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Quinine blocks the high conductance, calcium-activated potassium channel in rat pancreatic β-cells

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The $[Ca^{2+}]_i$-activated K^+-channel, one of the 3 K^+-channels described in pancreatic β-cells, is a high conductance, voltage-dependent K^+-channel. Quinine, known to block $[Ca^{2+}]_i$-activated K^+-channels in other cells, has been described to block the silent phase between the bursts of glucose-evoked electrical activity in mouse pancreatic β-cells, and to inhibit K^+ efflux from rat pancreatic islets. We report here that quinine blocks the $[Ca^{2+}]_i$-activated K^+-channel in rat pancreatic β-cells from the external side of the membrane. We also show that the blockade is characterized by fast flickering of the K^+-channel between the open and closed state. Mean open and closed times within bursts were found to be exponentially distributed, suggesting that the blockade by quinine involves obstruction on the K^+ flow through the open to be exponentially distributed, suggesting that the blockade by quinine involves obstruction on the K^+ flow through the open channel.

Quinine; Potassium-channel, $[Ca^{2+}]_i$-activated; β-Cell

1. INTRODUCTION

Three main types of K^+-channels in pancreatic β-cells have been described up to date [1,2]; a highly K^+-selective channel with a maximum conductance of ~300 pS (high K^+ in the pipette), which is activated by internal $[Ca^{2+}]$ and membrane depolarization, a glucose blockable channel which rectifies the K^+ current (high K^+ in the pipette) with conductances of ~55 pS and ~34 pS for inward and outward currents, respectively, whose gating properties are sensitive to internal ATP, and a small conductance channel (~10 pS), which also rectifies the K^+ current. The large conductance K^+-channel has been proposed to play a role in the electrical response to glucose in mouse pancreatic β-cells [3].

Quinine is known to block $[Ca^{2+}]_i$-dependent K^+-channels in red blood cells [4], medullary chromaffin cells [5], cultured medullary thick ascending limb cells [6] and an insulin secreting cell line [7]. Previous studies on mouse pancreatic β-cells have shown that, in the absence of glucose, quinone (100 μM) depolarizes the membrane and changes the glucose (11 mM)-induced burst pattern to continuous electrical activity [3]. Our objective was to further study the effect of quinine on the high conductance, calcium and membrane potential dependent K^+-channel known to be present in rat pancreatic β-cells in culture [8].

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2. MATERIALS AND METHODS

Rat β-cells were obtained from collagenase isolated islets, maintained in culture medium (CMRL 1066 Gibco) supplemented with 5.6 mM glucose for 3–5 days. Patch clamp experiments were done using glass microelectrodes made as described elsewhere [9]. Single K^+-channel measurements were done on excised outside-out patches. The solutions were as follows (mM): 140 KCl, 5 Hepes-KOH, Ca < 1 μM, pH = 7.4 in the pipette; 135 NaCl, 2.6 $CaCl_2$, 1 $MgCl_2$, 5 Hepes-KOH, pH = 7.4 outside the cells. Quinine hydrochloride (Sigma Chemical Co., St. Louis, MO) dissolved in water was used in all the experiments.

Single channel currents were recorded using an EPC-7 patch clamp amplifier (List Electronics, Darmstadt/Eberstadt, FRG), stored in digital form on VCR tapes using a video cassette recorder (PCM/VCR, Sony PCM 501-ES). Current records were digitized using a driver board (Labmaster, Scientific Solutions) installed in a microcomputer (AST 386/20 MHz) driving a 12-bit analog-to-digital converter (TI-1 interface, Axon Instruments Inc., Burlingame, CA). To acquire the records the tapes were played back and the signal was filtered (corner frequency set at 3 kHz), and sampled at 100 μs intervals. Single channel current records were later analyzed using commercially available programs (pClamp, Axon Instruments Inc., Burlingame, CA) to generate the corresponding histograms. Events were included in the database if they were greater than 50% of the set amplitude. The program measured the open and closed time intervals which were used for the histograms.

Amplitudes of the K^+-channel events were measured using a digital storage oscilloscope (model 4094, Nicolet Oscilloscope Division, Madison, WI) with a low-pass filter set at 3 kHz set before the input.

3. RESULTS

We identified β-cells by the presence of the 55 pS glucose-blockable channel in the cell-attached configuration, before breaking the patch to achieve an outside-out configuration [10].

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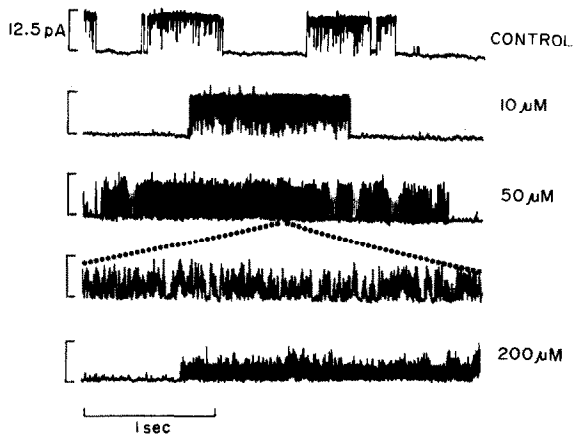

Fig.1. Effect of different concentrations of quinine on the calcium- and voltage-dependent K^+-channel. Upper record was made under control conditions. The second, third and fifth records were made 1–3 min after the addition of quinine to the bath at the concentrations indicated on the right side. The fourth record from the top is a segment of the third (with 50 μM quinine) on an 8-fold expanded time scale. The membrane potential was 30 mV in all the records.

Under our experimental conditions (excised outside-out configuration) we observed two potassium channels characterized by conductances of ca 20 and 155 pS, respectively. While the open probability of the 20 pS channel was rather insensitive to membrane potential, the 155 pS channel was voltage dependent; its fractional open time increased upon depolarization of the patch. The 155 pS channel (fig.1) was active in bursts in most of our membrane patches for long periods of time (~30 min). A burst was defined as a series of successive openings with closures shorter than 5 ms.

Quinine, at doses of 0.01–2 mM was used to block the higher conductance channel, producing a marked increase in the number of brief openings and closures as shown in fig.1 (fourth record from the top). On average, the burst duration increased and the number of bursts per unit time decreased as quinine concentration was augmented up to 0.2 mM. The fraction of time spent in the open bursting state increased substantially for concentrations in the millimolar range. The effect of quinine illustrated in figs 1 and 2 was observed seconds after exposure of the outside-out patch to the drug. The blockade was rapidly reverted when the bath solution containing the blocker was replaced by the control, quinine-free solution.

In addition to the profound increase in the frequency of closures induced by quinine, we detected a decrease in the amplitude of the events (fig.2). To quantify the effect of quinine, the mean amplitude of the single channel current as a function of concentration of the drug at a fixed potential (30 mV) was measured (fig.3). Fig.3 shows fraction of the single K^+-channel current plotted against the negative value of the logarithm of the quinine concentration in the bath. The fitted curve

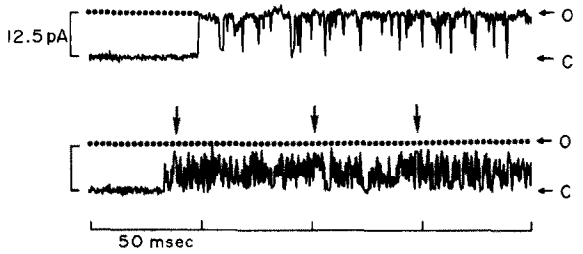

Fig.2. Channel flickering produced by quinine. The upper record was made in control conditions. The lower record was made 2 min after the addition of quinine (50 μM). The membrane potential was set at 0 mV in both cases. The scale is the same for both records. The open state current level of the channel in the absence of quinine is indicated by the dotted lines. Arrows depict complete channel openings.

was calculated using the following formula, based on Clark's occupancy theory [11]:

$$\text{fraction} = 1 - [Q]/(K_d + [Q])$$

[Q] represents the concentration of quinine and K_d the dissociation constant. A K_d of 155 μM gave the best fit of the experimental points.

One exponential function was required to fit the open and closed time histograms. This operation gave mean open and closed times within bursts. Notice in fig.4 the decrease in the mean open time, t_o, with increasing quinine concentration. The mean closed time, t_c, with increasing quinine concentration. The mean closed time, t_c, not shown in fig.4, did not vary significantly with the addition of quinine. The blocking and unblocking rates were calculated as described by Colquhoun [12] where $1/t_o$ is the blocking rate and $1/t_c$ is the unblocking rate in a model where the channel can be blocked only in the open state. That is,

$$\text{Shut} \underset{1/t_c}{\overset{1/t_o}{\rightleftharpoons}} \text{Open} \underset{1/t_o}{\overset{1/t_c}{\rightleftharpoons}} \text{Blocked}$$

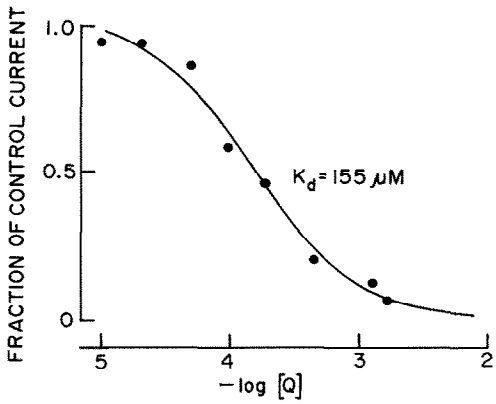

Fig.3. Dose-response curve of the effect of quinine on single channel current amplitude. Each point is the average of at least two experiments on different cells. Percent of control amplitude is plotted against −log[quinine]. Solid curve represents the least-squares regression fit of the data points by the model as explained in the text.

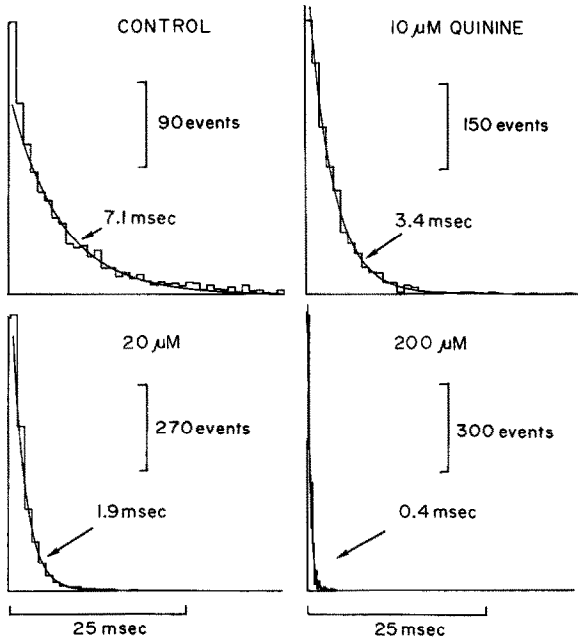

Fig.4. Mean open time histograms in control conditions and after the addition of 10, 20 and 200 μM quinine. Graphs were fitted with a single exponential. Mean open times are written in each figure. Membrane potential was 30 mV.

As shown in fig.5B, the blocking rate increased linearly with increasing concentration of quinine (the slope of the best fit was $2 \times 10^7\ M^{-1} \cdot s^{-1}$). The unblocking rate was not affected by the concentration of quinine (fig.5A). Because of our limited frequency response, our system could resolve event durations of 0.3 ms, therefore concentrations of quinine larger than 0.2 mM were not included in the analysis. Changes in membrane potential did not significantly affect the mean open-time within bursts of the channel in the presence of the drug.

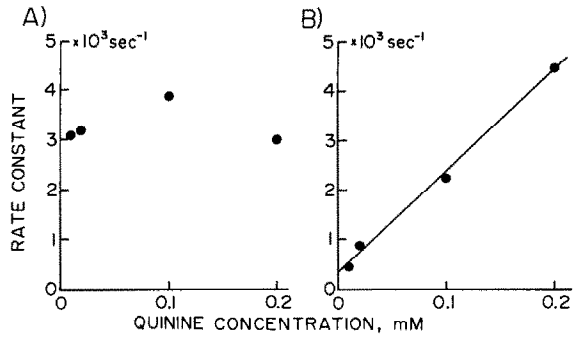

Fig.5. Channel blocking (A) and unblocking (B) rates. $1/t_o$ (blocking rate) and $1/t_c$ (unblocking rate) are plotted against quinine concentration. t_o represents the mean open time of the channel, and t_c, the mean closed time, within bursts. The straight line represents a least-squares regression fit of the linear function:

rate constant = $m[Q] + n$

where $m = 2 \times 10^7\ M^{-1} \cdot s^{-1}$ and $n = 3.3 \times 10^2\ s^{-1}$.

4. DISCUSSION

Our data show that the $[Ca^{2+}]_i$ and membrane potential dependent, high conductance K^+-channel in rat pancreatic β-cells is blocked by quinine from the external side of the membrane. We observed a marked, dose-dependent increase in its frequency of closures. In addition we observed that at a fixed patch potential there was a dose-related decrease in the amplitude of the single channel current. Open and closed time histograms were fitted with one exponential, suggesting a sequential block.

Our results are similar to those from previous work on inside-out membrane patches from medullary chromaffin cells. Indeed, as in chromaffin cells, the blocking rate increased linearly with quinine concentration and the unblocking rate remained constant [5]. However, our results differ in that the magnitude of the rate coefficient for pancreatic β-cells is larger suggesting that quinine is a better blocker of the $[Ca^{2+}]_i$-activated K^+-channel from the external side.

It should not be forgotten that quinine is not a specific blocker of the $[Ca^{2+}]_i$-activated K^+-channel [13]. Previous work in neonatal rat β-cells showed that externally applied quinine (100 μM) induces a marked reduction in the frequency of openings of the glucose blockable K^+-channel [10]. Furthermore, experiments in an insulin-secreting cell line suggested that quinine is a better blocker of the glucose-blockable K^+-channel than the $[Ca^{2+}]_i$-activated K^+-channel [7]. Quinine reduces K^+ efflux from rat islet cells [14], and depolarizes mouse β-cells. Our results suggest that the effect of quinine on intact β-cells could be due at least in part to the blockade of the $[Ca^{2+}]_i$-activated K^+-channel.

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