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Charybdotoxin-Sensitive $K_{(Ca)}$ Channel is not Involved in Glucose-Induced Electrical Activity in Pancreatic β-Cells

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Summary. The effects of charybdotoxin (CTX) on single $[Ca^{2+}]_i$-activated potassium channel ($K_{(Ca)}$) activity and whole-cell K^+ currents were examined in rat and mouse pancreatic β-cells in culture using the patch-clamp method. The effects of CTX on glucose-induced electrical activity from both cultured β-cells and β-cells in intact islets were compared. $K_{(Ca)}$ activity was very infrequent at negative patch potentials ($-70 < V_m < 0$ mV), channel activity appearing at highly depolarized V_m. $K_{(Ca)}$ open probability at these depolarized V_m values was insensitive to glucose (10 and 20 mM) and the metabolic uncoupler 2,4 dinitrophenol (DNP). However, DNP blocked glucose-evoked action potential firing and reversed glucose-induced inhibition of the activity of K^+ channels of smaller conductance.

The venom from *Leiurus quinquestriatus hebreus* (LQV) and highly purified CTX inhibited $K_{(Ca)}$ channel activity when applied to the outer aspect of the excised membrane patch. CTX (5.8 and 18 nM) inhibited channel activity by 50 and 100%, respectively. Whole-cell outward K^+ currents exhibited an early transient component which was blocked by CTX, and a delayed component which was insensitive to the toxin. The individual spikes evoked by glucose, recorded in the perforated-patch modality, were not affected by CTX (20 nM). Moreover, the frequency of slow oscillations in membrane potential, the frequency of action potentials and the rate of repolarization of the action potentials recorded from pancreatic islet β-cells in the presence of glucose were not affected by CTX.

We conclude that the $K_{(Ca)}$ does not participate in the steady-state glucose-induced electrical activity in rodent pancreatic islets.

Key Words calcium-activated potassium channels · charybdotoxin · pancreatic β-cell

Introduction

It is generally thought that the underlying mechanism for glucose sensing in pancreatic β-cells is the metabolic control of K^+ channels. In support of this idea is the observation that increasing glucose concentration from 0 up to 7 mM blocks K^+ permeability in islets of Langerhans (Atwater, Ribalet & Rojas, 1978; Henquin, 1978). Furthermore, the duration of the active phase of electrical activity induced by glucose is exquisitely modulated by the hexose. At least three classes of potassium channels are known to be present in pancreatic β-cells. The most studied, the ATP-sensitive channel, is blocked by ATP acting from the intracellular aspect of the membrane in excised patches (Cook & Hales, 1984) or by glucose metabolism in cell-attached patches (Ashcroft, Harrison & Ashcroft, 1984; Misler et al., 1986). The inhibition of the ATP-sensitive K^+ channel is linked to the depolarization of the membrane from −70 mV (in the absence of glucose) to −60 mV (in the presence of glucose) which is the threshold for electrical activity. Henquin (1988) proposed that this channel could modulate the duration of the active phase. The second class is the delayed rectifier K^+ channel which has been characterized using the patch-clamp technique both in the whole-cell and single-channel recording mode (Rorsman & Trube, 1986; Zunkler, Trube & Ohno-Shosaku, 1988).

The third class of K^+ channels is the high conductance K^+ channel which is activated by $[Ca^{2+}]_i$ and membrane depolarization ($K_{(Ca)}$) and found in pancreatic β-cells from rodents and insulin-secreting cell lines (Marty & Neher, 1982; Cook, Ikeuchi & Fujimoto 1984; Eddlestone, Ribalet & Ciani, 1989; Tabcharani & Misler, 1989). The existence of the $K_{(Ca)}$ channel was inferred from studies of the effects of quinine on glucose-evoked electrical activity in islet cells (Atwater et al., 1979*a*; Atwater, Rosario & Rojas, 1983). Studies conducted on insulin-secreting cell lines suggest a role for this channel in the glucose sensing over high glucose concentrations (Eddlestone et al., 1989). However, studies of single-channel currents in normal rodent β-cells have not provided definitive support for this hypothesis.

The main objective of this work was to re-evaluate the role of $K_{(Ca)}$ channels in the glucose-induced electrical activity in rodent pancreatic β-cells. We found and report here that although charybdotoxin blocks $K_{(Ca)}$ channels in pancreatic β-cells, the burst pattern of glucose-induced electrical activity is not affected in a noticeable manner.

Preliminary results have been reported elsewhere (Kukuljan & Goncalves, 1990).

Materials and Methods

Pancreatic β-Cell Cultures

For patch-clamp experiments, islets of Langerhans were isolated by collagenase digestion from the pancreases of male Wistar rats (10–16 weeks) and Swiss Webster albino mice (6–8 weeks). Islets were dispersed into single cells and small clusters of cells by further treatment with dispase. Enzymes were obtained from Boehringer Mannheim (Indianapolis, IN). Cells were suspended in RPMI 1066 tissue culture medium (GIBCO, Grand Island, NY) supplemented with 5.6 mM glucose, 10% fetal calf serum, 1000 U/ml penicillin and 1000 μg/ml streptomycin and incubated at 37°C in a humidified atmosphere with 5% CO_2. Cells were used 2 to 6 days after isolation.

Single-Channel and Whole-Cell Current Recordings

Patch-clamp experiments were conducted following standard procedures (Hamill et al., 1981). Electrodes were made from microhematocrit capillary glass using a microcomputer-controlled multi-step puller (Mecanex, Geneva, Switzerland). When filled with 140 mM KCl solution, electrode resistance ranged from 2 to 5 mΩ for pipets to be used in whole-cell current recordings and 4 to 8 MΩ for electrodes to be used in single-channel current recordings. Electrodes were coated with Sylgard (Dow Corning, Midland, MI) and the tip fire polished using a microforge (Narishige, Tokyo, Japan). Recordings started after a high resistance seal (>5 GΩ) formed. Single-channel and whole-cell current recordings were made using a EPC-7 amplifier (List, Darmstadt, FRG). For single-channel recording we used the cell-attached configuration and excised outside-out and inside-out patches. Permanent records of the signal were made continuously on a 4-channel magnetic tape recorder (Racal Recorders, Sarasota, FL). Whole-cell voltage-clamp records were made after breaking the membrane patch by gentle suction. In this configuration, voltage levels for holding potential and pulses were generated by a digital-to-analog converter under computer control (Compaq 386/25, Compaq, Houston, TX). Current transients in response to the voltage pulses were recorded using a 12-bit analog-to-digital converter (TL-1 DMA interface, Axon Instruments, Foster City, CA). Cell capacitance and series resistance were compensated. Current signals were digitized at 5 kHz and stored on the hard disk of the microcomputer for off-line analysis.

For analysis, single-channel records were digitized at 10 kHz and stored on the hard disk of the microcomputer. Commercially available software (pCLAMP, IPROC and LPROC, Axon Instruments) was used to measure the fractional open time from records exhibiting only one level of channel openings. In patches displaying two or more levels but of identical size, integration of the current over 15- to 20-sec segments was used to quantify the channel activity. Whole-cell records were corrected for linear components of leakage and capacity transients and displayed for measurements of current amplitude.

Membrane Potential Measurements

Membrane potential measurements from single cultured rat β-cells, were carried out using the "perforated-patch-clamp" technique (Horn & Marty, 1988) under current clamp. Conventional microelectrode membrane potential measurements from β-cells in mouse islets of Langerhans were made as described elsewhere (Atwater et al., 1978). Briefly, a single microdissected islet was mounted in a continuously perifused chamber and a β-cell impaled with a high resistance (150 to 300 MΩ) glass microelectrode filled with a 1 : 1 mixture of 1.5 M KCl and 0.5 M potassium citrate. The membrane potential was measured between two Ag/AgCl electrodes, one in the intraelectrode solution and the other in the solution flowing though the chamber. The rate of change of the action potentials during the falling phase was measured using segments of individual spikes digitalized at 1 kHz and selected for analysis with "pCLAMP." Statistical significance of results was determined by using two-tailed t test and one-way analysis of variance (ANOVA). Results are presented as mean ± SEM.

Solutions and Toxins

For patch-clamp experiments the culture medium was removed and the cells were washed three times with a modified Krebs buffer of the following composition (in mM): 130 NaCl, 4 KCl, 2.6 $CaCl_2$, 1 $MgCl_2$, 10 NaHEPES, pH 7.4 (solution I). For experiments in the cell-attached configuration the electrodes were filled with either solution I or a high potassium solution containing 140 KCl, 0.2 $CaCl_2$, 10 NaHEPES, pH 7.2 (solution II). This solution was also used to expose the inner aspect of the excised membrane patch in outside-out experiments. Whole-cell potassium currents were recorded with the following solution for dialysis: 90 potassium aspartate, 50 KCl, 10 NaHEPES, 1 $MgCl_2$, pH 7.1 (solution III). NaEGTA and calcium were added to obtain the desired free-calcium concentrations. Membrane potential measurements in isolated cells were obtained using electrodes filled with solution III plus 1 EBTA and 0.4 $CaCl_2$ (calculated free-Ca^{2+} concentration 100 nM) and 150 to 300 μg/ml nystatin dissolved in dimethyl sulfoxide (DMSO), final DMSO concentration 0.01%. Experiments using cultured cells were conducted at room temperature (20 to 22°C). Membrane potential recordings from mouse islet cells were carried out under continuous perifusion with a solution containing 120 NaCl, 5 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1.1 $MgCl_2$, equilibrated with a mixture of 95% O_2 and 5% O_2, pH 7.4 at 37°C.

Crude venom from *Leiurus quinquestriatus hebreus* (LQV) was purchased from Sigma (St. Louis, MO). Highly purified charybdotoxin (CTX) was obtained from Latoxan (Rosans, France).

Results

Effects of DNP on Glucose-Evoked Electrical Activity from Single Cultured β-Cells

Cell-attached membrane patches exhibited frequent openings of the ATP-sensitive K^+ channel. The

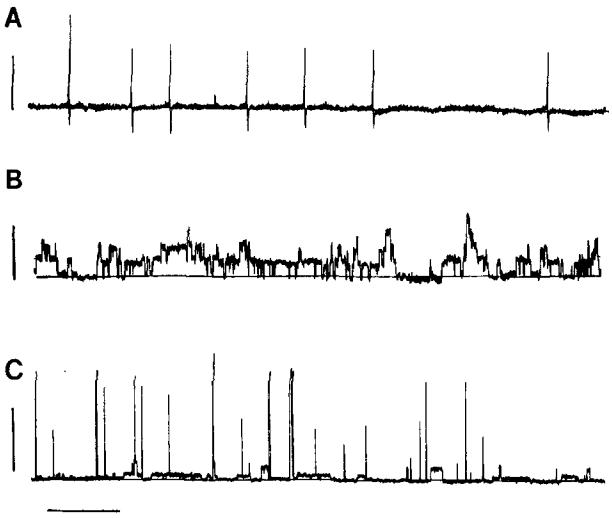

Fig. 1. Effects of DNP on electrical activity and K^+ channels. (*A*) Action potentials evoked by glucose (10 mM). (*B*) Two min after addition of 200 μM DNP. At least four 20-pS K channels were present in the patch. No high conductance $K_{(Ca)}$ channel activity was seen at this patch potential (V_p) = −40 mV). (*C*) Openings of $K_{(Ca)}$ channel in the same patch at V_p = −130 mV. Calibrations: vertical *A* and *B*, 1 pA; *C*, 3 pA. Horizontal, 50 msec. Pipette filled with solution I throughout

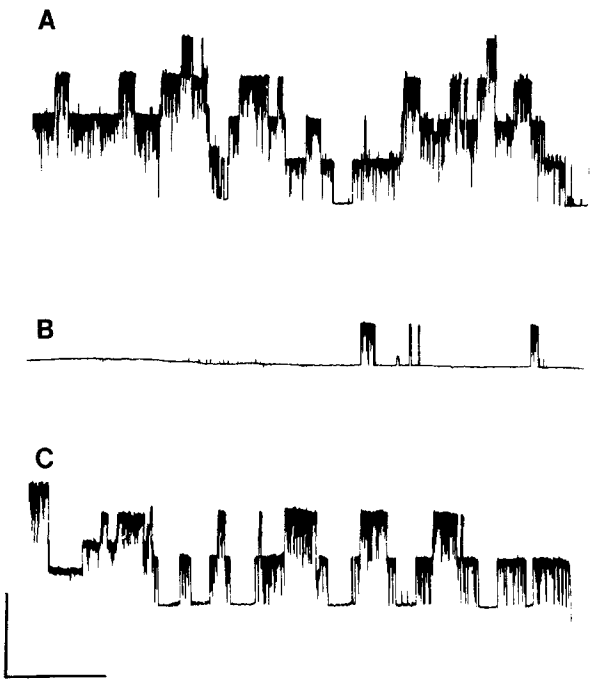

Fig. 2. Blockade of $K_{(Ca)}$ channel activity from outside-out patches by CTX. The membrane potential across the patch was held at V_m = 0 mV throughout. Control activity (*A*) was diminished by 96% within 5 sec of the addition of 10 μg/ml LQV. (*B*). $K_{(Ca)}$ channel activity 3 min after washing out the venom (*C*). Calibrations: vertical, 10 pA; horizontal, 10 sec

slope conductance and kinetic properties of this channel were as reported by others (Cook & Hales, 1984; Ashcroft et al., 1984). No other channel types were seen at pipette potentials (V_p) in the range from 50 to −80 mV in 20 cells. Setting V_p at potentials more negative than −90 mV caused the appearence of brief outward single K^+ channel currents. The frequency and duration of the openings increased in response to further depolarization of the membrane patch. Single-channel slope conductance (V_p −90 to −160 mV) was 204 ± 12 pS (n = 18). This conductance is characteristic of the large conductance calcium and voltage-gated K^+ channel ($K_{(Ca)}$).

$K_{(Ca)}$ channel openings were rare at pipette potentials close to the resting membrane potential of the cells. For this reason the effects of glucose were studied at V_p of −130 mV. At this potential fractional open time for the $K_{(Ca)}$ channel ranged from 0.02 to 0.1. Addition of glucose (10 or 20 mM) failed to change the activity of the channel in any significant manner and the fractional open time of the $K_{(Ca)}$ channel remained at its control level in 10 out 10 cells studied. However, augmenting the extracellular glucose concentration induced a reversible inhibition of the ATP-sensitive K^+ channel (20 pS for outward current at pipette potentials at which the $K_{(Ca)}$ channel activitated). In the presence of 10 mM glucose the ATP-sensitive K^+ channels were completely blocked and action potential firing occurred (Fig. 1*A*). The effect of the metabolic uncoupler 2,4-dinitrophenol (DNP) on K^+ channel activity was also tested. At intermediate V_p (−40 mV) 200 μM DNP abolished the generation of glucose-evoked action potentials and activated ATP-sensitive K^+ channels which had been previously inhibited by glucose (10 mM). Further depolarization of the patch membrane up to V_p = −120 showed that $K_{(Ca)}$ channels were present in the patch, but they were silent at lower membrane patch potentials (Fig. 1*C*).

CTX Blocks $K_{(Ca)}$ Channels

Outside-out patches exhibited the activity of the high amplitude $K_{(Ca)}$ (V_m = 0 mV). Multi-channel patches were the rule (mean number of channels = 3, n = 15) (Fig. 2*A*). The slope conductance for the $K_{(Ca)}$ channels was 154 ± 12 pS (n = 15). Channel activity was characterized by bursts of brief openings and closures separated by long-lasting closed intervals. This $K_{(Ca)}$ channel activity could be maintained for 30 to 60 min.

In 8 out of 8 outside-out patches studied 5 to 10 μg/ml of LQV rapidly increased the duration of the long-lived closed states, without apparent

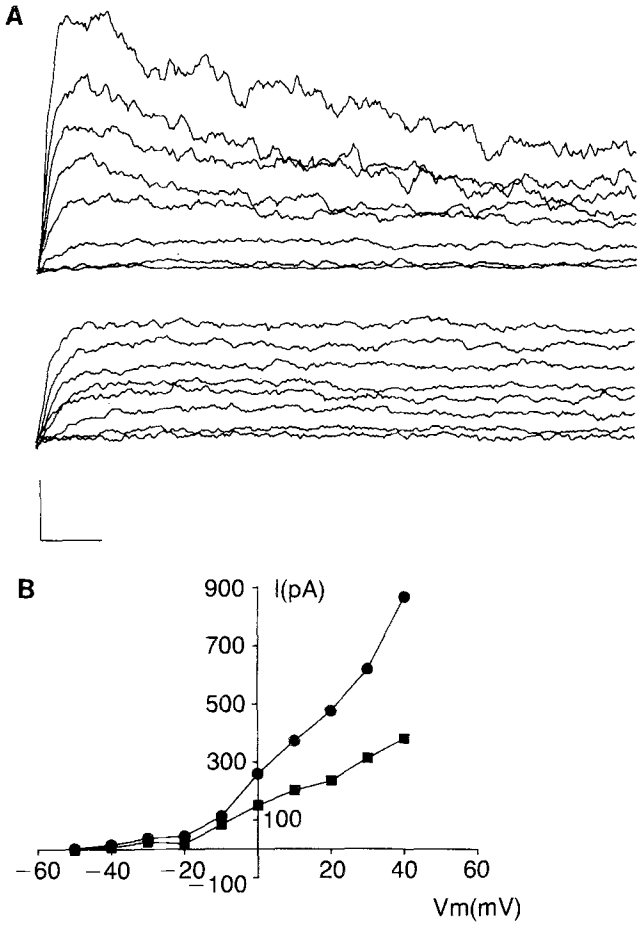

Fig. 3. Effects of CTX on K^+ currents. (*A*) Superimposed records of whole-cell K^+ currents elicited by depolarizing pulses taking the membrane potential from −80 mV to different levels indicated by the number near the corresponding record in 10-mV increments. Pipette solution contained 0.2 mM $CaCl_2$ and no EGTA. Lower panel shows the currents resulting from the same pulse protocol used for the control 1 min after adding 10 μg/ml LQV. Pulse frequency 0.2 Hz. Leak current was substracted using a P/N = −4 subpulses protocol. Vertical calibration: 200 pA, horizontal scale: 20 msec. (*B*) Current-voltage relationships for the control (circles) and in the presence of LQV (squares). Current was measured at 20 msec

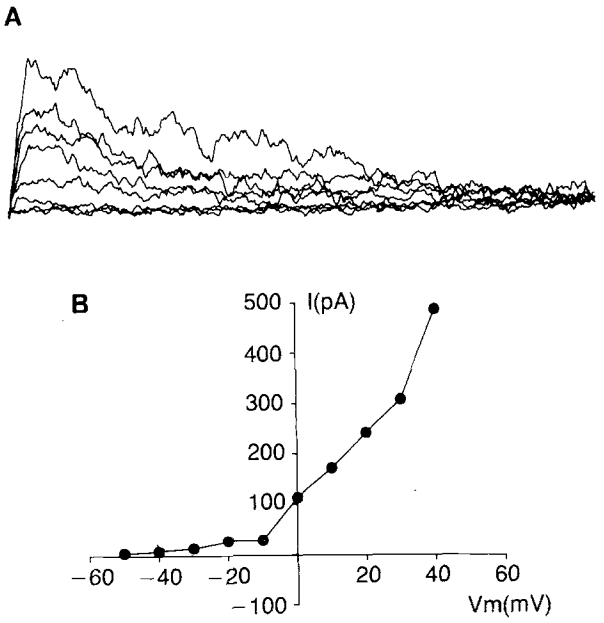

Fig. 4. LQV-blockable K^+ currents. (*A*) LQV-sensitive component of outward K^+ currents. (*B*) Current-voltage relationship at 20 msec after the onset of the pulse. Calibrations: vertical, 330 pA; horizontal, 40 msec

changes in the intraburst kinetics (Fig. 2*B*). The open-state amplitude and single-channel conductance were not modified by LQV. The blockade by LQV was almost completely reversed after washing the culture dish with modified Krebs buffer free of toxin (Fig. 2*C*). The addition of LQV to the intacellular aspect of the membrane patch, did not affect the open-time probability of the $K_{(Ca)}$ channel (*data not shown*).

Results with highly purified CTX were similar to those obtained with LQV when tested on $K_{(Ca)}$ channels from β-cells from both rat and mouse, verifying that the active toxin is in the LQV preparation. The half-maximal inhibition of channel activity was accomplished at a concentration of 5.8 nM CTX. Higher doses (14 nM) reduced the fractional open time to less than 5% of the control value. CTX at 18 nM suppressed $K_{(Ca)}$ channel activity.

CTX Blocks the $[Ca^{2+}]_i$-Dependent Transient Component of the Outward Currents

Whole-cell K^+ currents were also affected by CTX. In the absence of EGTA in the intracellular solution ($[Ca^{2+}]_i$ ca. 0.2 mM) outward currents exhibited a large transient component (Fig. 3*A*, upper panel). The observed decay in the currents was apparent at V_m positive to 20 mV in most of the cells studied. The addition of 10 to 20 μg/ml LQV or 10 to 20 nM CTX to the external solution induced a marked blockade of the decaying component of the K^+ current, leaving a sustained component (n = 9) (Fig. 3*A*, lower panel). Subtraction of this remaining component from the control (at the same potential during the pulses) yielded the CTX-sensitive current, in which the slow decay was the salient feature (Fig. 4*A*). It is apparent from the *I-V* curve (Fig. 4*B*) that the CTX-sensitive component of the K^+ current is turned on at membrane potentials positive to −20 mV (half-activation + 11.8 ± 8.9 mV in a series of six experiments in which the membrane was depolarized up to 40 mV in successive 10-mV increment pulses).

In the presence of EGTA 10 mM in the intracellu-

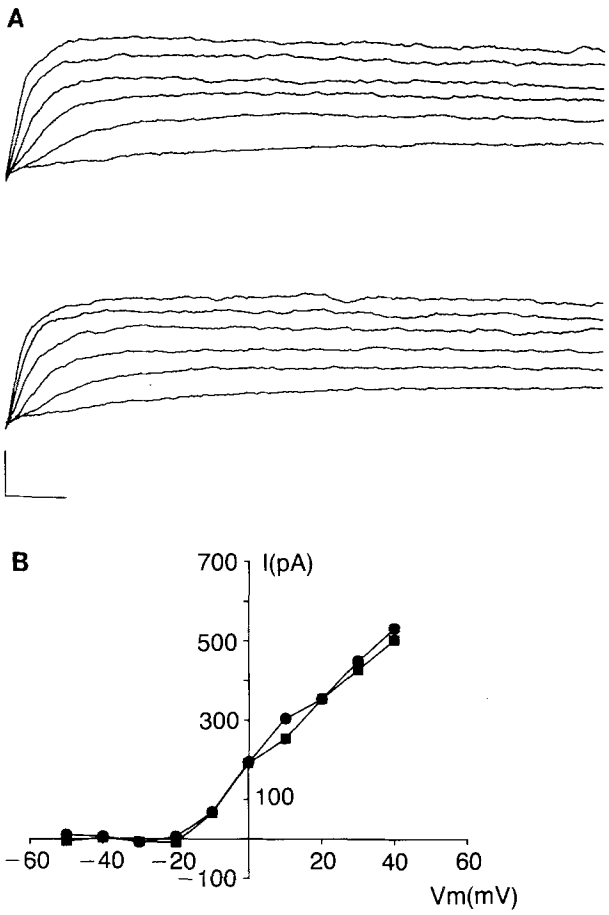

Fig. 5. Lack of effect of LQV on K^+ currents recorded with high intracellular calcium buffering capacity. (*A*) Superimposed K^+ currents recorded with 10 mM EGTA in the pipette solution (upper records) using a protocol as in experiment shown in Fig. 3. Note the absence of the early transient. Addition of CTX (up to 20 μg/ml LQV) did not affect the kinetic or the steady-state value of the current at each membrane potential from −10 to 40 mV. Calibrations: vertical, 200 pA; horizontal, 40 msec. (*B*) Current-voltage relationships measured in control (circles) and 2 min after addition of 20 μg/ml LQV (squares). Measurements made at 20 msec

lar solution (calculated free $[Ca^{2+}]_i$ in the range of 0.1 nM) outward K^+ currents did not decay along 200-msec pulses (Fig. 5*A*, upper panel). LQV (10 or 20 μg/ml) or CTX (18 nM) had no noticeable effects on these K^+ currents, as shown in Fig. 5*A*, lower panel.

Lack of Effect of CTX on Glucose-Evoked Electrical Activity

Glucose-induced electrical activity from cultured rat β-cells was measured under current-clamp mode. In the presence of glucose (15 or 20 mM) action potentials were always observed. Glucose-evoked

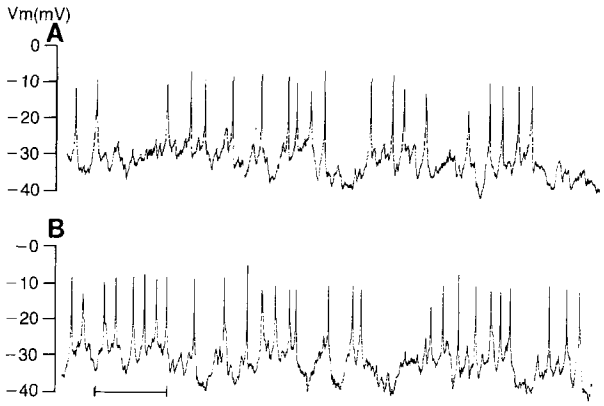

Fig. 6. Lack of effect of CTX on glucose-evoked electrical activity. Membrane potential records from a single rat β-cell using the perforated-patch technique. Extracellular glucose concentration: 20 mM. (*A*) Action potentials in the absence of CTX. (*B*) Action potentials in the absence of CTX (20 nM) unaffected by the toxin. Horizontal scale: 50 msec

electrical activity from single cultured β-cells was irregular, unlike the typical burst pattern from β-cells in intact islet of Langerhans. The addition of CTX (20 nM) did not change the pattern of glucose-induced spike firing, as shown in Fig. 6. Moreover, the shape of the action potential was not modified. Both spike duration and rate of repolarization were not changed in the presence of CTX. In the experiment shown in Fig. 6, the time constant for spike repolarization was 23.4 ± 2.1 msec in the control period and 22.2 ± 1.7 msec in the presence of CTX.

CTX Does Not Affect the Typical Burst Pattern of Glucose-Evoked Electrical Activity

The perifusion of single microdissected islets with crude venom (LQV 10 to 50 μg/ml, $n = 3$) induced a dose-dependent increase in the frequency of the bursts and a shortening of these, which resulted in no net change in the average time the islet cell spent in the active phase or in the overall frequency of the action potentials. Detailed analysis of the shape of the individual spikes revealed no alterations. The observed effects on burst frequency were poorly reversible even after 30 min of the removal of LQV.

In 5 out of 5 experiments in which CTX (20 nM) was applied to single microdissected islets for as long as 10 min, we were unable to induce steady alterations of the burst pattern of glucose-induced electrical activity from superficial β-cells (Fig. 7). The average frequency of slow waves in the presence of CTX was 109.2 ± 8.7% of the control value ($n = 5$, NS). The frequency of action potentials 5 to 10 min after the islet exposure to CTX was 97.9 ±

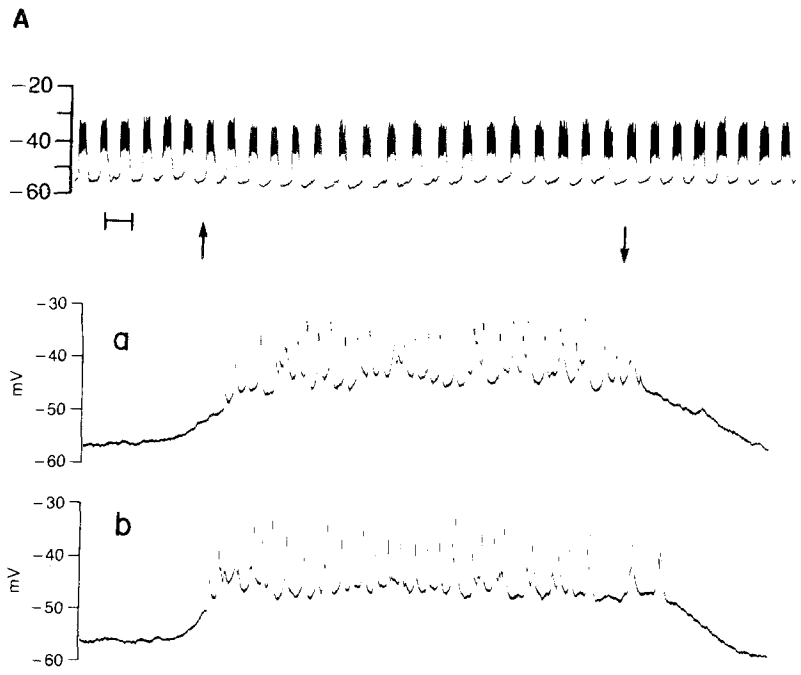

Fig. 7. Lack of CTX effect on glucose-evoked electrical activity in an islet β-cell. (*A*) Membrane potential record from a mouse islet β-cell. The typical burst pattern of glucose-induced electrical activity is not affected by the addition of CTX (20 nM) (arrows) to the perifusing solution. Time scale bar: 30 sec. (*B*) Time-expanded records of single bursts in the control (*a*) and in the presence of CTX (*b*)

1.3% of the control value (n = 4, NS). A transient hyperpolarization, lasting 30 to 50 sec, was observed during the first minute after the application of CTX to the perifusing solution (two out of the five experiments). After this transient effect the slow oscillations of membrane potential returned to the control pattern. CTX did not induce major modification in the shape of action potentials. Since wide variations in spike duration and amplitude occur among cells, we did not measure these parameters. We measured the time constants for spike repolarization by fitting an exponential decay function to the digitized experimental records. Thirty to fifty action potentials were analyzed for each control and each test condition. The decaying phase of the individual spikes was well fitted by a single exponential (in 95% of the action potentials analyzed) with time constants ranging from 4.35 to 22.31 msec. The time constant in the presence of CTX was 104.2 ± 13.5% of the control (n = 4, NS).

Disscussion

The high conductance calcium and voltage-activated K^+ channel from normal rat and mouse β-cells was totally inactive in the potentials expected to be reached by the β-cell membrane during a typical burst of electrical activity induced by glucose (11 mM). Activation of the $K_{(Ca)}$ by depolarization of the membrane patch became evident at estimated membrane potentials positive to 20 mV. This finding is in agreement with previously reported studies conducted in β-cells from rat (Ashcroft, Ashcroft & Harrison, 1988; Tabcharani & Misler, 1989) and ob/ob (obese) mouse (Kukuljan, Li & Atwater, 1990).

Involvement of the $K_{(Ca)}$ channel in the termination of the burst of glucose-evoked electrical activity would require modulation by the hexose of the open-time probability. Thus, in the presence of high glucose concentrations the diminished open-time probability would delay the repolarization which terminates the active phase, assuming a constant depolarizing (inward) current. This kind of glucose-sensitivity of the $K_{(Ca)}$ has been shown to be present in the HIT insulin-secreting cell line and has been contrasted to the nonglucose-sensitive gating of the $K_{(Ca)}$ in RINm5F cells (Eddlestone et al., 1989). However, our results in normal rat and mouse pancreatic β-cells, as well as in human β-cells (I. Atwater, E. Mancilla and M. Kukuljan, *unpublished data*), have not yielded results similar to those reported from HIT cells. Increasing extracellular glucose concentrations from zero to levels as high as 20 mM were ineffective in reducing the open-state probability of the $K_{(Ca)}$. Since initial V_m in the experiments reported by Eddlestone et al. (1989) was clamped at depolarized values by using a high potassium extracellular solution, our results are not completely comparable. Two results presented here, the voltage range of activation of the $K_{(Ca)}$ in cell-attached mode and the lack of effect of glucose argue against the involvement of the $K_{(Ca)}$ in the control of glucose-induced electrical activity. It should be

emphasized that glucose-evoked inhibition of K^+ permeability in mouse islets can be explained by assuming a marginal reduction of open probability for $K_{(Ca)}$ channels (Dawson et al., 1983).

Application of the metabolic uncoupler DNP to cultured β-cells exposed to glucose resulted in the activation of ATP-sensitive K^+ channels. In contrast the activity of the $K_{(Ca)}$ was not affected. The activation of the ATP-sensitive K^+ channels could result from the drop in intracellular ATP concentration and changes in cytosolic pH (Carroll et al., 1988; Misler, Gillis & Tabcharani, 1989). It may be that the ensuing increase in intracellular free calcium is insufficient to activate $K_{(Ca)}$ channels.

Patch-clamp studies have shown that in the presence of intracellular free-calcium concentrations ranging from 0.3 to 2 μM the $K_{(Ca)}$ channel can be activated by depolarization of excised membrane patches (Findlay, Dunne & Petersen, 1985*a*). However, in cell-attached patches this channel behaves as a channel with low sensitivity to calcium, as the one observed in human fibroblasts (Galietta, Galdzicki & Nobile, 1988).

Nonetheless, the different experimental conditions that are inherent to patch-clamp and intracellular membrane potential measurements might preclude a definitive interpretation of the results obtained with the different modalities of the patch-clamp technique.

Tetraethylammonium and quinine, blockers of K^+ currents, were historically considered to be specific blockers of the delayed rectifier and calcium-activated K^+ currents, respectively. The results obtained with these two K^+ channel blockers on glucose-induced electrical activity from mouse pancreatic β-cells provided the basis for the idea that the $K_{(Ca)}$ channel played a role in the genesis of slow oscillations of glucose-induced electrical activity (Atwater et al., 1979*a*; Atwater, Ribalet & Rojas, 1979*b*; Atwater et al., 1983). In addition to those observations, theoretical modeling of β-cell electrical activity provided further support for this hypothesis (Chay & Keizer, 1983; Sherman, Rinzel & Keizer, 1988). However, quinine is a rather nonspecific blocker of $K_{(Ca)}$ channels (Mancilla & Rojas, 1990; Bokvist, Rorsman & Smith, 1990*a*), ATP-sensitive K^+ channels (Findlay et al., 1985*b*; Bokvist et al., 1990*a*) and of the delayed rectifier current (Bokvist, Rorsman & Smith, 1990*b*). The identification of the peptide toxin charybdotoxin as a highly selective blocker of the high conductance $K_{(Ca)}$ channel (Miller et al., 1985) allowed us to resolve the controversy over the role of the $K_{(Ca)}$ in glucose-induced electrical activity in pancreatic β-cells.

Charybdotoxin is a potent blocker of the single $K_{(Ca)}$ currents, acting on the outer aspect of the membrane. The blockade by CTX has a fast onset and is almost completely reversible. Although only preliminary kinetic analysis of the $K_{(Ca)}$ channel activity was performed in the present work, it is apparent that CTX induces a marked increase of the long-lived shut states, without affecting the amplitude and the intraburst kinetics. This observation suggests that the mechanism of blockade is as proposed for the $K_{(Ca)}$ channel from sarcolemma. In this model CTX would plug into the outer mouth of the channel, blocking the passage of ions (Mackinnon & Miller, 1988). Our results contrast with the apparent insensitivity of the $K_{(Ca)}$ from pancreatic islets to LQV concluded on the basis of rubidium fluxes studies (Lebrun et al., 1988). This discrepancy can be explained by the low open probability of the channel in physiological conditions, which renders its contribution to potassium fluxes negligible.

Early measurements of whole-cell K^+ currents in mouse β-cells failed to show a calcium-dependent K^+ current (Rorsman & Trube, 1986). More recent work suggests this current can be seen only if the calcium-buffering capacity of the intracellular medium is kept low (Satin et al., 1989). Using no EGTA in the dialysis solution we were able to record K^+ currents with two components, namely an early transient component and a sustained, noninactivating delayed component similar to the single component observed when EGTA (10 mM) was added to the intracellular solution. The blockade of the transient component by LQV and CTX, at concentrations in the range used to block the single $K_{(Ca)}$ channels allowed the identificiation of this component as the $K_{(Ca)}$ current. The activation curve for the $K_{(Ca)}$ current was similar to that observed at the single-channel level in cell-attached mode. Since the CTX-insensitive remaining component is similar to the outward K^+ currents flowing through delayed rectifier channels, CTX appears to be a rather specific probe to assess the role of $K_{(Ca)}$ in more physiological settings.

It has been shown that the perforated-patch technique allows the measurement of potential or current in single cells circumventing the run-down of intracellular metabolic activity (Falke et al., 1989). Under these conditions, a single β-cell can fire action potentials continuously during long periods of time. Our observation that CTX does not affect spike activity evoked by glucose (spike duration and rate of repolarization) allows us to conclude that $K_{(Ca)}$ channels are not involved in the process of repolarization of the action potentials.

Furthermore, our results with microdissected islets do not differ from those obtained from single cells in culture.

The observations that highly purified CTX does

not modify the burst pattern, burst frequency or the shape of the action potential argue strongly against the participation of $K_{(Ca)}$ in the generation of the burst pattern of glucose-induced electrical activity in islets of Langerhans.

Early experiments with LQV showed an increase in the burst frequency induced by the venom, without affecting the average time spent in the active phase, the principal glucose-sensitive parameter. The effect on the burst frequency observed in the presence of LQV could result from the action of any of the other 50 to 60 components of the crude venom (Smith, Phillips & Miller, 1988).

Mechanisms other than activation of potassium permeability by calcium have been proposed to underlie the genesis of burst pattern, such as calcium-induced calcium channel inactivation (Satin & Cook, 1989) or further closure of ATP-sensitive K^+ channels (Henquin, 1988). However more work is required to distinguish between these alternatives.

The function of high conductance $K_{(Ca)}$ still remains unknown in striated muscle membrane, where a great deal of biophysical research has been carried out. On the other hand, the $K_{(Ca)}$ is thought to be involved in the regulation of secretion in a number of exocrine cells (Latorre et al., 1989; Marty, 1989). Our studies do not discard the possibility of a role for the $K_{(Ca)}$ in the modulation of electrical activity in vivo, when factors other than glucose (i.e., autonomic nervous system) participate in the control of insulin secretion. It is also possible to speculate that the $K_{(Ca)}$ plays a role in conditions leading the cell to a calcium overload. This is suggested by the increase in the $K_{(Ca)}$ open-time probability observed when insulin-secreting cells are exposed to a high extracellular potassium concentration (Smith et al., 1990). Withal, the results presented here indicate that the $K_{(Ca)}$ channel is not involved in the modulation of β-cell membrane potential induced by glucose in steady-state conditions.

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