ appropriately, as in other β-cells studies (20–22). At high magnification, many round or elongated fluorescent structures could be observed in confocal images, indicative of individual mitochondria (Fig. 1*A*) (5,22). As shown in Fig. 1*B*, the pattern of fluorescence we observed became much more uniform after treatment with the mitochondrial poison NaN_3, which dissipates the mitochondrial gradient and causes the release of Rh123 into the cytosol (Fig. 1*B*). In both clusters and islets under all experimental conditions, Rh123 was clearly excluded from the cell nucleus (see Fig. 1*A*– *C*).

By monitoring Rh123 fluorescence levels over time, changes in $\Delta\Psi_m$ were ascertained in response to changes in metabolic fuel availability and after the addition of mitochondrial poisons. Specifically, switching from a solution containing 2.8 mmol/l glucose to one containing 11.1 mmol/l glucose caused Rh123 levels to decrease by 9 ± 2% (Fig. 1*D*; representative of eight islets tested), consistent with hyperpolarization of $\Delta\Psi_m$ (20). In contrast, Rh123 fluorescence markedly increased (31 ± 4%; $n = 8$) in response to the mitochondrial poison NaN_3, which is consistent with a reduction or dissipation of $\Delta\Psi_m$, resulting in more visible dye in the cytosol. Similar results were obtained with the mitochondrial uncoupler FCCP (5 μmol/l; data not shown). These findings confirm those of previous studies (20–22) and support the validity of the technique to measure the metabolic activity of mitochondria.

Endogenous oscillations in $\Delta\Psi_m$. At stimulatory glucose levels, endogenous oscillations in $\Delta\Psi_m$ were detected in a majority of islets and clusters. Islets were maintained in 11.1 mmol/l glucose for 10–20 min to establish their baseline fluorescence. (Note that all remaining studies were performed using 11.1 mmol/l glucose.) During this control period, 17 of 30 islets displayed spontaneous oscillations in Rh123 fluorescence. The peak-to-peak amplitude of the oscillations was >5% of the total fluorescence signal (Fig. 2). The estimated period of the $\Delta\Psi_m$ oscillations was 218 ± 26 s (~3.6 min); oscillations ranged from 120 to 440 s (~2–7 min), consistent with previous reports of slow metabolic oscillations evoked by glucose stimulation in islets (21,22). Some β-cell clusters displayed similar patterns, but these were not examined in detail because the signal-to-noise ratio was generally lower in clusters and single β-cells due to the reduced area of their ROIs.

Insulin hyperpolarizes $\Delta\Psi_m$. Previous work from this laboratory has demonstrated that insulin opens plasma membrane K_{ATP} channels in β-cells, resulting in membrane hyperpolarization and an inhibition of islet calcium oscillations (14). In the present study, we wished to examine whether an identical insulin treatment also altered mitochondrial metabolic activity. Application of solutions containing 100 nmol/l insulin caused a clear hyperpolarization of ΔΨm in both islets and β-cell clusters, suggesting that

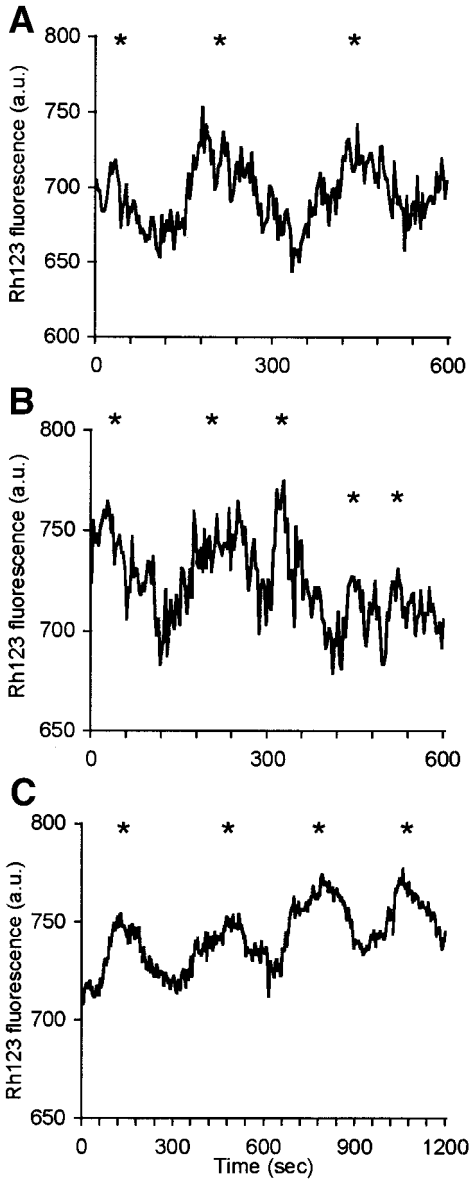

FIG. 2. Endogenous oscillations in ΔΨm in steady-state glucose. *A*–*C*: Plot of Rh123 fluorescence intensity versus time of sequential images from three islets displaying oscillations in fluorescence signal recorded in 11.1 mmol/l glucose. *Visually identified peaks in signal used to estimate the periodicity of activity. (Note that *x*- and *y*-axes differ among panels.)

insulin increased mitochondrial metabolism. As illustrated by the representative example shown in Fig. 3*A*, insulin reduced Rh123 fluorescence. This was the case in over half of the islets tested ($n = 8$ of 13; 12 ± 3% mean decrease in responders), whereas in the remaining islets, the effects of insulin were small (2 ± 1% decrease in fluorescence). The mean response among all islets was an 8 ± 2% decrease in fluorescence ($n = 13$; $P < 0.004$). Islets exposed to NaN_3 after insulin treatment responded with a brisk increase in fluorescence in all cases (29 ± 7% increase; $n = 9$). The effects of insulin on $\Delta\Psi_m$ were not reversed by washing the cells for 5 min ($n = 4$). Similar results were observed using small clusters of islet cells (<30 μm diameter), with 15 of 22 clusters responding with a mean fluorescence decrease of 13 ± 1% (Fig. 3*B*). Of the remaining clusters, six demonstrated a change of <4% in Rh123 fluorescence

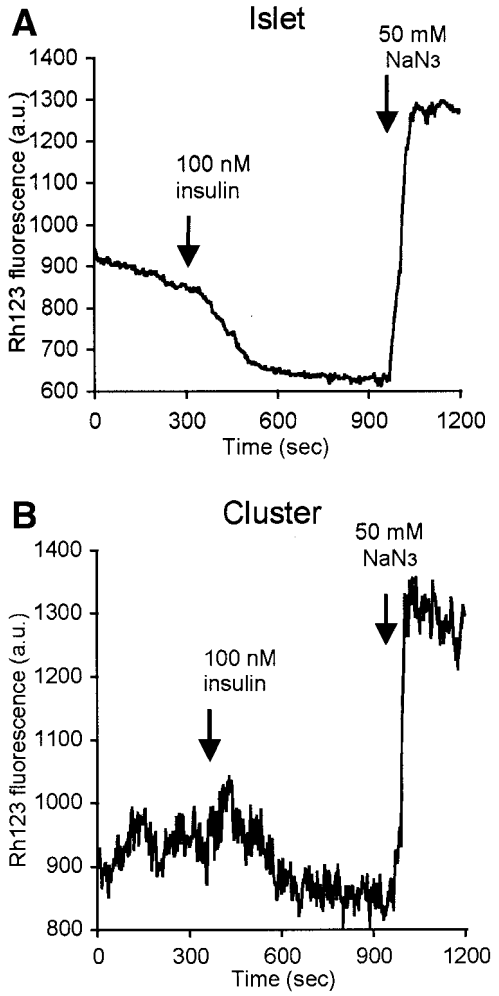

FIG. 3. Insulin hyperpolarizes ΔΨm in mouse islets and β-cells. Plot of fluorescence versus time for an islet (*A*) and a small cluster of cells (*B*) recorded in 11.1 mmol/l glucose and treated with insulin followed by NaN_3. Arrows indicate the start of drug treatments.

in response to insulin and one exhibited an anomalous 17% increase in fluorescence. The mean response among all clusters was a 9 ± 1% decrease in fluorescence ($n = 22$; $P < 0.00025$).

Insulin acts on mitochondria through K_{ATP} and calcium channels. Based on our previous findings that insulin inhibits islet electrical activity and reduces $[Ca^{2+}]_i$, we hypothesized that changes in K_{ATP} channel activity and concomitant changes in calcium channel activity were responsible for the effects of insulin on $\Delta\Psi_m$. We confirmed that the activation of K_{ATP} channels could, in principle, mediate changes in $\Delta\Psi_m$ using the K_{ATP} channel opener diazoxide. Thus the application of diazoxide (250 μmol/l) had a similar hyperpolarizing effect on $\Delta\Psi_m$ in islets as did insulin ($n = 8$) (Fig. 4*A*), although the effect was smaller (mean decrease of 6 ± 1% in eight islets; $P < 0.01$). To determine if closing K_{ATP} channels produced the opposite effect, the K_{ATP} channel blocker glyburide (100 nmol/l) was tested. Glyburide had little or no effect on Rh123 fluorescence levels (mean increase of 1 ± 2% in 18 clusters); however, this may have been due, in part, to a majority of K_{ATP} channels being closed in 11.1 mmol/l glucose.

Because the opening of K_{ATP} channels causes calcium channels to subsequently close, we hypothesized that pharmacologically blocking calcium should mimic insulin modulation of $\Delta\Psi_m$. Exposure of β-cells to the L-type channel blocker nifedipine (50 μmol/l) resulted in an insulin-like hyperpolarization of $\Delta\Psi_m$ in islets (10 ± 1% decrease; $n = 5$; $P < 0.002$) (Fig. 4*B*) and β-cell clusters (15 ± 8% decrease; $n = 20$; $P < 0.005$), thus supporting our hypothesis. These results suggest that changes in $\Delta\Psi_m$ brought about by opening K_{ATP} channels or closing calcium channels are mediated by $\Delta\Psi_m$ by reducing $[Ca^{2+}]_i$.

From these observations, we hypothesized that increased $[Ca^{2+}]_i$ would have the opposite effect on $\Delta\Psi_m$; therefore, islets were treated with 30 mmol/l KCl to depolarize the plasma membrane. Because K_{ATP} channels on mitochondria can be the direct targets of K_{ATP} channel drugs (31,32), diazoxide was also included in this study to test whether the hyperpolarization of $\Delta\Psi_m$ by diazoxide was due to the opening of K_{ATP} channels on the plasma membrane (thus reducing calcium) or to direct effects on the mitochondria (by modulating mitochondrial enzymes or mitochondrial K_{ATP} channels). Figure 5 is representative of the depolarizing effect of 30 mmol/l KCl + 250

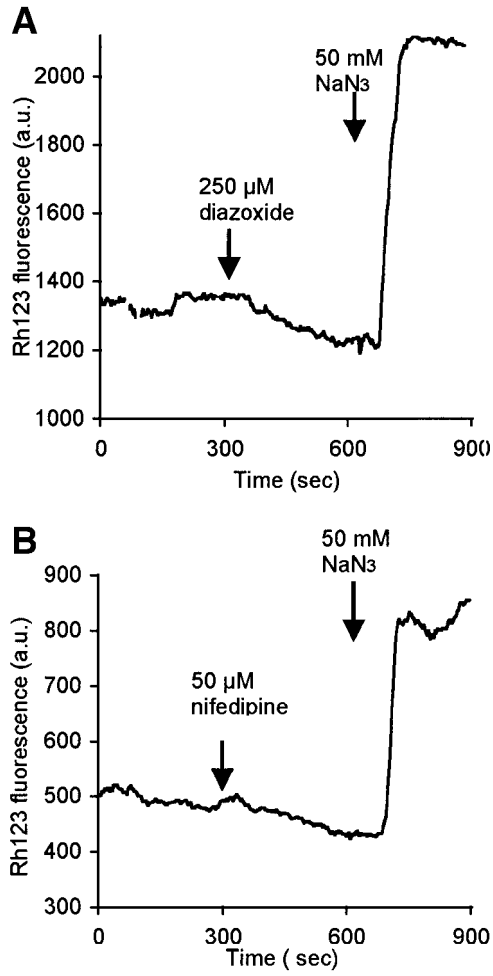

FIG. 4. The K_{ATP} channel opener diazoxide or the calcium channel blocker nifedipine mimics the effects of insulin. *A*: Plot of fluorescence versus time for an islet in 11.1 mmol/l glucose treated with 250 μmol/l diazoxide, followed by NaN_3. Similar results were observed in five of eight islets. *B*: Plot of fluorescence versus time for an islet treated with 50 μmol/l nifedipine, followed by NaN_3. Similar results were observed in five of five islets. Arrows indicate the start of drug treatments.

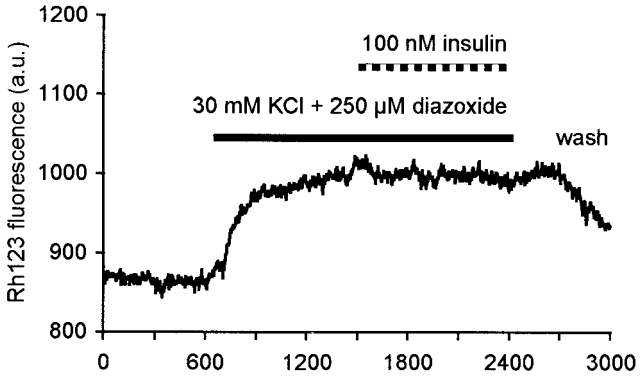

FIG. 5. Induced calcium influx depolarizes ΔΨm and prevents insulin action. Plot of fluorescence versus time for a representative islet in 11.1 mmol/l glucose treated with 30 mmol/l KCl + 250 μmol/l diazoxide, 100 nmol/l insulin + 30 mmol/l KCl + 250 μmol/l diazoxide, and washed. Representative of effects on six islets.

μmol/l diazoxide observed in six islets (mean depolarization 7 ± 2%). This result was consistent with actions of increased calcium influx and not of direct hyperpolarizing actions of diazoxide on mitochondria. We further demonstrated that insulin had no additional effect on $\Delta\Psi_m$ under these conditions. These findings support the interpretation that changes in $\Delta\Psi_m$ are a consequence of changes in plasma membrane potential and $[Ca^{2+}]_i$.

To further demonstrate that K_{ATP} channel activation mediates the actions of insulin on mitochondria, insulin was applied to islets or β-cell clusters after first blocking K_{ATP} channels with glyburide. By closing K_{ATP} channels before insulin treatment, the K_{ATP}-dependent pathway of insulin feedback onto mitochondria should have been blocked. As shown in Fig. 6, inhibiting K_{ATP} channels prevented the effects of insulin on Rh123 fluorescence in islets (n = 11) (Fig. 6*A*) and β-cell clusters (n = 12) (Fig. 6*B*). The mean fractional change in fluorescence among control, glyburide, and glyburide plus insulin phases for islets did not significantly differ ($P > 0.10$ for each comparison). This confirmed that insulin effects on mitochondria are indeed mediated by changes in K_{ATP} channel activity. Similar results were obtained when β-cell clusters were first exposed to nifedipine before insulin application. Among eight β-cell clusters tested, a mean decrease in Rh123 fluorescence of 20 ± 5% occurred during nifedipine pretreatment, followed by little or no change in $\Delta\Psi_m$ during insulin plus nifedipine treatment.

Effects of increasing endogenous insulin secretion on mitochondria. Although the data presented thus far indicate that β-cell exposure to exogenous insulin results in hyperpolarization of $\Delta\Psi_m$, we have not addressed whether endogenously secreted insulin can have the same effect. The most direct way to measure effects of secreted insulin is to acutely block the insulin receptor; however, there are currently no commercially available insulin receptor antagonists. We instead attempted to potentiate insulin secretion by using forskolin, a direct activator of adenylate cyclase that acts to increase granule exocytosis without profoundly affecting electrical activity in elevated glucose (33). Forskolin treatment (4 μmol/l) induced an 8 ± 3% decrease in Rh123, consistent with the hypothesis that endogenous insulin can indeed alter $\Delta\Psi_m$ in a qualitatively similar fashion to exogenous insulin (n = 5 islets; $P <$

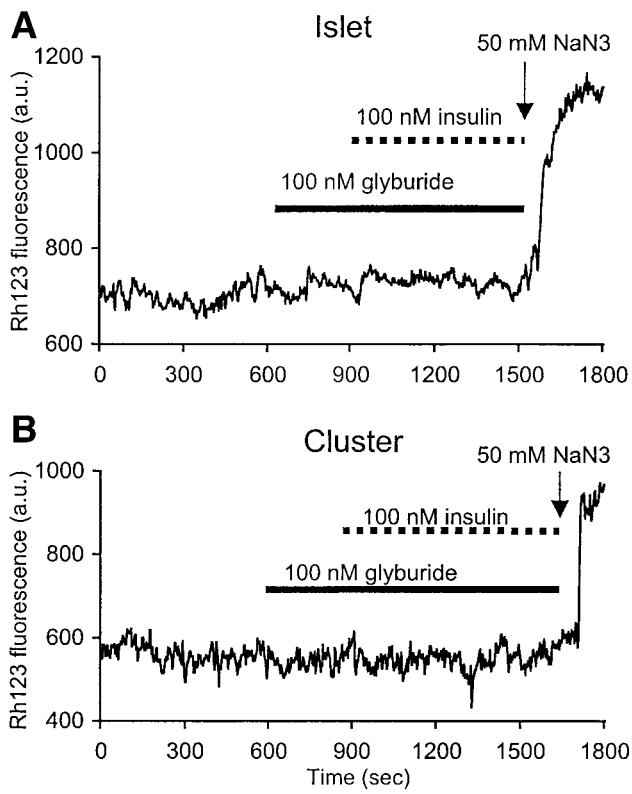

FIG. 6. Pretreatment with glyburide prevents the action of insulin on ΔΨm. Plot of fluorescence versus time for a representative islet (*A*) and a small cluster (*B*) in 11.1 mmol/l glucose treated with 100 nmol/l glyburide, 100 nmol/l glyburide + 100 nmol/l insulin, and then NaN_3. Solid bars indicate duration of glyburide treatment, dotted lines indicate duration of insulin treatment, and arrows indicate the start of NaN_3 treatment.

0.05) (Fig. 7). However, it should be noted that forskolin can increase calcium influx and alter other aspects of β-cell function (34,35), which could in turn influence $\Delta\Psi_m$ independently of secreted insulin. Nevertheless, this is the strongest evidence thus far for an autocrine action of insulin on islet mitochondrial function.

Time course of treatment effects on mitochondria. To compare the time course of the agents studied, we compared their latencies under comparable experimental con-

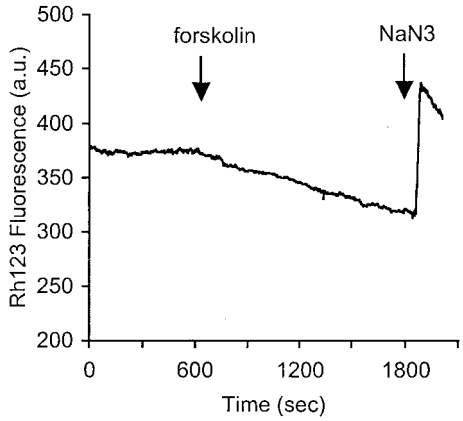

FIG. 7. Forskolin, an activator of adenylate cyclase, mimics the effects of insulin. *A*: Plot of fluorescence versus time for an islet in 11.1 mmol/l glucose treated with 4 μmol/l forskolin, followed by NaN_3. Arrows indicate the start of drug treatments. Representative of effects on five islets.

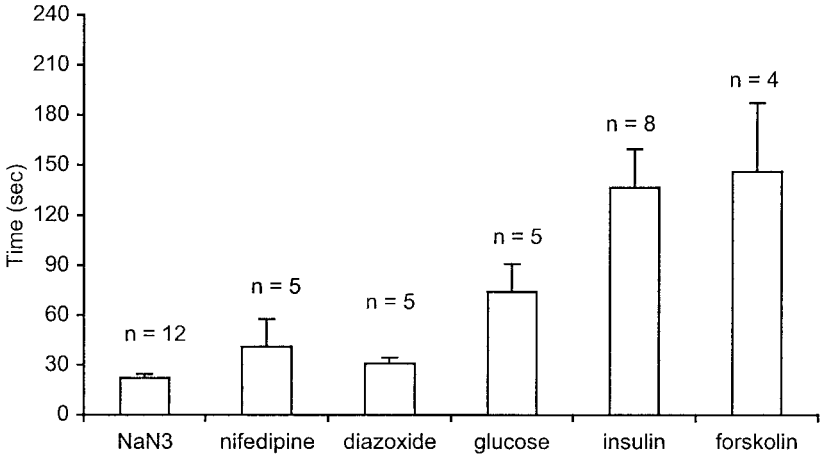

FIG. 8. Latencies of treatments that affect mitochondrial membrane potential. The times shown correspond to the mean latency between the treatment first entering the recording chamber and the first detected change in fluorescence from islets.

ditions (Fig. 8). The most rapidly acting agent was the mitochondrial poison NaN_3, which depolarized $\Delta\Psi_m$ in <30 s. Modulators of ion channel activity were found to be slightly slower, followed by changes brought about by higher levels of glucose, and finally insulin. These latencies suggest that changes in plasma membrane ion channels are closely linked to changes in mitochondria, and that the relatively longer times observed for glucose, insulin, and forskolin may logically follow, given the presence of several intervening steps in signaling. Thus, for glucose, it is likely that its transport, phosphorylation, and subsequent tricarboxylic acid cycle activation (36,37) must occur before altering $\Delta\Psi_m$. In the case of insulin, it is well known that its signal transduction pathway requires insulin receptor phosphorylation and subsequent phosphorylation of insulin receptor substrate proteins and PI 3-kinase activation, among other intermediate steps (38,39). Finally, forskolin must first stimulate cAMP production and downstream kinases before activation of the insulin receptor pathway can occur (34), thus explaining the long latency. The time course for mitochondrial effects is generally in agreement with the time of insulin-induced K_{ATP} channel opening (14) and the timing of the classic insulin receptor–mediated cascade in insulin target tissues as well as β-cells (40,41).

DISCUSSION

In the present study, we found that mitochondrial membrane potential was depolarized by raising $[Ca^{2+}]_i$ and hyperpolarized by decreasing calcium influx using the K_{ATP} channel opener diazoxide or the L-type calcium channel blocker nifedipine. Further, the application of exogenous insulin also hyperpolarized $\Delta\Psi_m$, an effect that was abolished in the presence of the K_{ATP} channel blocker glyburide. This insulin effect is consistent with our earlier report that insulin opens K_{ATP} channels in the β-cell plasma membrane, leading to hyperpolarization, a cessation of bursting activity, and reduced $[Ca^{2+}]_i$ (14). The present results confirm that insulin receptor activation stimulates mitochondrial activity in islets and β-cells by opening K_{ATP} channels and decreasing β-cell $[Ca^{2+}]_i$.

The observation that mitochondrial activity is calcium dependent is consistent with the effects of changing $[Ca^{2+}]_i$ on $\Delta\Psi_m$ in other reports using Rh123 (21,22) and in measures of the ATP/ADP ratio (42). This observation is also in line with studies showing that a rise in $[Ca^{2+}]_i$ due to a burst of action potentials or brief exposures to elevated potassium can lead to K_{ATP} channel activation in β-cells (10,43). Although the precise mechanism of $[Ca^{2+}]_i$-induced K_{ATP} channel activation is not known, it has been suggested that a decrease in mitochondrial respiration, reflected by a decrease in $\Delta\Psi_m$, in turn decreases the ATP/ADP ratio sufficiently to cause K_{ATP} channels to open. Keizer and Magnus (23,24) proposed a mathematical model based on this idea, in which increased calcium influx resulted in reduced ATP production, and demonstrated that K_{ATP} channel activation due to a decreased ATP/ADP ratio was feasible as a repolarization mechanism during glucose-induced electrical bursting. Alternatively, changes in β-cell $[Ca^{2+}]_i$ could, in theory, modulate $\Delta\Psi_m$ by affecting ATP consumption rather than production. In this view, an increase in $[Ca^{2+}]_i$ would result in increased ATP utilization via increased calcium ATPase activity to pump calcium, which would decrease $\Delta\Psi_m$ (42–44). Either scenario would be consistent with K_{ATP} channel activation due to a drop in the ATP/ADP ratio.

Although we have previously shown that depolarization-induced calcium influx in β-cells primarily activates another potassium channel, $K_{Ca,slow}$ (11), on occasion we observed an additional current that is activated after a burst of action potential–like depolarizations, which could reflect K_{ATP} channel activation (P. Goforth, unpublished observations). Indirect calcium-dependent activation of K_{ATP} channels may thus result from increased $[Ca^{2+}]_i$ acting on mitochondria or calcium ATPases, thus mediating a negative feedback loop to help repolarize β-cells (10,45).

A second way in which a rise in $[Ca^{2+}]_i$ could open K_{ATP} channels is by means of an exocytotic release of insulin. Secreted insulin would then activate the K_{ATP} channels of neighboring β-cells through a PI 3-kinase–dependent pathway, contributing to their repolarization (14). Our present data suggest that the decrease in $[Ca^{2+}]_i$ that would follow insulin-induced K_{ATP} channel activation could ultimately increase mitochondrial activity. Insulin signaling to the mitochondria could potentially mediate an increase in cellular ATP, which could contribute to a subsequent cycle of β-cell depolarization and/or secretion. The closure of K_{ATP} channels by this action would occur on a time scale of tens to hundreds of seconds, as compared with the calcium-induced opening of K_{ATP} channels described earlier (<10 s), as evidenced by the much longer latency of

insulin to mitochondrial changes compared with drugs that act directly on ion channels or mitochondria (Fig. 8). Although insulin is therefore unlikely to play a pacemaker role in islet burst firing patterns on the order of seconds, secreted insulin may instead act as a modulator of electrical activity on a minute-to-minute basis.

A general objection to the hypothesis that secreted insulin functionally modulates glucose-induced bursting via K_{ATP} channel activation is the observation that inhibiting islet insulin secretion by cooling islets from 37 to 20–27°C does not abolish glucose-induced bursting (46,47). This suggests that secreted insulin must therefore not be required for islet bursting to occur. However, when islets are cooled, there is a decrease in burst frequency and an increase in the fraction of time spent in the plateau or active phase of bursting. Although these changes may be due to the nonspecific effects of cooling, they would also, in fact, be expected if cooling inhibited K_{ATP} channel opening after the suppression of insulin secretion within the islet. We have quantitatively tested this hypothesis using a computer simulation in which the effect of cooling was modeled as an 8% reduction in maximal K_{ATP} conductance (R. Bertram and A. Sherman, unpublished observations) and found that indeed this resulted in qualitatively similar changes in bursting to those reported by Atwater et al. (46) and Debuyser et al. (47). Although this does not prove our hypothesis that secreted insulin activates K_{ATP} channels in mouse islets, these classic temperature experiments do not rule out the hypothesis.

In conclusion, our findings showed that insulin receptor activation by exogenous insulin can modulate metabolism in β-cells, suggesting that released insulin might thus be coupled to the cellular ATP/ADP ratio to calibrate the energy needs of the β-cell on a minute-to-minute basis. Further study will be required to determine if secreted insulin indeed plays a role in modulating mitochondrial, calcium, and/or electrical activities in islets. Alterations in these feedback processes may contribute to the dysfunctional insulin secretion, and especially abnormal insulin pulsatility, observed in patients with type 2 diabetes (48,49).

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