Oscillations in K_{ATP} channel activity promote oscillations in cytoplasmic free Ca^{2+} concentration in the pancreatic β cell

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ABSTRACT Pancreatic β cells exhibit oscillations in electrical activity, cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$), and insulin release upon glucose stimulation. The mechanism by which these oscillations are generated is not known. Here we demonstrate fluctuations in the activity of the ATP-dependent K^+ channels (K_{ATP} channels) in single β cells subject to glucose stimulation or to stimulation with low concentrations of tolbutamide. During stimulation with glucose or low concentrations of tolbutamide, K_{ATP} channel activity decreased and action potentials ensued. After 2–3 min, despite continuous stimulation, action potentials subsided and openings of K_{ATP} channels could again be observed. Transient suppression of metabolism by azide in glucose-stimulated β cells caused reversible termination of electrical activity, mimicking the spontaneous changes observed with continuous glucose stimulation. Thus, oscillations in K_{ATP} channel activity during continuous glucose stimulation result in oscillations in electrical activity and $[Ca^{2+}]_i$.

The resting conductance of the pancreatic β cell is predominantly determined by ATP-dependent K^+ channels (K_{ATP} channels) (1). Metabolism of glucose causes these channels to close, leading to membrane depolarization and initiation of electrical activity. This in turn causes an increase in cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$), which triggers insulin release (2). Stimulation of β cells with intermediate glucose concentrations (8–12 mM) results in a characteristic pattern of slow oscillations in membrane potential on which bursts of action potentials are superimposed (3). $[Ca^{2+}]_i$ oscillates in synchrony with electrical activity (4), and oscillations in $[Ca^{2+}]_i$ correspond to pulsatile insulin release (5). Absence of normal oscillations in plasma insulin levels is observed in maturity onset diabetes (6). The molecular mechanisms regulating glucose-induced oscillations in electrical activity, $[Ca^{2+}]_i$ and insulin release in β cells are not known, but the elucidation of these mechanisms is of importance for the understanding of impaired β-cell function in diabetes mellitus. In the present investigation, we have studied to what extent changes in the activity of the K_{ATP} channel could participate in the generation of glucose-induced oscillations in electrical activity and thereby $[Ca^{2+}]_i$.

MATERIALS AND METHODS

β cells were isolated from a local colony of obese (*ob/ob*) mice as described (7). Pancreatic islets from these mice contain more than 90% β cells (8), which have been shown to contain K_{ATP} channels displaying normal characteristics (9). Adult mice were killed by decapitation and islets were isolated using a collagenase technique. A cell suspension was prepared in a Ca^{2+}-free medium after which the cells were plated on coverslips or in Petri dishes in RPMI 1640 culture medium

containing 11 mM glucose, 10% fetal calf serum, 100 units of penicillin per ml, 100 μg of streptomycin per ml, and 60 μg of gentamycin per ml. Cells were used either 3–4 hr after isolation or following overnight incubation in RPMI 1640 medium. $[Ca^{2+}]_i$ was measured at 37°C in fura-2-loaded β cells. Before the experiments, cells were exposed to 1.5 μM fura-2/AM for 20 min at 37°C. The β cells, attached to coverslips, were then transferred to a perifusion chamber (7). The $[Ca^{2+}]_i$ measurements were carried out essentially as reported (7) using a Spex Industries (Edison, NJ) fluorolog-2 CM1T11I system connected to an inverted microscope (Zeiss, Axiovert 35 M). The medium used for isolation of cells, measurements of $[Ca^{2+}]_i$, and as bath solution in the patch clamp experiments was a Hepes buffer (pH 7.4) with Cl^- as the sole anion (10).

The patch-clamp technique was used for electrophysiological measurements (11). Current and voltage were recorded with an Axopatch 200 patch clamp amplifier (Axon Instruments, Foster City, CA). During the experiments the current and voltage signals were stored using a VR-100A digital recorder (Instrutech, Elmont, NY) and a high-resolution video cassette recorder (JVC). The β-cell membrane potential was monitored using the perforated patch configuration with the pipette solution consisting of 10 mM KCl, 76 mM K_2SO_4, 10 mM NaCl, 1 mM $MgCl_2$, and 10 mM Hepes-NaOH (pH 7.35) and 200 μg of amphotericin B per ml (dissolved in Me_2SO, final concentration of $Me_2SO < 0.1\%$). When estimating whole-cell currents, the signal was filtered at 1000 Hz (-3 dB value), using an 8-pole Bessel filter. Single K_{ATP} channel currents, filtered at 100 Hz, were monitored using the cell-attached configuration of the patch-clamp technique, with both extracellular and pipette solutions consisting of the Hepes buffer described above. Gigaohm seals were established at room temperature and the experiments were carried out at 33–35°C. Figures show representative results from series of at least three experiments.

RESULTS AND DISCUSSION

The K_{ATP} channel has a central role in maintaining the β-cell membrane potential. However, available experimental data do not allow a conclusive interpretation of the extent to which the channel has a role also in the regulation of the bursting pattern of electrical activity and thereby oscillations in $[Ca^{2+}]_i$. Hence, the present study was undertaken with the aim to disclose a possible regulatory function for the K_{ATP} channel in this process.

Glucose-induced oscillations in electrical activity (long bursts) and $[Ca^{2+}]_i$ (2, 7) in dispersed β cells are shown in Fig. 1 A and B. The glucose concentration that initiates electrical activity (7–10 mM) does not fully block the whole-cell K_{ATP} current, but remaining current can be almost totally inhibited by increasing the glucose concentration further. At 20 mM glucose, when channels are nearly completely blocked (12), electrical activity is continuous and $[Ca^{2+}]_i$ remains at a

Abbreviations: ATP-dependent K^+ channel (K_{ATP} channel); cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$).
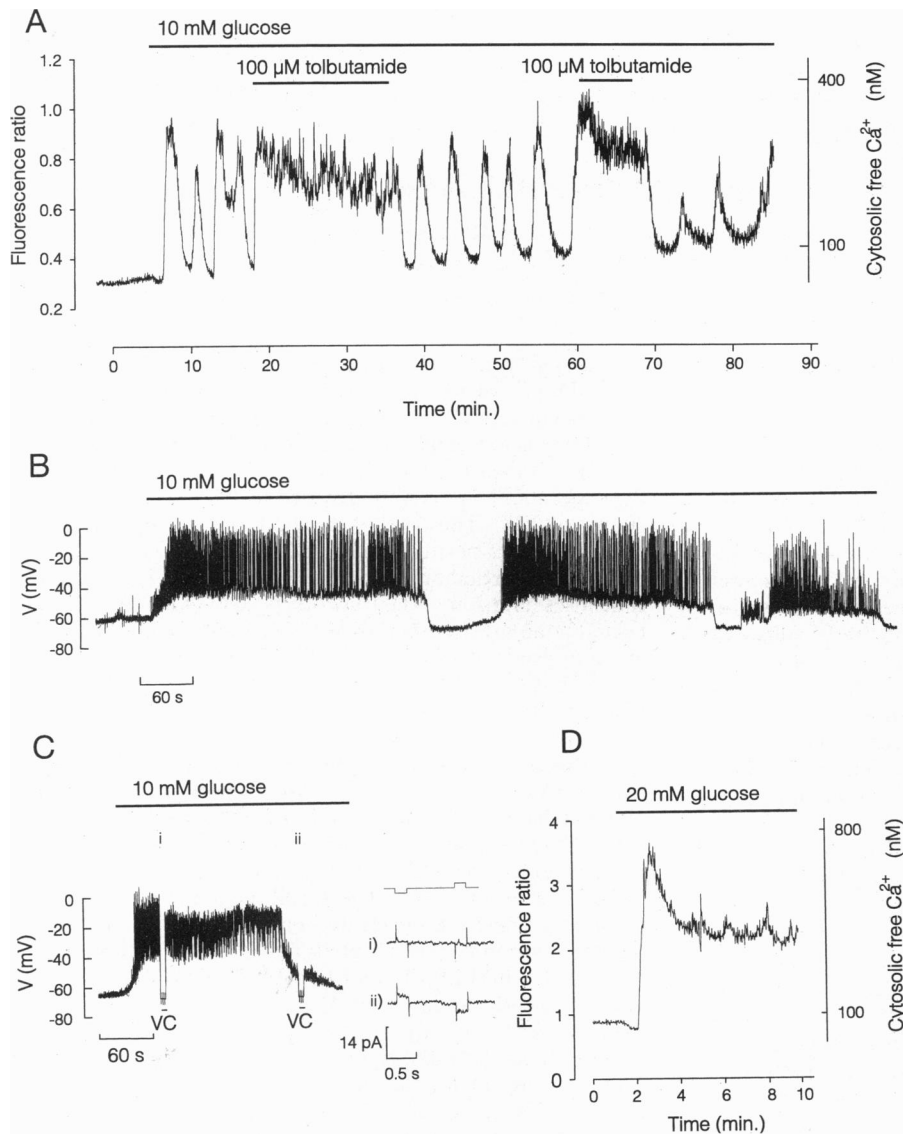†To whom reprint requests should be addressed.

FIG. 1. Studies of glucose-induced oscillations in $[Ca^{2+}]_i$ and membrane potential in pancreatic β cells. (*A*) Glucose (10 mM) was added to an aggregate of β cells, which responded with oscillations in $[Ca^{2+}]_i$. When $[Ca^{2+}]_i$ reached near basal values after an oscillation, 100 μM tolbutamide was added (first addition) and the cell was continuously exposed to the sulfonylurea for $\approx$15 min. Tolbutamide was then discontinued and the cell resumed oscillations in $[Ca^{2+}]_i$. When $[Ca^{2+}]_i$ reached near peak values, 100 μM tolbutamide was again added (second addition). The cell was continuously exposed to the sulfonylurea for $\approx$10 min. Then tolbutamide was discontinued and the cell exposed only to 10 mM glucose. (*B*) Membrane potential was recorded from a β cell. Glucose (10 mM) was added and the cell depolarized and displayed action potentials. After $\approx$5 min, action potentials ceased and the cell spontaneously repolarized, despite the continued presence of the hexose. The cycle with depolarization and repolarization was repeated another two times. (*C*) Membrane potential recording from a β cell. Addition of 10 mM glucose caused depolarization and the appearance of action potentials. To estimate to what extent the K_{ATP} channels were open at various times in the experiment, voltage excursions of $\pm$10 mV were performed when the cell was temporarily voltage clamped at -70 mV. (*i* and *ii*) Different time points at which the voltage-excursion protocol was used. Resulting currents from the voltage-excursion protocols are shown to the right. (*i*) Current generated during a depolarized phase. (*ii*) Current generated during a spontaneous repolarization. (*D*) Effect of addition of 20 mM glucose on $[Ca^{2+}]_i$.

sustained elevated level (Fig. 1*D*). Thus, there is a correlation between the pattern of β-cell electrical activity and the magnitude of the whole-cell K_{ATP} current, such that the time spent at the depolarized plateau potential varies inversely with the magnitude of the K_{ATP} current (1).

Closure of K_{ATP} channels by glucose induces a high resistance of the β-cell membrane. Therefore, only small changes in K_{ATP} current are needed to markedly affect β-cell membrane potential, electrical activity, and $[Ca^{2+}]_i$. Consequently, minor fluctuations in K_{ATP} channel activity may generate long bursts. During glucose-induced $[Ca^{2+}]_i$ oscillations, inhibition of K_{ATP} channels with the sulfonylurea compound tolbutamide clamped $[Ca^{2+}]_i$ at a sustained elevated level and $[Ca^{2+}]_i$ remained elevated for the duration of tolbutamide stimulation (Fig. 1*A*). When the sulfonylurea drug was removed, the β cell $[Ca^{2+}]_i$ decreased and oscillations in $[Ca^{2+}]_i$ reappeared. This indicates that the fluctuations in $[Ca^{2+}]_i$ observed during glucose stimulation are caused by spontaneous oscillations in K_{ATP} channel activity, resulting in periodic changes in membrane ionic currents and electrical activity. These putative oscillations in K_{ATP} channel activity were susceptible to tolbutamide inhibition.

We tested the hypothesis that oscillations in electrical activity are paralleled by spontaneous oscillations in K_{ATP} channel activity by estimating the input conductance, under temporary voltage-clamp conditions at -70 mV, during the pla-

teau and silent phases of electrical activity. In the presence of 10 mM glucose, the input conductance (measured from the current response to 10 mV excursions from a holding potential of -70 mV) was assessed to 22 pS during the depolarized phase. During the silent phase, the conductance increased to 350 pS (Fig. 1*C*). In a series of experiments, input conductance increased from 43 ± 31 pS to 240 ± 78 pS (mean $\pm$ SD, $P < 0.05$, $n = 4$) when the cell spontaneously repolarized in the continuous presence of 10 mM glucose.

To directly study the relationship between openings of the K_{ATP} channel and electrical activity, we performed single K_{ATP} channel recordings in intact β cells with preserved metabolism by using the cell-attached configuration of the patch-clamp technique. Fig. 2 shows a typical recording from one β cell in a small cluster of cells. This cell displayed frequent openings of K_{ATP} channels at a nonstimulatory concentration of glucose. As the concentration of glucose was raised from 3 to 10 mM, channel activity subsided and action potentials were observed. After 1–3 min, however, the action potentials ceased and abundant openings of K^+ channels were again observed, despite the continuous presence of glucose. These channels are K_{ATP} channels, because 100 μM tolbutamide, added to the perifusion medium, caused an immediate block of channel activity and induced a continuous train of action potentials. Together these results provide good evidence that the mechanism behind the oscillatory electrical activity in single β cells,
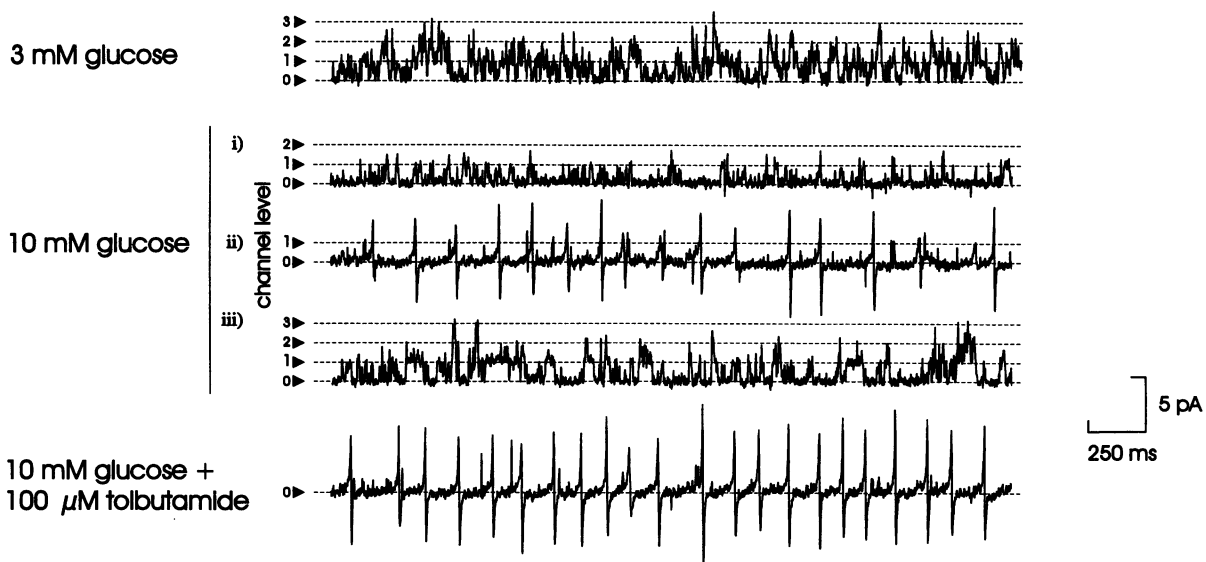
FIG. 2. Channel activity was recorded from a β cell in a cell cluster using the cell-attached mode of the patch-clamp technique. Results from one particular β cell are shown. Openings of K_{ATP} channels can be observed at 3 mM glucose. (*i*) After increasing the glucose concentration to 10 mM, K_{ATP} channel activity decreases. (*ii*) Later, the picture is dominated by the biphasic action potentials. (*iii*) In the continuous presence of 10 mM glucose, action potentials subside and openings of K_{ATP} channels can again be seen. Finally, the β cell was stimulated with 100 μM tolbutamide, resulting in closure of K_{ATP} channels and the appearance of action potentials. Filled triangles denote current levels corresponding to different numbers of simultaneously open channels.

in the continuous presence of a stimulatory concentration of glucose, is spontaneous oscillations in K_{ATP} channel activity.

Blockers of the K_{ATP} channel have previously been reported to alter the duration of the bursts of action potentials in

recordings from intact islets in a manner similar to glucose (13, 14) and tolbutamide is able to induce oscillations in $[Ca^{2+}]_i$ in the presence of a nonstimulatory concentration of the sugar (Fig. 3*A*; ref. 15). Furthermore, single-channel recordings
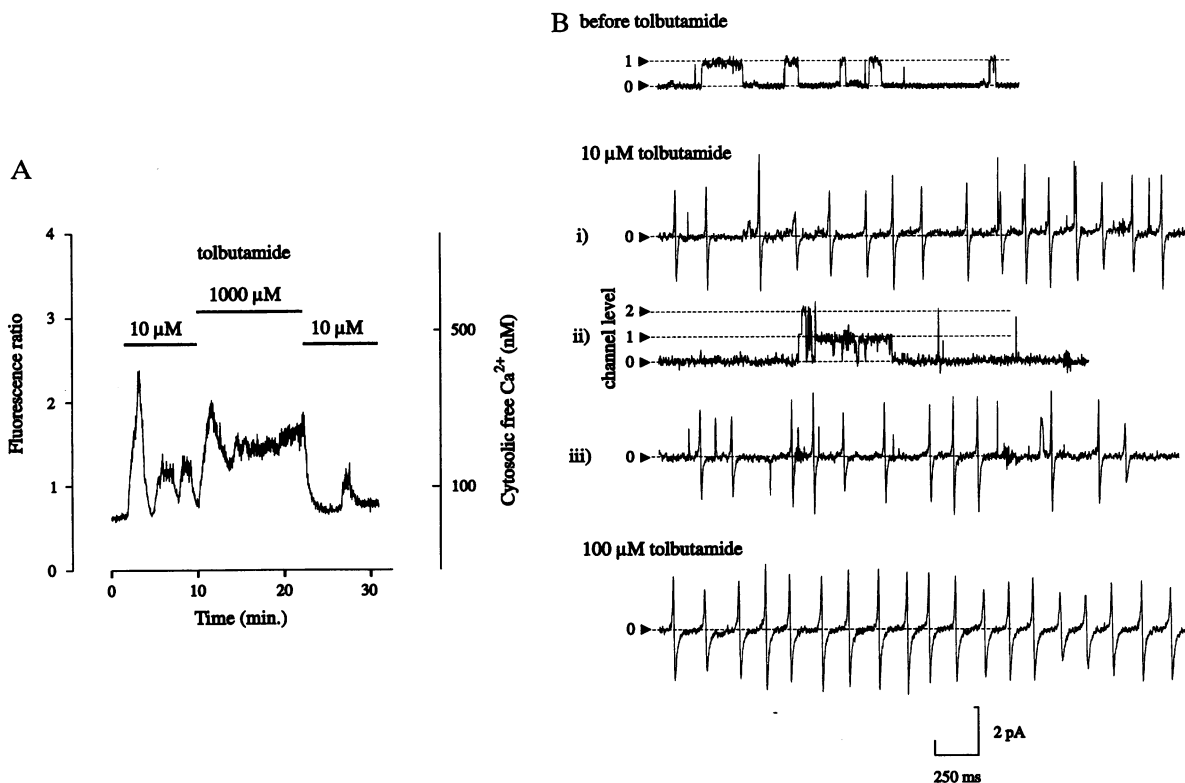

FIG. 3. Effect of tolbutamide on $[Ca^{2+}]_i$ and K_{ATP} channel activity in mouse β cells. (*A*) A single β cell was exposed to 10 μM tolbutamide and $[Ca^{2+}]_i$ was monitored. $[Ca^{2+}]_i$ started to oscillate. After three oscillations, 1000 μM tolbutamide was added, and this clamped $[Ca^{2+}]_i$ at an elevated level. After ≈10 min, the tolbutamide concentration was lowered to 10 μM. $[Ca^{2+}]_i$ then decreased and another oscillation was observed. (*B*) Channel activity was recorded from a β cell in a cell cluster using the cell-attached mode of the patch-clamp technique. Openings of K_{ATP} channels can be observed before addition of tolbutamide. After addition of the sulfonylurea, very few openings of K_{ATP} channels can be seen and the picture is dominated by biphasic action potentials (*i*). Then, in the continuous presence of tolbutamide, action potentials subside and openings of K_{ATP} channels can again be seen (*ii*). Thereafter, the K_{ATP} channels close and action potentials return (*iii*). Addition of 100 μM tolbutamide leads to a complete inhibition of K_{ATP} channels and a continuous train of action potentials.

showed that periods of K_{ATP} channel openings alternated with periods of action potentials in the continuous presence of 10 μM tolbutamide (Fig. 3B).

It was reported that K_{ATP} channels remain closed during the repolarized phase of glucose-induced oscillations in membrane potential (16). In contrast, our results show that the open probability of the K_{ATP} channel undergoes spontaneous fluctuations during glucose stimulation. Glucose metabolism is known to oscillate in various systems (17–19). In pancreatic islets, oscillations in oxygen consumption have been reported, paralleling oscillations in $[Ca^{2+}]_i$ (20). Accordingly, it has been proposed that oscillations in $[Ca^{2+}]_i$ in the pancreatic β cell could be caused by fluctuations in the activity of the K_{ATP} channel due to underlying oscillations in intracellular metabolism (21). Our results are consistent with this view. The open probability of the K_{ATP} channel increases when the ATP/ADP ratio is low (1). Such a situation can be created by inducing a transient suppression of metabolism by exposing the β cell to the metabolic inhibitor sodium azide (NaN_3), which blocks cytochrome a_3 (22). NaN_3 has indeed been shown to decrease the ATP/ADP ratio in the β cell (23) and would therefore be expected to cause an increased number of openings of the K_{ATP} channel. As seen in Fig. 4A, 20 mM glucose induced

continuous electrical activity, which immediately ceased as NaN_3 was added. The changes in input resistance, reflecting current through K_{ATP} channels, measured before, during, and after perifusion with NaN_3 (Fig. 4A ii–iv), mimicked the spontaneous fluctuations in input resistance observed during continuous perifusion with 10 mM glucose. The effect of the metabolic inhibitor was readily reversed upon withdrawal of the compound. A strong repolarizing effect of NaN_3 was also seen during stimulation with 100 μM tolbutamide in the presence of 3 mM glucose (Fig. 4B). During cell-attached recordings of K_{ATP} channel activity, a clearcut increase in channel openings occurred in the presence of the metabolic inhibitor (Fig. 4C), thus explaining the repolarizing effect of NaN_3. In a series of experiments, a more than 5-fold increase in mean K_{ATP} current was observed [575 ± 33% (mean ± SD), $P < 0.01$, $n = 5$] during addition of the metabolic inhibitor in the presence of 3 mM glucose.

These observations of oscillations in K_{ATP} channel activity, mimicked by transient suppression of metabolism, are in good agreement with recent results obtained from heart muscle cells, which display metabolically driven oscillations in K_{ATP} channel currents with concomitant oscillations in $[Ca^{2+}]_i$ during severe conditions of fuel deprivation (18). In the
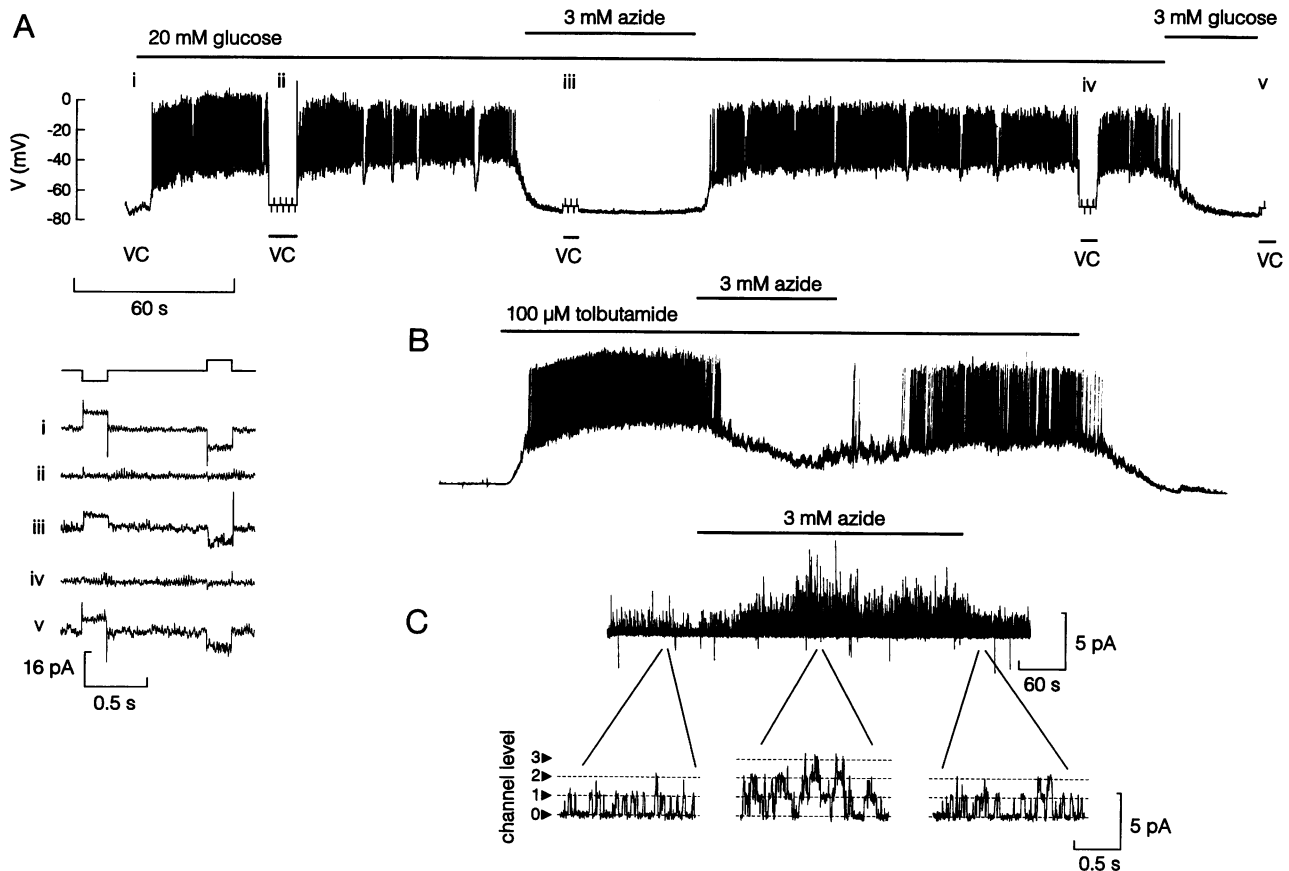

FIG. 4. Effects of sodium azide on membrane potential or channel activity recorded in different β cells. (A) Glucose (20 mM) was added to one β cell and this caused the cell to depolarize and display continuous action potentials. Sodium azide (3 mM) was then added, and this caused cessation of electrical activity and repolarization. Sodium azide stimulation was then terminated. Since glucose stimulation continued, the cell again depolarized and resumed electrical activity. To estimate to what extent the K_{ATP} channels were open at various times in the experiment, voltage excursions of ±10 mV were performed when the cell was temporarily voltage clamped at −70 mV. (i–v) Different time points at which the voltage-excursion protocol was used. Resulting currents from the voltage-excursion protocols are shown below. Current generated during combined glucose and sodium azide perifusion (iii) is similar to the current generated before and after glucose stimulation (i and v). (B) Tolbutamide (100 μM) was added to one β cell, which depolarized and displayed action potentials. Addition of 3 mM sodium azide caused cessation of electrical activity and repolarization. Sodium azide stimulation was then terminated. Because tolbutamide stimulation continued, the cell depolarized again and resumed electrical activity. (C) Sodium azide (3 mM) was added to a β cell at 3 mM glucose and channel openings were registered with the cell-attached configuration of the patch-clamp technique. This protocol demonstrates that the characteristics of the channels that open during azide perifusion are typical of K_{ATP} channels. (Upper) An entire representative experiment is shown. (Lower) Three selected segments using a different time scale are shown. Details of channel openings can be seen in these segments.

pancreatic β cell, under physiological conditions, electrical activity is induced when a sufficient number of K_{ATP} channels are closed, driving the membrane potential to the threshold potential for the activation of the voltage-activated Ca^{2+} channels. At this critical level of membrane resistance, minor changes in K_{ATP} channel current, induced by fluctuating metabolism-generated signals—e.g., oscillations in the ATP/ADP ratio—may give rise to membrane potential fluctuations. This would force the Ca^{2+} channels to alternate between activated and inactivated states, leading to the described long bursts of electrical activity and oscillations in $[Ca^{2+}]_i$. Our results are compatible with the notion that stimulation with increasing concentrations of sulfonylurea at basal glucose closes a progressively greater fraction of K_{ATP} channels, thereby causing a depolarization of the membrane potential. This enables existing fluctuating metabolic signal(s), present both at basal (24) and stimulating glucose concentrations, to have an influence on the remaining K_{ATP} channel current and thus generate oscillations in electrical activity and $[Ca^{2+}]_i$. At continuous electrical activity, induced by high glucose concentrations, low doses of the K_{ATP} channel opener diazoxide have been shown to restore slow wave activity in intact islets (13).

The long bursts displayed in dispersed β cells have a duration ranging between 1 and 4 min, which is approximately one order of magnitude longer than the oscillations in the intact islet (2, 4). It is uncertain whether or not these oscillatory patterns are related and share the same underlying mechanisms. Nevertheless, there are similarities between the oscillatory patterns in dispersed β cells and islets, in that slow and fast oscillations in $[Ca^{2+}]_i$ correspond to long burst and slow wave oscillations in electrical activity, respectively (2, 4, 16).

Changes in the ATP/ADP ratio may not be the only metabolic signal to affect the K_{ATP} channel. Other potential regulatory signals include changes in redox potential (25), pyridine (26), and guanine (27) nucleotides and intracellular pH (28). The present study shows that the number of active K_{ATP} channels undergoes spontaneous oscillations in single β cells subsequent to glucose stimulation and that this gives rise to the well-documented oscillations in electrical activity and $[Ca^{2+}]_i$. Whether these oscillations promote pulsatile insulin secretion merits further investigation.

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