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Abstract
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Feedback Control of the ATP-Sensitive K⁺ Current by Cytosolic Ca²⁺ Contributes to Oscillations of the Membrane Potential in Pancreatic β-Cells

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During glucose stimulation, pancreatic β-cells display membrane potential oscillations that correspond to intermittent Ca²⁺ influx, leading to oscillations of the cytosolic free calcium concentration ([Ca²⁺]ₜ) and insulin secretion. The role of ATP-sensitive K⁺ (K⁺-ATP) channels in the control of these oscillations was investigated by measuring the K⁺-ATP current (IₚATP) with the perforated mode of the patch-clamp technique. No oscillations of IₚATP were observed when glucose-stimulated β-cells were kept hyperpolarized, thus with low and stable [Ca²⁺]ₜ. However, increasing [Ca²⁺]ₜ, by Ca²⁺ influx (depolarizing pulses) or Ca²⁺ mobilization (acetylcholine) transiently augmented IₚATP. This effect was abolished by tolbutamide, attenuated by increasing the glucose concentration in the medium, and prevented by abrogation of the [Ca²⁺]ₜ rise, which demonstrates that the current is really IₚATP and that its increase is Ca²⁺-dependent. Injection of a current of a similar amplitude to that of the Ca²⁺-induced increase in IₚATP was sufficient to repolarize glucose-stimulated β-cells. These results suggest that, in the absence of [Ca²⁺]ₜ oscillations, no metabolic oscillations affect IₚATP in pancreatic β-cells. In contrast, [Ca²⁺]ₜ oscillations evoke IₚATP oscillations. This mechanism may constitute the feedback loop controlling the glucose-induced oscillating electrical activity in β-cells. Diabetes 51: 376–384, 2002

Pancreatic β-cells are electrically excitable. Their membrane potential and electrical activity are finely controlled by glucose, the most important stimulus of insulin secretion. These effects have mainly been characterized in mouse islets (1–4). In the absence of glucose or in the presence of a nonstimulating concentration of glucose (≤6 mmol/l), the membrane potential is at the resting level. When the glucose concentration increases (≥7 mmol/l), the plasma membrane depolarizes and an oscillating electrical activity starts (1). Each oscillation of the membrane potential, usually referred to as a slow wave, consists of a depolarized phase on top of which a train of action potentials appears and a repolarized phase without action potentials. Glucose modulates the duration of the slow waves that become longer, with little change in their frequency, as the glucose concentration increases (between 7 and 25 mmol/l). When this concentration exceeds 25 mmol/l, slow waves are transformed into a sustained depolarization with continuous spike activity. The changes in membrane potential are crucial for the control of β-cell function because each depolarization induces a concomitant rise in the cytosolic free Ca²⁺ concentration ([Ca²⁺]ₜ) (5,6), which is the signal that triggers insulin secretion.

The resting membrane potential of β-cells is mainly controlled by an unknown depolarizing current and a hyperpolarizing current carried by ATP-sensitive K⁺ (K⁺-ATP) channels (7). When the glucose concentration is low, the ATP-to-ADP ratio is low, and many K⁺-ATP channels are open; therefore, K⁺-ATP current (IₚATP) overwhelms the depolarizing current and keeps the potential close to the equilibrium potential of K⁺. When the glucose concentration increases, cell metabolism is stimulated and the ATP-to-ADP ratio rises (8), leading to closure of K⁺-ATP channels (9,10). The resulting decrease in IₚATP permits the depolarizing current to move the membrane potential further away from the equilibrium potential of K⁺. When the threshold potential of activation of voltage-dependent Ca²⁺ channels is reached, Ca²⁺ influx starts (reflected by the appearance of electrical activity), [Ca²⁺]ₜ increases, and insulin secretion is stimulated. Whereas it is unanimously admitted that the rise in the ATP-to-ADP ratio triggers the initial depolarization, the mechanisms driving the oscillations of the membrane potential remain incompletely understood. The opening of voltage-dependent Ca²⁺ channels undoubtedly underlies the depolarizing phase, but the mechanism(s) causing the repolarization at the end of each slow wave has escaped identification. Several hypotheses have been put forward. They include activation of Ca²⁺-dependent K⁺ channels (11–14) different from the charbdotoxin-sensitive K⁺ channel (15); slow inactivation of voltage-dependent Ca²⁺ channels (3,16); decrease of cell-to-cell coupling (17) or of a store-operated current (18,19); and increase of IₚATP (20–22). According to this last hypothesis, cyclic closure and opening of K⁺-ATP channels cause oscillations of membrane potential that, in turn, repetitively open and close Ca²⁺ channels. Theoretically, such cycles could result from intrinsic Ca²⁺-independent metabolic oscillations.

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ACh, acetylcholine; [Ca²⁺]ₜ, cytosolic free Ca²⁺ concentration; IₚATP, K⁺-ATP current; IP₃, Ins(1,4,5)P₃, K⁺-ATP channel, ATP-sensitive K⁺ channel; PIP₂, phosphatidylinositol 4,5-bisphosphate.
(23,24) or be driven by Ca^{2+} in a sort of negative feedback control (20,21,25–27).

In the present study, we used the perforated mode of the patch-clamp technique to monitor I_{KATP} in single mouse β-cells. We investigated whether oscillations of I_{KATP} exist when [Ca^{2+}]_{e} is either kept low and stable (reflecting intrinsic metabolic oscillations) or is repetitively increased (reflecting Ca^{2+}-dependent activation of the channel). Some of the results have been presented in abstract form (28).

**RESEARCH DESIGN AND METHODS**

**Solutions and drugs.** The medium used for the preparation of islet cells was a bicarbonate-buffered solution that contained (in mmol/l): 120 NaCl, 4.8 KCl, 2.5 CaCl_{2}, 1.2 MgCl_{2}, 24 NaHCO_{3}, 5 HEPES, and 10 mmol/l glucose (pH adjusted to 7.40 with NaOH). The Ca^{2+}-free medium used to disrupt the islets into single cells had the following composition (in mmol/l): 138 NaCl, 5.6 KCl, 1.2 MgCl_{2}, 5 HEPES, and 1 mmol/l EGTA (pH adjusted to 7.1 with KOH). The electrical contact was established by adding a pore-forming antibiotic, amphotericin B or nystatin, to the pipette solution. Amphotericin (stock solution of 60 mg/ml in DMSO) was used at a final concentration of 300 μg/ml. Nystatin (stock solution of 10 mg/ml in DMSO) was used at a final concentration of 200 μg/ml. The tip of the pipette was filled with antibiotic-free solution, and the pipette was then back-filled with the amphotericin- or nystatin-containing solution. The voltage clamp was considered satisfactory when the series conductance was >50–40 nS.

**Preparation of cells.** The pancreases were taken from Naval Medical Research Institute mice killed by cervical dislocation. Pancreatic islets were isolated enzymatically by collagenase digestion followed by hand selection. To obtain single cells, the islets were first incubated for 5 min in a Ca^{2+}-free medium. After a brief centrifugation, this solution was replaced by culture medium, and the islets were disrupted by gentle pipetting through a sili-conized glass pipette. The cells were plated on 22 mm–diameter glass coverslips and maintained for 1–4 days in RPMI 1640 tissue culture medium containing 10 mmol/l glucose, 10% heat-inactivated FCS, 100 IU/ml penicillin, and 100 μg/ml streptomycin.

**Electrophysiological recordings.** Two criteria were used to identify β-cells. The capacitance of mouse α-, δ-, and β-cells averages 4.4, 5, and 7.4 pF, respectively (29). Therefore, only large cells with a capacitance >5 pF were chosen for the present study. For 150 randomly chosen cells, the average capacitance was 7.6 ± 0.2 pF. After verification of the capacitance, a depolarizing protocol was applied to identify the properties of the voltage-dependent Na\(^+\) current, which is known to be inactivated at resting potential in β-cells but not in α- and δ-cells (30). Thus, cells in which a Na\(^+\) current could be activated by a small depolarizing pulse from a prolonged holding potential of -70 mV were discarded. By contrast, cells that displayed a Na\(^+\) current only after a hyperpolarizing pulse to -140 mV were considered to be β-cells (30,31) and were used for the experiments.

**Patch-clamp measurements.** Currents were recorded using the perforated whole-cell mode of the patch-clamp technique at 33–35°C, using an EPC-9 patch-clamp amplifier (Heka Electronics, Lambrrecht/Pfalz, Germany) and the software Pulsefit, or an Axopatch 200 B patch-clamp amplifier (Axon Instruments, Foster City, CA) and the software pClamp 8. Patch pipettes were pulled from borosilicate glass capillaries (World Precision Instruments, Hertfordshire, U.K.) to give a resistance of 4–5 MΩ. I_{KATP} was monitored by 100 ms–duration pulses of ±20 mV from a holding potential of -70 mV (Figs. 1–4) or pulses of ±20 mV from a holding potential of -60 mV (Figs. 5 and 6). In the latter protocol, -60 mV was chosen because it corresponds best to the interburst potential in spontaneously oscillating cells within an islet, whereas the depolarizing pulses were omitted to avoid activation of voltage-dependent Ca^{2+} channels. To prevent the capacitive transient due to electrical charge of the pipette, which might complicate I_{KATP} measurements, each change in voltage was preceded by 100-ms ramps (except for in Fig. 1D). Two protocols of depolarization were used: either a single 30-s pulse to 0 mV (Figs. 2 and 3) or a train of depolarizations mimicking the action potentials of the patch-clamp technique. A–C: The amplitude of I_{KATP} is reflected by the size of the vertical bars around the continuous thick line representing the holding current at -70 mV. The glucose concentration (G) was either 10 mmol/l throughout (A) or was alternated between 8 and 12 mmol/l (B). C: The average amplitude of I_{KATP} in the presence of G8 and G12 was measured during the last 12 test pulses at each glucose concentration in the experiments illustrated in B. *P < 0.05 vs. G8 by unpaired t test. D: Azide was added when indicated. Traces A and D are representative of results obtained in five cells. Trace B is the mean of results obtained in four cells.

**[Ca^{2+}]_{i} measurements.** Islet cells were loaded with 1 μmol/l fura-2/AM (Molecular Probes, Eugene, OR) for 45 min at 37°C in a bicarbonate-buffered solution containing 10 mmol/l glucose. The glass coverslips onto which the loaded cells were attached constituted the bottom of a temperature-controlled perfusion chamber (Intracell, Royston, Herts, U.K.) mounted on the stage of an inverted microscope. The Ca^{2+} probe within the cells was excited at 340 and 380 nm, and the fluorescence emitted at 510 nm was captured at 20 Hz by a photometric-based system (PTL Lawrenceville, N.J.) [Ca^{2+}], was calculated by comparing the ratio of the 510-nm signals successively acquired at 340 and 380 nm with a calibration curve based on the equation of Grynkiewicz et al. (32) and established by filling the chamber with an intracellular-type solution containing 10 μmol/l fura-2-acid free, and either 10 mmol/l free Ca^{2+} or <1 mmol/l free Ca^{2+}. A K_{i} for the fura-2-Ca^{2+} complex of 224 nmol/l was used.
cultures but different from those of series C. The cell total I_{KATP} estimated by the combined application of diazoxide and azide to open K^+ -ATP channels was already closed at 10 mmol/l glucose, as previously reported (34).

During constant stimulation by 10 mmol/l glucose, no oscillations of I_{KATP} could be detected over a period of ~8 min (Fig. 1A), which is approximately twice as long as the period of the spontaneous oscillations of [Ca^{2+}]_c induced by the sugar in single \( \beta \)-cells (33). In contrast, I_{KATP} fluctuations were detected when cell metabolism was changed by alternating the glucose concentration of the perfusion medium between 12 and 8 mmol/l (Fig. 1B). Average I_{KATP} was two times larger in the presence of 8 mmol/l glucose than in the presence of 12 mmol/l glucose (Fig. 1C). Moreover, decreasing the ATP-to-ADP ratio with a low concentration of azide (36), a mitochondrial poison, reversibly increased I_{KATP} (Fig. 1D). Therefore, the absence of apparent oscillations of I_{KATP} at stable glucose and [Ca^{2+}]_c suggests that no intrinsic metabolic oscillations, independent from changes in [Ca^{2+}]_c, exist in \( \beta \)-cells.

**Influence of a depolarization-induced [Ca^{2+}]_c rise on I_{KATP}.** The alternative hypothesis, suggesting that metabolic oscillations in \( \beta \)-cells are driven by [Ca^{2+}]_c oscillations, was tested by measuring the effect of an imposed increase in [Ca^{2+}]_c on I_{KATP}. In this series, [Ca^{2+}]_c and I_{KATP} were measured simultaneously in the same single \( \beta \)-cells perfused with 10 mmol/l glucose and submitted to a 30-s depolarizing pulse to 0 mV from a holding potential of ~70 mV (Fig. 2A and C). In \( \beta \)-cells held hyperpolarized at ~70 mV, [Ca^{2+}]_c was low and stable, and I_{KATP} was small. Depolarizing the cells to 0 mV rapidly increased [Ca^{2+}]_c, which slowly returned to basal levels upon repolarization to ~70 mV. I_{KATP} was 276 ± 70% larger just after compared with before the depolarizing pulse. This increase was transient, with I_{KATP} decreasing with time to similar values as those before the depolarizing pulse. To ascertain that the increased current observed after the depolarizing pulse corresponds well to I_{KATP}, the same experiment was repeated in the presence of 250 μmol/l tolbutamide, a potent blocker of K^+ -ATP channels (Fig. 2B and D). As expected, tolbutamide reduced I_{KATP} in the presence of 10 mmol/l glucose (compare the beginning of Fig. 2C and D). This inhibition amounted to 63% (0.60 ± 0.01 pA/pF, n = 5, vs. 1.62 ± 0.03 pA/pF, n = 7, in the presence and absence of tolbutamide, respectively; P <
In contrast, tolbutamide did not affect the rise in 
\([\text{Ca}^{2+}]_c\) produced by the depolarizing pulse to 0 mV. However, the increase in current observed after the depolarizing pulse was abolished (compare Fig. 2C and D).

If the current activated by the depolarizing pulse is \(I_{\text{KATP}}\), one could anticipate that it will be decreased by high glucose. This finding was tested by applying a 30-s depolarizing pulse to cells perfused with 3 or 25 mmol/l glucose (Fig. 2E and F). As expected, \(I_{\text{KATP}}\) measured before the depolarization to 0 mV was reduced by 45% in the presence of the high concentration of glucose (1.86 ± 0.02 pA/pF in G3, \(n = 6\), vs. 1.03 ± 0.02 pA/pF in G25, \(n = 7\), respectively; \(P < 0.001\)). Importantly, the increase in current observed after the 30-s depolarization to 0 mV was threefold smaller in 25 mmol/l glucose than in 3 mmol/l glucose (1.70 ± 0.26 pA/pF in G25, \(n = 7\), vs. 5.10 ± 0.94 pA/pF, \(n = 6\), in G3; \(P < 0.01\)), although the rise in \([\text{Ca}^{2+}]_c\) was similar at both glucose concentrations. The time for \(I_{\text{KATP}}\) to return to basal levels was also much reduced in the presence of 25 mmol/l glucose. Altogether, these experiments demonstrate that the increased current observed after the pulse in control cells does correspond to \(I_{\text{KATP}}\) and that the negative feedback effect of \([\text{Ca}^{2+}]_c\) on \(I_{\text{KATP}}\) can be modulated by glucose.

To ascertain that the increase in \(I_{\text{KATP}}\) is the consequence of the rise in \([\text{Ca}^{2+}]_c\), produced by the depolarizing pulse, the same protocol was repeated under conditions where \([\text{Ca}^{2+}]_c\) influx was prevented. In the absence of external \([\text{Ca}^{2+}]_c\), \([\text{Ca}^{2+}]_c\) did not increase upon depolarization, and \(I_{\text{KATP}}\) was of similar amplitude before and after the pulse (Fig. 3A). In the presence of 2.5 mmol/l \([\text{Ca}^{2+}]_c\) and 10 \(\mu\text{mol/l}\) nimodipine, an L-type \([\text{Ca}^{2+}]_c\) channel blocker, the depolarizing pulse to 0 mV, increased \([\text{Ca}^{2+}]_c\), only marginally (Fig. 3B). This small elevation may be attributed to the activity of the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger working in reverse mode at 0 mV or to an incomplete blockade of L-type \([\text{Ca}^{2+}]_c\) channels. However, it was too small to affect \(I_{\text{KATP}}\) (Fig. 3B).

If a rise in \([\text{Ca}^{2+}]_c\) is really the cause of the increase in \(I_{\text{KATP}}\), mobilization of intracellular \([\text{Ca}^{2+}]_c\) should produce a similar effect as that of \([\text{Ca}^{2+}]_c\) influx. Application of 100 \(\mu\text{mol/l}\) acetylcholine (ACh), a potent Ins(1,4,5)P₃ (IP₃)-producing agent, to hyperpolarized β-cells reversibly augmented \(I_{\text{KATP}}\) (Fig. 4A and B). To ascertain whether this effect resulted from a rise in \([\text{Ca}^{2+}]_c\), the same protocol was repeated after treatment of the cell with thapsigargin, a potent and specific inhibitor of the sarco-endoplasmic reticulum \([\text{Ca}^{2+}]_c\)-ATPase. Thapsigargin depletes the endoplasmic reticulum \([\text{Ca}^{2+}]_c\) in β-cells (37) without impairing the production of IP₃ in response to phospholipase C–linked agonists. Addition of ACh to thapsigargin-pre-treated cells did not affect \(I_{\text{KATP}}\) (Fig. 4C). Altogether, these experiments demonstrate that the rise in \([\text{Ca}^{2+}]_c\) is the mechanism that increases \(I_{\text{KATP}}\).

**Effect of imposed \([\text{Ca}^{2+}]_c\) oscillations on \(I_{\text{KATP}}\).** Because 30-s depolarizations to 0 mV might be stronger than spontaneous depolarizations, single cells were depolarized by a voltage clamp protocol mimicking the spontaneous electrical activity in islets. Cycles of 6 s depolarization and 18 s hyperpolarization were chosen to reproduce the durations of the depolarization and repolarization phases elicited by 10 mmol/l glucose (38). During depolarization, the cell was submitted to short depolarizing pulses resembling the burst of action potential of the slow waves (see research design and methods). The left part of Fig. 5 shows spontaneous oscillations of \([\text{Ca}^{2+}]_c\) induced by 10 mmol/l glucose in a single β-cell. The right part shows \([\text{Ca}^{2+}]_c\) oscillations imposed by the voltage clamp protocol in the same cell, ~5 min after establishment of the seal. The imposed \([\text{Ca}^{2+}]_c\) oscillations were similar to those occurring spontaneously in that cell. The average peak of \([\text{Ca}^{2+}]_c\) oscillations in several cells was not different during spontaneous oscillations (1.053 ± 91 nmol/l, \(n = 23\)) or during oscillations imposed by the pulse protocol (802 ± 132 nmol/l, \(n = 12\)) or 30-s depolarizations to 0 mV (823 ± 103 nmol/l, \(n = 7\)). Imposed \([\text{Ca}^{2+}]_c\) oscillations are thus within the physiological range.

The same pulse protocol as that used in Fig. 5 was then applied to measure the influence of \([\text{Ca}^{2+}]_c\) oscillations on \(I_{\text{KATP}}\) (Fig. 6). The cells were initially perfused with 6 mmol/l glucose, a subthreshold concentration at which the islets are electrically silent (1). Increasing glucose from 6 to 10 mmol/l decreased \(I_{\text{KATP}}\), from 1.57 to 0.89 pA/pF (\(n = 8\)). This difference in current is probably larger than that occurring in a cell that would not be voltage-clamped and in which the decrease in \(I_{\text{KATP}}\) produced by the acceleration of ATP production in response to the elevation of the glucose concentration is normally counterbalanced by the
increase in $I_{KATP}$ due to the concomitant rise in $[Ca^{2+}]_c$. In cells voltage-clamped between $-60$ and $-80$ mV (Fig. 6), $I_{KATP}$ is only influenced by the change in glucose metabolism but not by the rise in $[Ca^{2+}]_c$ that is prevented by the hyperpolarization. Application of trains of depolarization repetitively increased $I_{KATP}$ (Fig. 6). The average increase was such that the current after each train was similar ($1.68 \pm 0.09$ pA/pF) to that measured in the presence of 6 mmol/l glucose. This increase in $I_{KATP}$ might thus be sufficient to repolarize the membrane below the threshold potential for activation of voltage-dependent $Ca^{2+}$ channels. The changes in current induced by the rise of the glucose concentration and by the pulse protocol were all prevented by 250 $\mu$mol/l tolbutamide, demonstrating that they really correspond to variations in $I_{KATP}$ ($n = 5$, not shown).

**Effect of injection of a hyperpolarizing current equivalent to the $Ca^{2+}$-induced increase in $I_{KATP}$ on the $\beta$-cell membrane potential.** We next verified whether the $Ca^{2+}$-induced increase in $I_{KATP}$ is sufficient to repolarize the plasma membrane to the resting potential. This increase ($\Delta I_{KATP}$) was calculated by averaging the difference in $I_{KATP}$ after and before the last four trains of depolarizing pulses ($\Delta I_{KATP,4}$ in Fig. 6). It amounted to $0.59 \pm 0.06$ pA/pF. A current of similar amplitude, adjusted for cell size (0.59 multiplied by the capacitance of the tested cell), was then injected into $\beta$-cells studied in the current-clamp mode and stimulated by 10 mmol/l glucose. Figure 7A shows the electrical activity induced by glucose in one of these cells. Injection of a hyperpolarizing current corresponding to the average $\Delta I_{KATP}$ ($-5$ pA in this cell) suppressed the electrical activity and repolarized the plasma membrane to the resting level. Removal of this current was accompanied by the immediate resumption of action potentials. In other experiments (Fig. 7B), the hyperpolarizing current was increased stepwise by increments corresponding to one-sixth of the average $\Delta I_{KATP}$. As shown in Fig. 7B, 50% of average $\Delta I_{KATP}$ was sufficient to repolarize the cell below the threshold for activation of voltage-dependent $Ca^{2+}$ channels. This result strongly suggests that the $Ca^{2+}$-induced rise in $I_{KATP}$ might control the oscillations of the membrane potential.

**DISCUSSION**

Oscillations of the membrane potential are one of the major characteristics of the pancreatic $\beta$-cell response to glucose. They underlie the periodic influx of $Ca^{2+}$ that
triggers oscillations of insulin secretion. Understanding their fine control is thus of utmost importance. The present study demonstrates that [Ca\(^{2+}\)]\(_c\) oscillations in pancreatic \(\beta\)-cells rhythmically increase \(I_{\text{KATP}}\) and provide direct support to the proposal (20) that such an effect constitutes a feedback control of the oscillations of membrane potential.

**Intrinsic metabolic oscillations do not drive membrane potential oscillations.** It has been suggested that intrinsic Ca\(^{2+}\)-independent metabolic oscillations exist in \(\beta\)-cells (24) and that they lead to cycles of K\(^+-\)ATP channel activity (23). To verify this hypothesis, single metabolically intact \(\beta\)-cells were hyperpolarized to keep [Ca\(^{2+}\)]\(_c\) at basal and stable levels, and \(I_{\text{KATP}}\) was continuously monitored during perfusion with a stimulatory glucose concentration. In no cell did we find \(I_{\text{KATP}}\) oscillations under these conditions. This suggests that either no intrinsic metabolic oscillations exist, or they are unable to modulate K\(^+-\)ATP channel activity and membrane potential because of their nature or small amplitude (smaller than those imposed by 4 mmol/l glucose changes). Experiments monitoring O\(_2\) consumption (39), glucose consumption (27), and the fluorescence of reduced pyridine nucleotides [NAD(P)H] in single islets (6) have also concluded to the absence of Ca\(^{2+}\)-independent metabolic oscillations in \(\beta\)-cells.

**I\(_{\text{KATP}}\) oscillations driven by [Ca\(^{2+}\)]\(_c\) oscillations.** When [Ca\(^{2+}\)]\(_c\) was increased by stimulating Ca\(^{2+}\) influx or mobilizing Ca\(^{2+}\) from intracellular Ca\(^{2+}\) stores, \(I_{\text{KATP}}\) increased. There is no doubt that this increase resulted from the [Ca\(^{2+}\)]\(_c\) rise because \(I_{\text{KATP}}\) did not change when Ca\(^{2+}\) influx was prevented by omission of external Ca\(^{2+}\) and blockade of voltage-dependent Ca\(^{2+}\) channels or when Ca\(^{2+}\) mobilization was prevented by pretreatment with thapsigargin. It is also clear that the current increased by the rise in [Ca\(^{2+}\)]\(_c\) is \(I_{\text{KATP}}\) because it was attenuated by a rise in ambient glucose concentration and completely inhibited by tobutamide, a blocker of K\(^+-\)ATP channels. K\(^+\) channels sensitive to sulfonylureas but distinct from K\(^+-\)ATP channels have been described in some systems (40,41), but not in \(\beta\)-cells. It is likely that the current that
study. Others did not find any direct effect of Ca\(^{2+}\) on K\(^{+}\)-ATP channels in \(\beta\)-cells (9). It is worth noting that the K\(^{+}\)-ATP channels of \(\beta\)-cells and muscle cells have different subunit compositions (SUR1 and Kir6.2 for \(\beta\)-cells and SUR2 and Kir6.2 for muscle cells) (47), which might confer different sensitivities to Ca\(^{2+}\).

Several Ca\(^{2+}\)-dependent processes influencing K\(^{+}\)-ATP channels have been described in pancreatic \(\beta\)-cells or muscle cells. They involve cytoskeletal proteins (44), the Ca\(^{2+}\)-dependent protein phosphatase type 2B (48), or other proteins (49). Activation of phospholipase C by Ca\(^{2+}\), with subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)), is unlikely to be involved for two reasons. First, acceleration of PIP\(_2\) breakdown would be expected to decrease I\(_{KATP}\) (44), which is opposite to the effect of a rise in [Ca\(^{2+}\)]\(_c\) observed in the present study. Second, ACh, a potent Ca\(^{2+}\)-independent activator of phospholipase C, was without effect on \(\beta\)-cell I\(_{KATP}\) when Ca\(^{2+}\) mobilization was prevented by thapsigargin pretreatment.

Metabolic oscillations might be driven by [Ca\(^{2+}\)]\(_c\) oscillations. Indeed, each rise in [Ca\(^{2+}\)]\(_c\) could stimulate ATP production (50) and increase the ATP-to-ADP ratio by activating mitochondrial dehydrogenases (51,52). Oscillations of oxygen consumption driven by [Ca\(^{2+}\)]\(_c\) oscillations have recently been reported in islets (27). Our data do not exclude this possibility. Alternatively, each rise in [Ca\(^{2+}\)]\(_c\) could decrease the ATP-to-ADP ratio. This hypothesis is supported by direct measurements of adenine nucleotide levels within mouse islets (25) or of ATP concentration in INS-1 cells expressing luciferase (53). These studies demonstrated that, at a fixed glucose concentration, the ATP-to-ADP ratio and the ATP concentration decreased when [Ca\(^{2+}\)]\(_c\) was raised by high K\(^{+}\). By demonstrating that a rise in [Ca\(^{2+}\)]\(_c\) increases I\(_{KATP}\), the present study supports this proposal. The drop in the ATP-to-ADP ratio could either result from inhibition of ATP production (26,54) or stimulation of ATP consumption (25,53).

**Physiological implications for the control of membrane potential oscillation.** In glucose-stimulated \(\beta\)-cells, I\(_{KATP}\) was found to be larger during the interburst intervals than during the depolarizing phases (22). These fluctuations were tentatively ascribed to metabolic oscillations, but no mechanistic explanation was provided. The present study strongly suggests that the rise in [Ca\(^{2+}\)]\(_c\) might be the feedback mechanism controlling I\(_{KATP}\) and hence the oscillations of the membrane potential. Thus, during a voltage clamp protocol mimicking the repetitive changes in electrical activity induced by 10 mmol/l glucose in islets, each imposed [Ca\(^{2+}\)]\(_c\) oscillation evoked a transient increase in I\(_{KATP}\). This increase had a similar amplitude to that of the difference in I\(_{KATP}\) measured at substimulating (6 mmol/l) and stimulating (10 mmol/l) glucose concentrations. Theoretically, this current should be able to repolarize the membrane to a potential more negative than that of the activation threshold of voltage-dependent Ca\(^{2+}\) channels. This finding was amply supported by current-clamp experiments. Injection of current corresponding to 50% of the Ca\(^{2+}\)-induced I\(_{KATP}\) increase was sufficient to repolarize the \(\beta\)-cell membrane in the presence of 10 mmol/l glucose. Because the voltage-
dependent Ca$^{2+}$ current is larger in β-cells within intact islets than in isolated single cells (29), it is possible that the amplitude of the Ca$^{2+}$-induced increase in $I_{\text{KATP}}$ in whole islets exceeds our estimate. We have no explanation why, in a previous report, no oscillations of $I_{\text{KATP}}$ were detected in single β-cells displaying membrane potential oscillations (55). The reported experimental procedures were indeed similar to those used in the present study.

The negative feedback effect of $[\text{Ca}^{2+}]_c$ on $I_{\text{KATP}}$ might be important for the control of oscillations of the β-cell membrane potential according to the following model. Acceleration of glucose metabolism in β-cells increases the ATP-to-ADP ratio, which closes K$^+$/ATP channels. This leads to depolarization of the plasma membrane and opening of voltage-dependent Ca$^{2+}$ channels. Ca$^{2+}$ influx then raises $[\text{Ca}^{2+}]_c$, which decreases the ATP-to-ADP ratio (25) and leads to reopening of K$^+$/ATP channels, partial repolarization of the plasma membrane, arrest of Ca$^{2+}$ influx, and a drop in $[\text{Ca}^{2+}]_c$. The eventual restoration of a high ATP-to-ADP ratio then initiates a new cycle. Our observation that the negative feedback effect of $[\text{Ca}^{2+}]_c$ on $I_{\text{KATP}}$ is largely attenuated by glucose can explain the lengthening of the depolarized phases and shortening of the repolarized intervals occurring when the glucose concentration is raised within the stimulatory range. Indeed, as the glucose concentration increases, the ATP-to-ADP ratio rises and closes more K$^+$/ATP channels. The depolarizing phase progressively becomes longer because a stronger feedback effect of Ca$^{2+}$ on $I_{\text{KATP}}$ is required to counteract the effects of increased metabolism on $I_{\text{KATP}}$. Continuous electrical activity occurs at glucose concentrations that reduce $I_{\text{KATP}}$ to such an extent that it is no longer counteracted by the $[\text{Ca}^{2+}]_c$ rise unless the latter is increased by augmenting the extracellular Ca$^{2+}$ concentration (20).

The central role of K$^+$/ATP channels in membrane potential oscillations suggested in our model are compatible with those in most studies on K$^+$/ATP-deficient mice. Thus, pancreatic β-cells from SUR1 or Kir6.2 knockout mice display a continuous spike activity (56,57) and a sustained and stable elevation of $[\text{Ca}^{2+}]_c$ (56,58) at both nonstimulating and stimulating glucose concentrations. Only one abstract reported $[\text{Ca}^{2+}]_c$ oscillations in β-cells from SUR1 knockout mice, but it is not known whether these oscillations resulted from concomitant changes in membrane potential (59). In view of the important role played by K$^+$/ATP channels in the control of pancreatic β-cell membrane potential, further studies should now elucidate the interplay between $[\text{Ca}^{2+}]_c$ and ATP turnover.

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