"Feedback control of the ATP-sensitive K(+) current by cytosolic Ca(2+) contributes to oscillations of the membrane potential in pancreatic beta-cells"

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ABSTRACT

During glucose stimulation, pancreatic beta-cells display membrane potential oscillations that correspond to intermittent Ca(2+) influx, leading to oscillations of the cytosolic free calcium concentration ([Ca(2+)](c)) 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 (I(KATP)) with the perforated mode of the patch-clamp technique. No oscillations of I(KATP) were observed when glucose-stimulated beta-cells were kept hyperpolarized, thus with low and stable [Ca(2+)](c). However, increasing [Ca(2+)](c) by Ca(2+) influx (depolarizing pulses) or Ca(2+) mobilization (acetylcholine) transiently augmented I(KATP). This effect was abolished by tolbutamide, attenuated by increasing the glucose concentration in the medium, and prevented by abrogation of the [Ca(2+)](c) rise, which demonstrates that the current is really I(KATP) and that its increase is Ca(2+)-dependent. Injection of a current of a similar amplitude to that of the Ca(2+)-induced increase in I(KATP) was sufficient to repolarize glucose-stimulated beta-cells. These results suggest that, in the absence of [Ca(2+)](c) oscillations, no metabolic oscillations affect I(KATP) in pancreatic beta-cells. In contrast, [Ca(2+)](c) oscillations evoke I(KATP) oscillations. This mechanism may constitute the feedback loop controlling the glucose-induced oscillating electrical activity in beta-cells.

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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

Jean-François Rolland, Jean-Claude Henquin, and Patrick Gilon

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⁺]c) 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 (I_KATP) with the perforated mode of the patch-clamp technique. No oscillations of I_KATP were observed when glucose-stimulated β-cells were kept hyperpolarized, thus with low and stable [Ca²⁺]c. However, increasing [Ca²⁺], by Ca²⁺ influx (depolarizing pulses) or Ca²⁺ mobilization (acetylcholine) transiently augmented I_KATP. This effect was abolished by tolbutamide, attenuated by increasing the glucose concentration in the medium, and prevented by abrogation of the [Ca²⁺]c rise, which demonstrates that the current is really I_KATP and that its increase is Ca²⁺-dependent. Injection of a current of a similar amplitude to that of the Ca²⁺-induced increase in I_KATP was sufficient to repolarize glucose-stimulated β-cells. These results suggest that, in the absence of [Ca²⁺]c oscillations, no metabolic oscillations affect I_KATP in pancreatic β-cells. In contrast, [Ca²⁺], oscillations evoke I_KATP 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⁺]c) (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 (I_KATP) 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 I_KATP 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⁺]c 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 mechanisms(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 charybdotoxin-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 I_KATP (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.
(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_{K_{ATP}} in single mouse β-cells. We investigated whether oscillations of I_{K_{ATP}} exist when \([Ca^{2+}]_{\text{c}}\) 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 7.0 with NaOH). All solutions used for tissue preparation were gassed with O_2:CO_2 (94:6%). All solutions used for electrophysiological recordings were gassed with O_2:CO_2 (94:6%).

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FIG. 1. Lack of oscillations of I_{K_{ATP}} at stable \([Ca^{2+}]_{\text{c}}\), and glucose concentrations in single mouse β-cells. I_{K_{ATP}} was monitored by pulses of ±20 mV from a holding potential of −70 mV using the perforated mode of the patch-clamp technique. A-C: The amplitude of I_{K_{ATP}} 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_{K_{ATP}} 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.
cultures but different from those of series
the cell total I_{KATP} estimated by the combined application
izing step from
tolbutamide (Tolb) (25)
25
[Ca^{2+}]_{c} concentration (10 mmol/l) that produces spontaneous
The cells were continuously perifused with a glucose
abolically intact
mally (34). This result suggests that no intrinsic metabolic oscilla-
tions, independent from changes in [Ca^{2+}]_{c}, exist in
β-cells.
Influence of a depolarization-induced [Ca^{2+}]_{c} rise on
I_{KATP}. The alternative hypothesis, suggesting that meta-
bolic oscillations in β-cells are driven by [Ca^{2+}]_{c}, oscilla-
tions, 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 β-cells perifused 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 β-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 re-
polation to −70 mV. I_{KATP} was 276 ± 70% larger just after
compared with before the depolarizing pulse. This in-
crease 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 <

FIG. 2. Effects of a 30-s depolarization on [Ca^{2+}]_{c} and I_{KATP} measured simultaneously in single mouse β-cells. Single β-cells loaded with
fura-2 were perifused with a medium containing 3 (E), 10 (A and C), or
25 (F) mmol/l glucose (G) alone or 10 mmol/l glucose + 250 μmol/l
tolbutamide (Tolb) (B and D). They were submitted to a 30-s depolar-
izing step from −70 to 0 mV (ΔV_{m}) during the period shown by the thick
horizontal bar. I_{KATP} could not be monitored during the depolarization.
A and B show representative traces, C-F show mean traces ± SE. Series E (n = 6) and F (n = 7) were performed with cells from the same
cultures but different from those of series C (n = 7) and D (n = 5).

Presentation of results. The experiments are illustrated by traces that are
means or representative traces of results obtained with the indicated number
of cells from at least three different cultures. The statistical significance of
differences between means was assessed by paired or unpaired Student's t test
as appropriate. Differences were considered significant at P < 0.05.

RESULTS
Measurements of I_{KATP} at stable and low [Ca^{2+}]_{c}. To
search for the existence of intrinsic Ca^{2+}-independent metabolic oscillations, I_{KATP} was measured in single metab-
olically intact β-cells hyperpolarized at −70 mV (Fig. 1).
The cells were continuously perifused with a glucose
concentration (10 mmol/l) that produces spontaneous
[Ca^{2+}]_{c} oscillations in unclamped β-cells (33). In the
present experiments, [Ca^{2+}]_{c} was low because of the
hyperpolarization and not affected by the 20-mV hyperpo-
larizing and depolarizing pulses used to monitor I_{KATP} (see
the beginning of the recording in Fig. 2A). I_{KATP} was small
(1.6 ± 0.3 pA/pF, n = 10), corresponding to 3.4 ± 0.6% of
the cell total I_{KATP} estimated by the combined application
diazoxide and azide to open K^{+}-ATP channels maxi-
mally (34). This result suggests that >95% of K^{+}-ATP
channels were already closed at 10 mmol/l glucose, as
previously reported (35).

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 β-cells (33). In contrast, I_{KATP}
fluctuations were detected when cell metabolism was
changed by alternating the glucose concentration of the
perifusion 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 oscilla-
tions, independent from changes in [Ca^{2+}]_{c}, exist in
β-cells.

FIG. 3. Effects of a 30-s depolarization on [Ca^{2+}]_{c} and I_{KATP} measured simultaneously in single mouse β-cells when Ca^{2+} influx was pre-
vented. Single β-cells loaded with Fura-2 were perifused with a Ca^{2+}-
free medium (A) or a Ca^{2+}-containing medium supplemented with
10 μmol/l nifedipine (Nimo) (B). They were submitted to a 30-s depolar-
ization to 0 mV (ΔV_{m}), as in Fig. 2. The glucose concentration of the
medium was 10 mmol/l throughout. The traces are means ± SE of
results obtained in three (A) and four (B) cells.

Average I_{KATP} was 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 oscilla-
tions, independent from changes in [Ca^{2+}]_{c}, exist in
β-cells.
pulse, the same protocol was repeated under conditions where \( \text{Ca}^{2+} \), influx was prevented. In the absence of external \( \text{Ca}^{2+} \), \( [\text{Ca}^{2+}]_c \), 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 \( \text{Ca}^{2+} \) and 10 \( \mu \text{mol/l} \) nimodipine, an L-type \( \text{Ca}^{2+} \) 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 \( \text{Ca}^{2+} \) 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 \( \text{Ca}^{2+} \) should produce a similar effect as that of \( \text{Ca}^{2+} \) influx. Application of 100 \( \mu \text{mol/l} \) acetylcholine (ACh), a potent \( \text{Ins}(1,4,5)\text{P}_3 \) (IP3)-producing agent, to hyperpolarized \( \beta \)-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 \( \text{Ca}^{2+}\text{-ATPase} \). Thapsigargin depletes the endoplasmic reticulum \( \text{Ca}^{2+} \) in \( \beta \)-cells (37) without impairing the production of \( \text{IP}_3 \) 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 \( \beta \)-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 mmol/l, \( n = 23 \)) or during oscillations imposed by the pulse protocol (802 ± 132 mmol/l, \( n = 12 \)) or 30-s depolarizations to 0 mV (823 ± 103 mmol/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 $I_{\text{KATP}}$ due to the concomitant rise in $[\text{Ca}^{2+}]_c$. In cells voltage-clamped between −60 and −80 mV (Fig. 6), $I_{\text{KATP}}$ is only influenced by the change in glucose metabolism but not by the rise in $[\text{Ca}^{2+}]_c$ that is prevented by the hyperpolarization. Application of trains of depolarization repetitively increased $I_{\text{KATP}}$ (Fig. 6). The average increase was such that the current after each train was similar (1.68 ± 0.09 pA/pF) to that measured in the presence of 6 mmol/l glucose. This increase in $I_{\text{KATP}}$ might thus be sufficient to repolarize the membrane below the threshold potential for activation of voltage-dependent $\text{Ca}^{2+}$ channels. The changes in current induced by the rise of the glucose concentration and by the pulse protocol were all prevented by 250 μmol/l tolbutamide, demonstrating that they really correspond to variations in $I_{\text{KATP}}$ ($n = 5$, not shown).

**Effect of injection of a hyperpolarizing current equivalent to the $\text{Ca}^{2+}$-induced increase in $I_{\text{KATP}}$ on the β-cell membrane potential.** We next verified whether the $\text{Ca}^{2+}$-induced increase in $I_{\text{KATP}}$ is sufficient to repolarize the plasma membrane to the resting potential. This increase ($\Delta I_{\text{KATP}}$) was calculated by averaging the difference in $I_{\text{KATP}}$ after and before the last four trains of depolarizing pulses ($\Delta I_{\text{KATP}1-4}$ in Fig. 6). It amounted to 0.59 ± 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 β-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 $\Delta I_{\text{KATP}}$ (−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 $\Delta I_{\text{KATP}}$. As shown in Fig. 7B, 50% of average $\Delta I_{\text{KATP}}$ was sufficient to repolarize the cell below the threshold for activation of voltage-dependent $\text{Ca}^{2+}$ channels. This result strongly suggests that the $\text{Ca}^{2+}$-induced rise in $I_{\text{KATP}}$ might control the oscillations of the membrane potential.

**DISCUSSION**

Oscillations of the membrane potential are one of the major characteristics of the pancreatic β-cell response to glucose. They underlie the periodic influx of $\text{Ca}^{2+}$ that
triggers oscillations of insulin secretion. Understanding their fine control is thus of utmost importance. The present study demonstrates that \([\text{Ca}^{2+}]\) 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 \(\text{Ca}^{2+}\)-independent metabolic oscillations exist in \(\beta\)-cells (24) and that they lead to cycles of \(\text{K}^{+}\)-ATP channel activity (23). To verify this hypothesis, single metabolically intact \(\beta\)-cells were hyperpolarized to keep \([\text{Ca}^{2+}]\) 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 \(\text{K}^{+}\)-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 \(\text{O}_2\) 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 \(\text{Ca}^{2+}\)-independent metabolic oscillations in \(\beta\)-cells.

**FIG. 6. Increase of \(I_{\text{KATP}}\) by imposed \([\text{Ca}^{2+}]\) oscillations mimicking spontaneous \([\text{Ca}^{2+}]\) oscillations induced by glucose.** Single \(\beta\)-cells were initially perfused with a medium containing 6 mmol/l glucose (G6). After 1 min of recording \(I_{\text{KATP}}\), the glucose concentration was increased to 10 mmol/l (G10). Two minutes later, the cell was submitted to the same pulse protocol as that used in Fig. 5 and designed to mimic spontaneous \([\text{Ca}^{2+}]\) oscillations induced by 10 mmol/l glucose. The mean difference in \(I_{\text{KATP}}\) before and after the depolarizing pulses (average \(\Delta I_{\text{KATP}}\)) was calculated by averaging the increase in \(I_{\text{KATP}}\) occurring after each of the last four trains of depolarizations (\(\Delta I_{\text{KATP1-4}}\)). This trace is the mean of results obtained in eight single cells.
we studied here is similar to the voltage-independent Ca\textsuperscript{2+}-activated K\textsuperscript{+} current previously described in \(\beta\)-cells (14). This current, which was originally thought to not involve K\textsuperscript{+}-ATP channels (14), was recently found to be largely sensitive to tolbutamide by the same authors (42). **Mechanisms by which a rise in [Ca\textsuperscript{2+}]\textsubscript{c} increases \(I_{\text{KATP}}\)**: A rise in [Ca\textsuperscript{2+}]\textsubscript{c} could increase \(I_{\text{KATP}}\) by different mechanisms, including a direct action of Ca\textsuperscript{2+} on K\textsuperscript{+}-ATP channels, an indirect action through Ca\textsuperscript{2+}-sensitive regulators of the channels, and an indirect action through changes in cell metabolism. A direct effect of Ca\textsuperscript{2+} on K\textsuperscript{+}-ATP channels has been reported in inside-out patches of membranes of normal \(\beta\)-cells or tumoral insulin-secreting RINm5F cells in which application of Ca\textsuperscript{2+} inhibited K\textsuperscript{+}-ATP channels (millimolar range of Ca\textsuperscript{2+}) (43) and attenuated the ADP-induced channel activation (micromolar range of Ca\textsuperscript{2+}) (44). Ca\textsuperscript{2+} increased the ability of ATP to block K\textsuperscript{+}-ATP channels or inactivated these channels in inside-out patches of skeletal muscle (45) and ventricular (46) membranes. However, all these effects are opposite to the Ca\textsuperscript{2+}-induced increase in \(I_{\text{KATP}}\) observed in the present study. Others did not find any direct effect of Ca\textsuperscript{2+} on K\textsuperscript{+}-ATP channels in \(\beta\)-cells (9). It is worth noting that the K\textsuperscript{+}-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\textsuperscript{2+}.

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

Metabolic oscillations might be driven by [Ca\textsuperscript{2+}]\textsubscript{c} oscillations. Indeed, each rise in [Ca\textsuperscript{2+}]\textsubscript{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\textsuperscript{2+}]\textsubscript{c} oscillations have recently been reported in islets (27). Our data do not exclude this possibility. Alternatively, each rise in [Ca\textsuperscript{2+}]\textsubscript{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\textsuperscript{2+}]\textsubscript{c} was raised by high K\textsuperscript{+}. By demonstrating that a rise in [Ca\textsuperscript{2+}]\textsubscript{c} increases \(I_{\text{KATP}}\), 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, \(I_{\text{KATP}}\) 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\textsuperscript{2+}]\textsubscript{c} might be the feedback mechanism controlling \(I_{\text{KATP}}\) 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\textsuperscript{2+}]\textsubscript{c} oscillation evoked a transient increase in \(I_{\text{KATP}}\). This increase had a similar amplitude to that of the difference in \(I_{\text{KATP}}\) 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\textsuperscript{2+} channels. This finding was amply supported by current-clamp experiments. Injection of current corresponding to 50% of the Ca\textsuperscript{2+}-induced \(I_{\text{KATP}}\) increase was sufficient to repolarize the \(\beta\)-cell membrane in the presence of 10 mmol/l glucose. Because the voltage-
dependent \(\text{Ca}^{2+}\) current is larger in \(\beta\)-cells within intact islets than in isolated single cells (29), it is possible that the amplitude of the \(\text{Ca}^{2+}\)-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 \(\beta\)-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 \([\text{Ca}^{2+}]_\text{c}\) on \(I_{\text{KATP}}\) might be important for the control of oscillations of the \(\beta\)-cell membrane potential according to the following model. Acceleration of glucose metabolism in \(\beta\)-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 \(\text{Ca}^{2+}\) channels. \(\text{Ca}^{2+}\) influx then raises \([\text{Ca}^{2+}]_\text{c}\), which decreases the ATP-to-ADP ratio (25) and leads to reopening of \(K^+\)-ATP channels, partial repolarization of the plasma membrane, arrest of \(\text{Ca}^{2+}\) influx, and a drop in \([\text{Ca}^{2+}]_\text{c}\). The eventual restoration of a high ATP-to-ADP ratio then initiates a new cycle. Our observation that the negative feedback effect of \([\text{Ca}^{2+}]_\text{c}\) 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 \([\text{Ca}^{2+}]_\text{c}\) 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 \([\text{Ca}^{2+}]_\text{c}\) rise unless the latter is increased by augmenting the extracellular \(\text{Ca}^{2+}\) 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 channel-deficient mice. Thus, pancreatic \(\beta\)-cells from SUR1 or Kir 6.2 knockout mice display a continuous spike activity (56,57) and a sustained and stable elevation of \([\text{Ca}^{2+}]_\text{c}\) (56,58) at both nonstimulating and stimulating glucose concentrations. Only one abstract reported \([\text{Ca}^{2+}]_\text{c}\) oscillations in \(\beta\)-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 played by \(K^+\)-ATP channels in the control of pancreatic \(\beta\)-cell membrane potential, further studies should now elucidate the interplay between \([\text{Ca}^{2+}]_\text{c}\) and ATP turnover.

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