J. Membrane Biol. 120, 105–114 (1991)
002226319100037N

The Journal of
Membrane Biology

Specificity of Tetraethylammonium and Quinine for Three K Channels in Insulin-Secreting Cells

Sahba Fatherazi and Daniel L. Cook

Division of Metabolism, Seattle Veterans Administration Medical Center, Seattle, Washington 98108, and Departments of Physiology and Biophysics, and of Medicine, University of Washington School of Medicine, Seattle, Washington 98195

Summary. The effects of tetraethylammonium (TEA) and quinine on Ca-activated [K(Ca)], ATP-sensitive [K(ATP)] K channels and delayed-rectifier K current [K(dr)] have been studied in cultured insulin-secreting HIT cells using the patch-clamp technique. K(Ca) and K(ATP) channels were identified in excised, outside/out patches using physiological solutions and had unitary conductances of 60.8 ± 1.3 pS (n = 31) and 15.4 ± 0.3 pS (n = 40), respectively. Macroscopic K(dr) current (peak current = 607 ± 100 pA at +50 mV, n = 14) were recorded in the presence of 100 μM cadmium and 0.5 μM tetrodotoxin. Tetraethylammonium (TEA) blocked all three channel types but was more effective on K(Ca) channels (EC_{50} = 0.15 mM) than on K(ATP) channels (EC_{50} = 15 mM) or K(dr) currents (EC_{50} = 3 mM). Quinine also blocked all three currents but was less effective on K(Ca) channels (EC_{50} = 0.3 mM) while equally effective against K(ATP) channels and K(dr) currents (EC_{50} = 0.025 mM). TEA blocked K(Ca) and K(ATP) channels by reducing their single-channel conductances and decreasing the probability of K(ATP) channel opening. Quinine blocked K(Ca) channels by reducing the single-channel conductance, but blocked K(ATP) channels by reducing the probability of channel opening. Reinterpretation of previous microelectrode studies in light of these findings suggest that, (*i*) only K(ATP) channels are active in low glucose, (*ii*) both K(Ca) and K(dr) channels may assist Ca-spike repolarization, and (*iii*) K(Ca) channels play no role in forming the burst pattern of Ca spiking in the B cell.

Key Words Ca-activated K channels · ATP-sensitive K channels · delayed rectifier · tetraethylammonium · quinine · pancreatic islets

Introduction

Microelectrode studies have demonstrated the importance of membrane potassium permeability in the regulation of membrane electrical activity (Meissner, Henquin & Preissler, 1978; Atwater et al., 1979*a*; Atwater, Ribalet & Rojas, 1979*b*; Henquin & Meissner, 1984) in pancreatic islet B cells. With the advent of the patch-clamp method, three types of K-selective channels have been identified in insulin-secreting cells: ATP-sensitive K channels [K(ATP); Ashcroft, Harrison & Ashcroft, 1984; Cook & Hales, 1984], Ca-activated K channels [K(Ca); Cook, Ikeuchi & Fujimoto, 1984] and delayed-rectifier K channels [K(dr); Rorsman & Trübe, 1986; Zünkler, Trübe & Ohno-Shosaku, 1988]. Before these studies, however, various K channel blockers were used in B cells as probes to pharmacologically determine the possible roles of K currents in creating the complex B-cell electrical response to glucose and other modulators. Quinine, for instance, was first used to implicate Ca-activated K current in B-cell electrical activity (Atwater et al., 1979*a*) based on a report that quinine inhibited Ca-dependent K fluxes in red blood cells (Armando-Hardy et al., 1975) while TEA sensitivity was interpreted as evidence for delayed-rectifier current activation (Atwater et al., 1979*b*). More recently, Findlay et al. (1985*b*) questioned these interpretations of TEA and quinine action by showing that 0.1 mM quinine actually blocks K(ATP) ("Ca-independent") K channels, but not K(Ca) channels. They also showed that 2 mM TEA can block K(Ca) channels, although effects on K(dr) currents were not reported.

In this study, we have determined the dose responses of TEA and quinine to block the three B-cell K currents in HIT cells for two reasons. First, aside from certain peptide toxins (Moczydlowski, Lucchesi & Ravindrau, 1988; Castle, Haylett & Jenkinson, 1989), K channel blockers are generally not specific for particular K channels, and their potency varies from tissue to tissue (Hille, 1984; Cook, 1988). Second, the interpretation of a great deal of published work and the utility of these blockers in future studies depends on a clear definition of the pharmacology of these channels. In the course of these studies we unexpectedly found that while TEA reduces the apparent open-channel conductance of both K(ATP) and K(Ca) channels, it had the additional effect of inhibiting the opening of K(ATP)

channels. Furthermore, we have found that quinine blocks K(Ca) channels by reducing channel conductance while it blocks K(ATP) channels only by reducing the probability of opening. Preliminary accounts of this work have appeared (Fatherazi & Cook, 1989) which parallel recent findings in mouse pancreatic B cells (Bokvist, Rorsman & Smith, 1990*a*,*b*; Henquin, Nenquin & Schmeer, 1990).

Materials and Methods

Cell Culture

HIT cells (Santerre et al., 1981) were obtained at passage 47, maintained in Ham's F12 medium and used at passages 58–80 (as in Satin & Cook, 1989). Single isolated HIT cells were visualized using an inverted phase-contrast microscope at room temperature (22–25°C).

Outside/Out Patch-Clamp Technique

Electronics

Single-channel currents from K(ATP) and K(Ca) channels were recorded from excised outside/out patches using the patch-clamp technique (Hamill et al., 1981) with a Dagan 8900 patch-clamp amplifier (Dagan Instruments, Minneapolis, MN). Patch electrodes were fabricated using soda glass hematocrit microcapillary tubing. Signals were filtered with an 8-pole Bessel filter at 1–2 kHz (Frequency Devices, Haverhill, MA), digitized (MacADios II, G-W Instruments, Somerville, MA; Macintosh IIcx, Apple Computer, Cupertino, CA) and analyzed with amplitude histograms from 5–10 sec data sweeps. Average channel currents were determined and used to plot the dose-response curves. Single-channel conductances (γ) were determined from single channel *I-V*'s based on the amplitude histograms.

Solution

Solutions were designed to mimic the ionic gradients used in microelectrode studies of perifused islet electrical activity while isolating either K(ATP) or K(Ca) channels. The extracellular solution (bath) had the following composition (in mM): 120 NaCl, 5 KCl, 3 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, pH = 7.4. Tetraethylammonium hydrochloride (Sigma, St. Louis, MO) and quinine chloride (Sigma, St. Louis, MO) were dissolved in the bath solution. For studying K(ATP) channels, the intracellular (pipette) solution contained (in mM): 140 KCl, 2 $MgCl_2$, 10 HEPES, 1 EGTA and no added calcium, pH = 7.2. For K(Ca) channels: 140 KCl, 2 $MgCl_2$, 10 ATP, 10 HEPES, 0.2 $CaCl_2$, pH = 7.2.

Whole-Cell Clamp Technique

Whole-cell K(dr) currents were recorded in the presence of 0.1 mM Cd and 0.5 μM tetrodotoxin (TTX) to block inward Ca and Na currents, respectively (Satin et al., 1989). Since the outward current in most cases was >200 pA in amplitude, series resistance compensation (to 80% of control value) was used. Voltage pulses (duration = 100 msec) were used to measure peak outward current and determine current-voltage (*I-V*) relationships. In many cells, a voltage-independent outward current increased slowly with perfusion time indicating activation of K(ATP) channels. This was prevented, without affecting K(dr) current (Trübe, Rorsman & Ohno-Shosaku, 1986) by adding 1 mM tolbutamide to intracellular solutions (Sturgess et al., 1985; Ashford et al., 1986). Data were filtered at 1–3 kHz using an 8-pole Bessel filter and digitized at 1 kHz. Current were linear-leak subtracted and analyzed as described previously (Satin & Cook, 1988).

Solutions

The external bath solution was identical to that used above except that it included 11.1 mM glucose, 0.1 mM Cd to block inward Ca current, and 0.5 μM TTX (Sigma, St. Louis, MO) to block Na current. The internal (pipette) solution contained (in mM): 114 KOH, 114 aspartic acid, 20 KCl, 20 HEPES, 2 $MgCl_2$, 2 ATP, 1 ethyleneglycol-*bis* (B-amino ethyl-ether)-N,N′ tetracetic acid (EGTA; Sigma, St. Louis, MO) and 1 tolbutamide to block K(ATP) current.

Data Analysis

To control for time-dependent changes in channel activity for establishing dose-response curves of channel-blocking effects, test currents were normalized to pre- and post-control values.

Results

Channel and Current Characteristics Under Control Conditions

ATP-Sensitive K Channels

K(ATP) channels with single-channel conductances of 50–65 pS in symmetrical 140 mM K^+ were first described in cardiac muscle (Noma, 1983), and subsequently in neonatal rat pancreatic B cells (Cook & Hales, 1984), mouse B cells (Ashcroft et al., 1984), RINm5F cells (Findlay, Dunne & Petersen, 1985*a*; Ashford et al., 1986) and HIT cells (Ribalet, Ciani & Eddlestone, 1989). In this study, K(ATP) channels had a single-channel conductance of 50–70 pS in symmetrical 140 mM KCl and 15.4 ± 0.3 pS (n = 40) in the extracellular physiological solution (Fig. 1*A*, top panel). The activity of K(ATP) channels of HIT cells also decreased with time, i.e., "ran down," as reported in other insulin-secreting cell (Findlay et al., 1985*a*,*b*; Trübe et al., 1986; Ohno-Shosaku, Zünkler & Trübe, 1987) and cardiac cells (Kakei & Noma, 1984; Trübe & Hescheler, 1984). K(ATP) channel rundown lasted from a few seconds

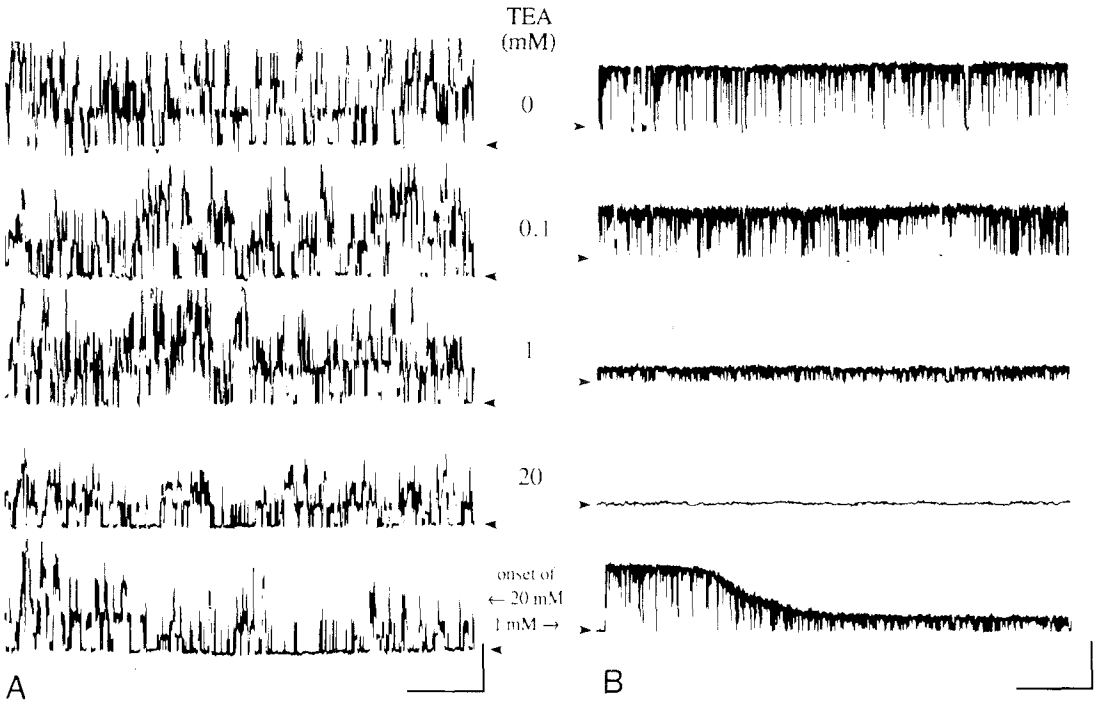

Fig. 1. Dose-dependent effects of TEA on (*A*) K(ATP) and (*B*) K(Ca) channel activity in excised, outside/out patches of HIT cells. Patches were held at 0 mV membrane potential while exposed to 0, 0.1, 1 and 20 mM TEA in the physiological extracellular buffer. To record K(Ca) channels, the intracellular solution (pipette) contained 200 μM Ca and 10 mM ATP. For K(ATP) channels, the intracellular solution contained 1 mM EGTA and no added Ca. Bottom records show the time course of the onset of the TEA effect for each channel as TEA was exchanged in the bath. Vertical scale: *A*: 2 pA; *B*: 4 pA. Horizontal scale: 1 sec. Arrows mark the current level where all channels are closed

to several minutes in different experiments. In some cells, the channel rundown was stable after a few minutes and channels could be recorded for 30–40 min while in other cases rundown rapidly went to completion. To control for rundown in the data analysis, control measures of channel activity were taken before and after perfusion of blockers and the controls averaged to normalize the response.

Ca-Activated K Channels

K(Ca) channels were identified in excised outside/out patches as in rat neonatal B cells (Cook et al., 1984) of the basis on their voltage sensitivity and single-channel conductance (Fig. 1*B*, top panel). The opening probability of this channel is increased by depolarization and increasing intracellular Ca (Cook et al., 1984). In HIT cells, in the presence of 1 mM EGTA with no added Ca (free $[Ca^{2+}] < 1$ nM), activation of this channel required membrane depolarization beyond +10 mV. In order to record this channel at membrane potentials near the range of B-cell spikes (−60 to −10 mV), 0.2 mM Ca was used in EGTA-free intracellular solution. In symmetrical 140 mM K^+, these channels had open-channel conductances (γ) of 200–220 pS which was reduced to 60.8 ± 1.3 pS (n = 31) by using physiological extracellular solutions. The dose-dependent effects of TEA are plotted in Fig. 2, as discussed below.

Delayed-Rectifier K Currents

At least two K(dr) channels (*not shown;* Zünkler, Trübe & Ohno-Shosaku, 1988) exist in HIT cells and contribute to the whole-cell current that we have recorded. We chose to study the aggregate current (Fig. 3), rather than single K(dr) channels, for three reasons. First, K(dr) single-channel currents are small, difficult to resolve and coexist in single patches. Therefore, determining dose responses for individual K(dr) channel subtypes would be extremely difficult if their blocker sensitivities differ. Second, we cannot guarantee that all channels which contribute to whole-cell delayed-rectifier current can be identified and studied at the single-channel level. Third, directly recording the whole-cell current avoids the serious possible errors in reconstructing the whole-cell current from estimates of

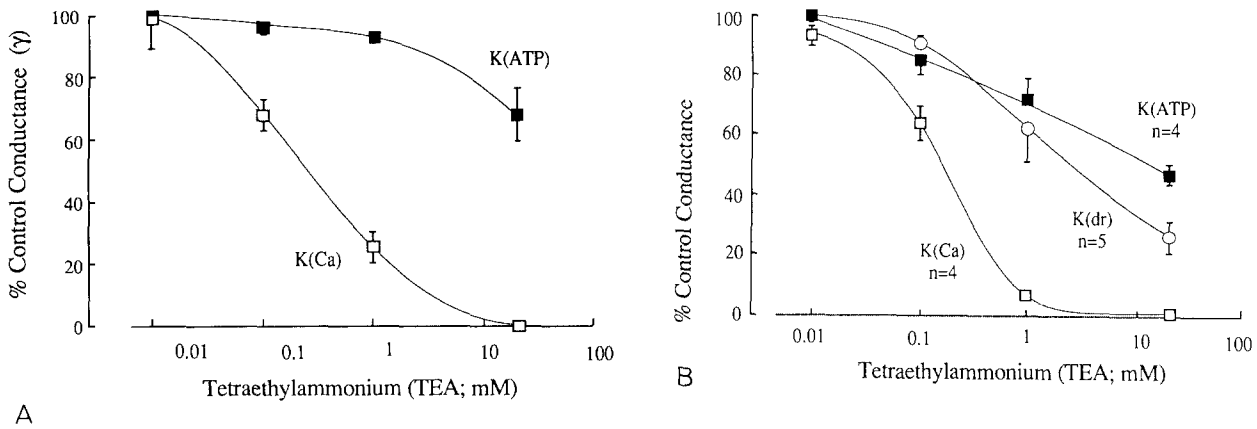

Fig. 2. (*A*) Effects of TEA on the unit conductances of K(ATP) and K(Ca) channels normalized to their control values in zero TEA. Single-channel conductances of K(ATP) and K(Ca) channels were determined from the single-channel *I–V*'s obtained from amplitude histogram analysis. (*B*) Concentration-dependent inhibition of K(ATP), K(Ca) and K(dr) currents by TEA normalized to control responses. K(ATP) and K(Ca) currents were averaged from integration of amplitude histogram peaks. K(dr) currents were measured 100 msec following depolarization to +50 mV. The bars indicate the mean ± SEM from at least four cells in each case

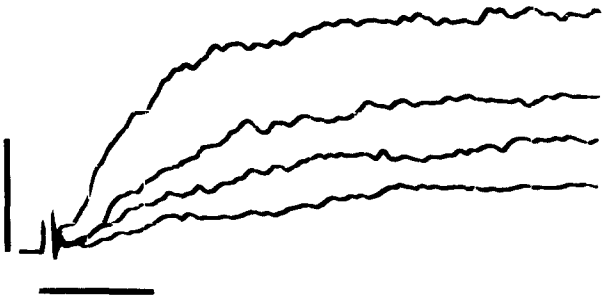

Fig. 3. Whole-cell outward currents in HIT cells in the presence of 0.1 mM Cd to block inward Ca current and subsequent activation of K(Ca) current as used previously (Satin et al., 1989). Currents are activated by 100-msec depolarizing pulses to 0, +5, +10 and +20 from a holding potential of −100 mV to demonstrate the typical delay and sigmoid time course of activation at low voltages with the more rapid activation seen with stronger depolarizations. Vertical scale: 200 pA. Horizontal scale: 20 msec

individual channel conductance, voltage-dependent open probability and channel number.

We have identified K(dr) current as the depolarization-activated outward current seen when Ca uptake through Ca channels is blocked by 0.1 mM Cd (Fig. 3) as used previously in a comparison of HIT cells and mouse B cells (Satin et al., 1989). Under these conditions, the outward current activates at a higher voltage threshold than K(Ca) current and activates with a delay and sigmoid time course unlike the more rapid activation of K(Ca) current (Satin et al., 1989). Despite these consistent differences in voltage- and time-dependent kinetics, we cannot rule out possible contamination of K(dr) current by K(Ca) current activated by prevailing intracellular Ca levels. Such a possibility seems unlikely however because the K_d's for both TEA and quinine turned out to be markedly different for the two channel types (Figs. 2 and 6). For 14 cells, the average K(dr) current amplitude was 607 ± 100 pA at a test potential of +50 mV.

The Effects Tetraethylammonium (TEA)

ATP-Sensitive K Channels

The effects of three concentrations of TEA (0.1, 1 and 20 mM) on K(ATP) channel activity are seen in Fig. 1*A*. A low concentration (0.1 mM) had little effect on K(ATP) channels while 20 mM reduced the open-channel conductance but did not completely block K(ATP) channel activity. The onset of the TEA effect was nearly as rapid as the exchange of the bath solutions (i.e., several seconds; Fig. 1*A*, bottom panel). The effect of TEA was as rapidly reversible where full-channel amplitude and activity were restored within seconds of switching to the TEA-free control solution. There was no visible increase in channel noise in records filtered at 1 kHz as seen with K(Ca) channels (*see below*). Figure 2*A* shows that 1 mM TEA reduced the K(ATP) open-channel conductance (γ) by only 7% (from 14.6 ± 0.9 to 13.6 ± 1.2 pS, n = 3) while the half-maximal inhibitory level was well above 20 mM. TEA was, however, more effective in blocking total patch K(ATP) current (Fig. 2*B*) since 1 mM TEA reduced this current by about 25%. The dose-inhibition curve was, however, very shallow and the half-maximal blocking dose was about 15 mM. The blocking effect

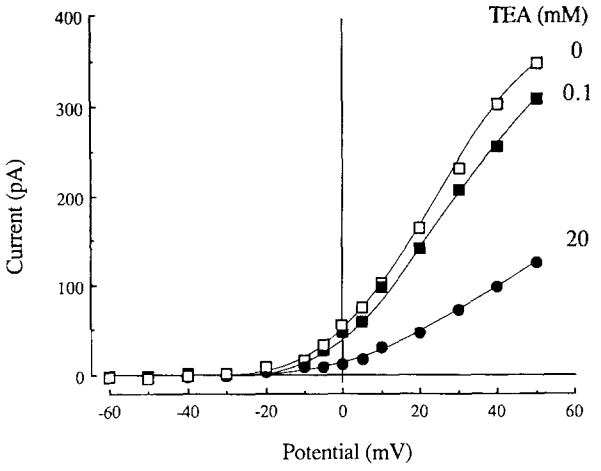

Fig. 4. Effect of TEA on macroscopic K(dr) current a HIT cell. Current-voltage curves measured at 100 msec in the presence of 0, 0.1 and 20 mM TEA

of submillimolar TEA on the total K(ATP) current must reflect inhibition of number of active channels or probability of channel opening although this was not directly measured in this study.

Ca-Activated K Channels

The same doses of TEA were much more effective against K(Ca) channels. The block appeared to be a "flicker block" with increased open-channel noise and reduced single-channel conductance (Fig. 1*B*, middle panels). The onset of the TEA effect was at least as rapid as for K(ATP) channels (Fig. 1*B*, bottom panel) and showed a graded decrease of single-channel conductance as TEA concentration approached its final level in the bath. Channel current amplitude recovered immediately after TEA withdrawal. The effect on open-channel conductance can be seen in Fig. 2*A*, in which 1 mM TEA reduced the K(Ca) single-channel amplitude (0 mV test potential) by 75% (from 51.3 ± 4.4 to 14.0 ± 9.2 pS, $n = 3$) with a half-maximal effect at about 0.25 mM. TEA did not appear to affect the number and probability of channel opening since its effect on total K(Ca) channel current (Fig. 2*B*) nearly coincided (EC_{50} = 0.15 mM) with the effect on open-channel conductances.

Delayed-Rectifier K Current

In doses comparable to those required to block K(ATP) channels, TEA reversibly reduced macroscopic delayed-rectifier current. The low concentration (0.01 mM) had a small effect while 20 mM reduced K(dr) current by only 60–70% (Fig. 4). TEA had no effect on the kinetics of K(dr) activation (*data not shown*). The TEA dose-inhibition curve for whole-cell K(dr) current was moderately shallow (Fig. 2*B*) and approximated the curve for K(ATP) except that its half-maximal level was closer to 3 mM.

The Effects of Quinine

ATP-Sensitive K Channels

Quinine (0.01–1 mM) reversibly reduced the opening probability of K(ATP) channels and possibly the number of active channels (Fig. 5*A*; middle panels) but did not affect K(ATP) channel conductance. In contrast to the effects of TEA, the onset of quinine block required several minutes (Fig. 5*A*; bottom panel; note that the time scale is 25 times slower than on the right). Recovery from quinine often required longer times (*not shown*). The half-maximal inhibitory dose of quinine on average K(ATP) channel current was about 0.025 mM (Fig. 6*B*) and appeared to specifically reflect a reduction of channel-opening probability since there was no effect on K(ATP) channel conductance (Fig. 6*A*).

Ca-Activated K Channels

Quinine blocked K(Ca) channels (Fig. 5*B*; middle panels) but was less effective than on K(ATP) channels. As for TEA, quinine increased open-channel noise while reducing the apparent open-channel conductance, suggesting a flicker block of open channels. The block was dose dependent and nearly complete in 1 mM quinine. Onset and recovery from the quinine effect was rapid (within seconds; Fig. 5*B*; bottom panel). The quinine dose-inhibition curves for both total patch current and open-channel conductance were superimposable (half-maximal at about 0.3 mM; Fig. 6) indicating that quinine simply blocks K(Ca) channel conductance without affecting gating, unlike its effects on K(ATP) channels.

Delayed-Rectifier K Current

Quinine blocked macroscopic delayed-rectifier K currents in the same concentration range that blocked K(ATP) channels (Fig. 7). As for K(ATP) channels, this effect required many minutes to develop and was even more slowly reversible. The half-maximal dose of quinine required to block K(dr) currents was identical to that required to block K(ATP) channel currents (about 0.025 mM; Fig. 6*B*).

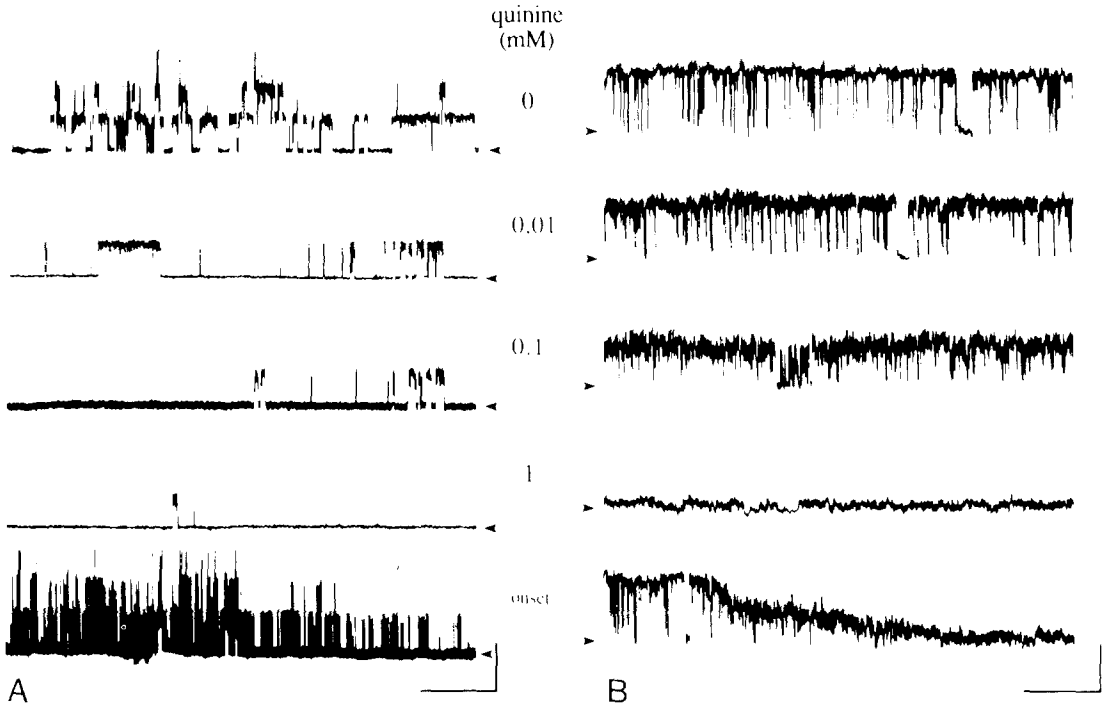

Fig. 5. Effect of quinine (0.01, 0.1, and 1 mM) on (*A*) K(ATP) and (*B*) K(Ca) channels recorded from excised outside/out patches of HIT cells under the same conditions as for Fig. 1. The bottom record shows that the onset of quinine's effect was about 25 times slower for K(ATP) channels (effect of 0.1 mM is shown) than for K(Ca) channels (effect of 1 mM is shown). Vertical scale: *A*: 2 pA; *B*: 4 pA. Horizontal scale: *A*: 25 sec; *B*: 1 sec. Arrows mark the current level where all channels are closed

Discussion

The goal of this study was to determine the dose responses and specificity of TEA and quinine for the three types of B-cell K channels. The unexpected findings were that (*i*) the mechanism of block by quinine was distinctly different for K(Ca) and K(ATP) channels, and (*ii*) that TEA may act in two ways on HIT cell K(ATP) channels as in skeletal muscle K(ATP) channels (Davies et al., 1989). Our observations have implications for the interpretation of previously microelectrode studies of mouse islet cell electrical activity and coincide very well with similar very recent findings in mouse B cells (Bokvist et al., 1990*a*,*b*).

Mechanisms of K Channel Block

Extracellular TEA appears to block open K(Ca) channels by a flicker block with no effect on channel gating. It is thought that such blockers intermittently enter the channel's open pore but, being unable to pass through, prevent the passage of K ions. Transitions between blocked and unblocked states are so fast as to be filtered by the recording apparatus (Hille, 1984). This leads to an apparent reduction of the single-channel conductance and, in most cases, excess open-channel noise (as in Fig. 1*B*).

Higher levels of TEA (20 mM) also reduced the apparent open-channel conductance of K(ATP) channels, suggesting that they too, undergo a flicker block although the flicker noise was of such high frequency as to be entirely filtered during recording (as in Davies et al., 1989). Unexpectedly, TEA had an additional effect to directly reduce the probability of K(ATP) channel opening. This was apparent since total K(ATP) current flow was reduced at levels of TEA (e.g., 0.1 mM) which did not reduce open-channel conductance. This combined effect of TEA differs from the linked effects seen by Davies et al. (1989) in skeletal muscle K(ATP) channels where binding of TEA sustained K(ATP) channel openings while blocking channel conductance. Our results suggest that TEA may interact with one site to inhibit or shorten channel openings and another site for blocking K flux through the open channel.

Quinine also had different effects on K(Ca) and K(ATP) channels. External quinine produced a flicker block of K(Ca) channels, similar to that seen with TEA, which was similarly rapid in onset and recovery. This parallels recent findings with K(Ca)

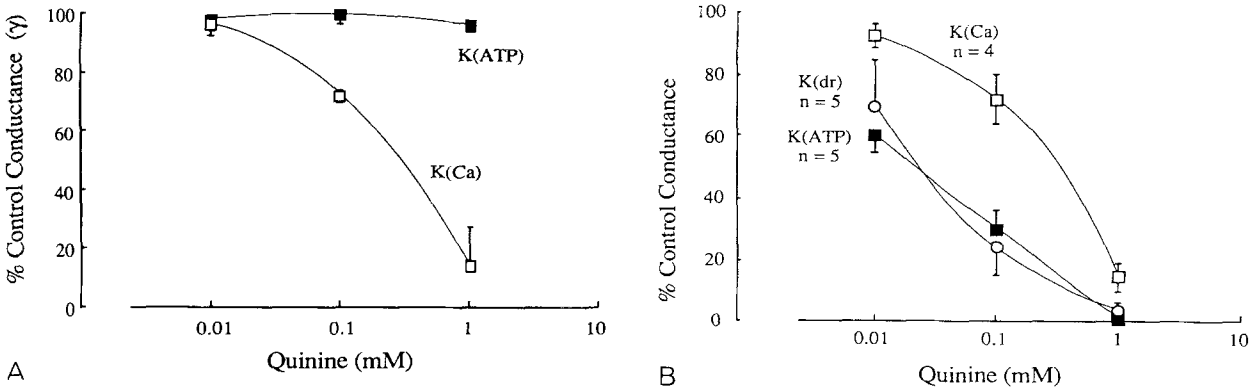

Fig. 6. (*A*) Effects of quinine on the unit conductances of K(ATP) and K(Ca) channels normalized to their control values. (*B*) Concentration-dependent inhibition of K(ATP), K(Ca) and K(dr) currents by quinine analyzed as described in Fig. 2. Values are mean ± SEM for at least four cells

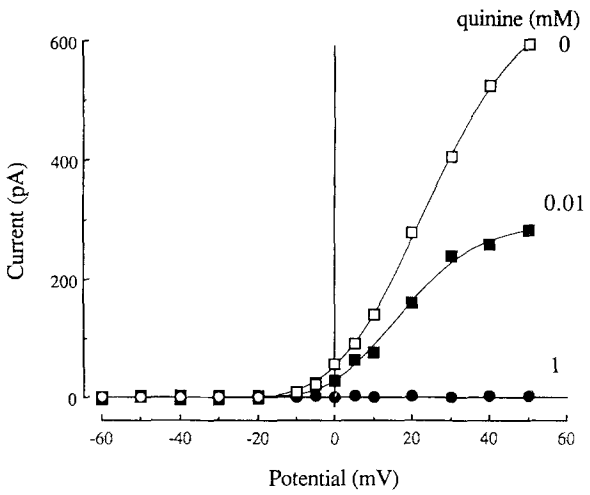

Fig. 7. Effect of quinine (0.1 and 1 mM) on a HIT cell whole-cell K(dr) current-voltage curve in the presence of 100 μM Cd as described in Fig. 3

channels in rat B cells (Mancilla & Rojas, 1990), mouse B cells (Bokvist et al., 1990*a*), bovine chromaffin cells (Glavinovic & Trifaro, 1988) and dissociated gastric smooth muscle cells (Wong, 1989). In contrast (Figs. 5*A* and 6*A*), quinine, to 1 mM, had no effect on K(ATP) channel conductance, but reduced the probability of channel opening as in mouse B-cell K(ATP) channels (Bokvist et al., 1990*a*). A similar effect of internal 0.1 mM quinine has been seen in neonatal rat B-cell membrane patches (D.L. Cook & C.N. Hales; *unpublished observations*). These effects developed and then recovered on a very slow time scale (in minutes) suggesting that quinine may need to accumulate in a pool, perhaps within the cell membrane, before acting on the channels.

While single K(dr) channel characteristics were not reported in this study, we found several parallels between the pharmacology of K(dr) and of K(ATP) channels. In contrast to K(Ca) channels, they both showed the same slow onset and recovery from the quinine effect, and they had virtually the same dose sensitivities to either TEA and quinine. Why these channels share these properties while differing substantially in others (voltage dependence, unitary conductance, sensitivity to intracellular nucleotides, etc.) remains to be determined.

Similar effects have been reported for quinidine, a stereoisomer of quinine, on a variety of K currents: block of total outward K current and single K(Ca) channels in pig pancreatic acinar cells (Iwatsuki & Petersen, 1985), block of delayed-rectifier K current in molluscan pacemaker neurons (Hermann & Gorman, 1984) and block of voltage-dependent K current in *Myxicola* giant axon (Wong, 1981).

Specificity of TEA and Quinine as K Channel Blockers in Insulin-Secreting Cells

In the insulin-secreting HIT cells we have studied, TEA was most effective in blocking K(Ca) channels (half-maximal inhibitory dose about 0.15 mM) compared with K(ATP) (15 mM), and K(dr) current (3 mM). These results confirm and extend those of Findlay et al. (1985*b*), that 2 mM TEA blocked K(Ca) channels with little effect on K(ATP) channels in rat insulinoma RINm5F cells. The dose-response curves of Fig. 2*B* show that moderately specific block of K(Ca) channels occurs only at TEA levels <1 mM. In this range, K(Ca) channels are blocked 90–100% while K(ATP) and K(dr) currents are each blocked less than 30%. The overlap of the dose re-

sponses indicates, however, that caution is required in interpreting TEA effects.

Quinine was not much more selective (Fig. 6*B*). It was nearly equally effective on K(ATP) channels and K(dr) current (half-maximal inhibitory dose 0.025 mM) which were 10-fold more sensitive than K(Ca) channels (0.3 mM). This also agrees with the finding of Findlay et al. (1985*b*) in RINm5F cells that 0.1 mM quinine blocked K(ATP) channels with little effect on K(Ca) channels. Low doses near 0.01 mM would be expected to block 30–40% of these currents while leaving K(Ca) largely unaffected. Doses approaching 1 mM substantially block all three B-cell K channel types.

Use of Quinine and TEA as K Channel Probes in Islet Cell Electrical Activity

By defining the pharmacology of K channels in insulin-secreting cells, the present results, in conjunction with previous (Findlay et al., 1985*b*) and more recent results (Bokvist et al., 1990*a*,*b*; Henquin et al., 1990), can be used to reinterpret previous studies to help answer three important questions. The key doses are 100 μM quinine and 1 mM TEA as used in the previous studies. With a measured K_d for quinine of about 25 μM, 100 μM quinine provides about a 70% block of HIT cell K(ATP) channels (Fig. 6). It may be, then, that in HIT cells these channels are slightly less sensitive to quinine than they are in RINm5F cells (where 100 μM blocks completely; Findlay et al., 1985*b*) or mouse B cells (where 10–20 μM provided nearly complete block; Bokvist et al., 1990*a*) although averaged, complete dose responses were not done in these studies. TEA effects on K(Ca) channels in HIT cells (K_d = 150 μM; Fig. 2) were nearly identical to those seen in mouse B cells (K_d = 140 μM; Bokvist et al., 1990*a*). TEA was nearly equally ineffective against HIT cell K(ATP) channels (K_d = 15 mM; Fig. 2*B*) as against mouse B-cell K(ATP) channels (K_d = 22 mM; Bokvist et al., 1990*a*).

Which K Channels Contribute to and Control the B-Cell Resting Potential in Low (<5 mM) Glucose?

Since their discovery in pancreatic B cells (Ashcroft et al., 1984; Cook & Hales, 1984), a consensus has emerged that K(ATP) channels dominate the resting K conductance in low glucose levels (Petersen & Findlay, 1987; Ashcroft, 1988; Cook et al., 1988). This is supported by the observation that 0.1 mM quinine, which provides a 70% block of K(ATP) channels in HIT cells (Fig. 6*B*) and probably complete block in mouse B cells (*see above*) is sufficient to depolarize B cells, induce Ca spiking (Atwater et al., 1979*a*) and stimulate insulin secretion (Henquin, 1982), even in the absence of glucose. Recent work (Henquin, 1988; Cook & Ikeuchi, 1989) with tolbutamide, which specifically blocks K(ATP) channels, also indicates the block of K(ATP) channels is sufficient to depolarize the cell and trigger spiking in the absence of glucose. The question remains, however, whether K(Ca) channels also contribute to resting K conductance. This appears to be unlikely since Atwater et al. (1979*b*) found that, in the absence of glucose, even 4 mM TEA (sufficient to completely block K(Ca) channels; Fig. 2*B*) did not affect the B-cell resting membrane potential.

Do K(dr) and K(Ca) Channels Contribute to Ca-Spike Repolarization?

Although rapid inactivation of Ca currents (Satin & Cook, 1989) may account for termination of spikes, voltage-activated K currents through K(Ca) and/or K(dr) channels may also contribute. This seems likely since the relatively K(Ca)-selective level of 1 mM TEA increased Ca-spike amplitude (Atwater et al., 1979*b*) while the spikes triggered (in zero glucose) by 0.1 mM quinine [relatively selective for K(dr) *vs.* K(Ca); Fig. 6*B*] are larger than those triggered by glucose-induced depolarization without quinine (Atwater et al., 1979*a*). Furthermore, a parallel effect on spike amplitude of 0.1 mM quinidine (Ribalet & Beigelman, 1980) appears with the same slow (minutes) onset as we have seen for K(dr), but not K(Ca), channels.

Do K(Ca) Channels Control the Clustering of Spikes into Bursts, or Control the Pacing of Bursts?

It is possible (Atwater et al., 1979*a*) that activation of K(Ca) channels by accumulation of free calcium during repetitive Ca spiking is responsible for repolarizing the membrane and terminating the burst of spikes. Our results would argue against this since Atwater et al. (1979*b*) found that 1 mM TEA, which blocks ≈95% of K(Ca) current (Fig. 2*B*; Bokvist et al., 1990*a*), was sufficient to increase spike amplitude, presumably by blocking K(Ca) channels, but did not affect either the burst duration or the interburst interval. Furthermore, recent measurements of intracellular free calcium with the fluorescent dye, Indo-1 (Valdeolmillos et al., 1989), indicate that free calcium level rapidly reaches a stable pla-

teau with the onset of spiking and does not, in fact, continue to increase during the burst of spikes.

We thank Dr. Leslie S. Satin for help with the histogram analysis program and Mr. Louis Stamps for culturing the cells. We also thank Dr. Wil Fujimoto and Ms. Jeanette Teague for generously supplying stock HIT cells obtained from Eli Lilly. S.F. is a fellow of the Juvenile Diabetes Foundation. This work was supported by the Veterans Administration and the NIH.

References

Armando-Hardy, M., Ellory, J.C., Ferreira, H.G., Fleminger, S., Lew, V.L. 1975. Inhibition of the calcium-induced increase in the potassium permeability of human red blood cells by quinine. *J. Physiol.* **250:**32–33

Ashcroft, F.M. 1988. Adenosine 5′-triphosphate sensitive potassium channels. *Annu. Rev. Neurosci.* **11:**97–118

Ashcroft, F.M., Harrison, D.E., Ashcroft, S.J.H. 1984. Glucose-induces closure of single potassium channels in isolated rat pancreatic B-cell. *Nature* **312:**446–448

Ashford, M.L., Sturgess, N.C., Cook, D.L., Hales, C.N. 1986. K-channels in an insulin secreting cell line: Effects of ATP and sulfonylureas. *Adv. Exp. Med. Biol.* **211:**69–76

Atwater, I., Dawson, C.M., Ribalet, B., Rojas, E. 1979*a*. Potassium permeability activated by intracellular calcium ion concentration in the pancreatic beta-cell. *J. Physiol.* **288:**575–585

Atwater, I., Ribalet, B., Rojas, E. 1979*b*. Mouse pancreatic beta-cell: tetraethylammonium blockage of the potassium permeability increase induced by depolarization. *J. Physiol.* **288:**561–574

Bokvist, K., Rorsman, P., Smith, P.A. 1990*a*. Block of ATP-regulated and Ca^{2+}-activated K^+ channels in mouse pancreatic β-cells by external tetraethylammonium and quinine. *J. Physiol.* **423:**327–342

Bokvist, K., Rorsman, P., Smith, P.A. 1990*b*. Effects of external tetraethylammonium and quinine on delayed rectifying K^+ channels in mouse pancreatic β-cells. *J. Physiol.* **423:**311–325

Castle, N.A., Haylett, D.G., Jenkinson, D.H. 1989. Toxins in the characterization of potassium channels. *Trends Neurosci.* **12:**59–65

Cook, D.L., Hales, N.C. 1984. Intracellular ATP directly blocks K channels in pancreatic B-cells. *Nature* **311:**271–273

Cook, D.L., Ikeuchi, M. 1989. Tolbutamide mimics the effect of glucose on B-cell electrical activity: ATP-sensitive K channels may be a common pathway for both stimuli. *Diabetes* **38:**416–421

Cook, D.L., Ikeuchi, M., Fujimoto, W.Y. 1984. Lowering of pH_i inhibits Ca^{2+}-activated K^+ channels in pancreatic B-cells. *Nature* **311:**269–271

Cook, D.L., Satin, L.S., Ashford, M.L.J., Hales, C.N. 1988. ATP-sensitive K^+ channels in pancreatic β-cells. Spare channel hypothesis. *Diabetes* **37:**495–498

Cook, N.S. 1988. The pharmacology of potassium channels and their therapeutic potentials. *Trends Pharmacol. Sci.* **9:**21–28

Davies, N.W., Spruce, A.E., Standen, N.B., Stanfield, P.R. 1989. Multiple blocking mechanisms of ATP-sensitive potassium channels of frog skeletal muscle by tetraethylammonium ions. *J. Physiol.* **413:**31–48

Fatherazi, S., Cook, D.L. 1989. The effects of tetraethylammonium and quinine on ATP-sensitive and Ca-activated K channels in HIT cells. *Diabetes* **38(Suppl. 1):**94*a*

Findlay, I., Dunne, M.J., Petersen, O.H. 1985*a*. ATP-sensitive inward rectifier and voltage- and calcium-activated K^+ channels in cultured pancreatic islet cells. *J. Membrane Biol.* **88:**165–172

Findlay, I., Dunne, M.J., Ullrich, S., Wollheim, C.B., Petersen, O.H. 1985*b*. Quinine inhibits Ca-independent K^+ channels whereas tetraethylammonium inhibits Ca^{2+}-activated K^+ channels in insulin-secreting cells. *FEBS Lett.* **185:**4–8

Glavinovic, M.I., Trifaro, J.M. 1988. Quinidine blockade of currents through Ca-activated K channels in bovine chromaffin cells. *J. Physiol.* **399:**139–152

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Henquin, J.C. 1982. Quinine and the stimulus-secretion coupling in pancreatic B-cells: Glucose-like effects on potassium permeability and insulin release. *Endocrinology* **110:**1325–1332

Henquin, J.C. 1988. ATP-sensitive K^+ channels may control glucose-induced electrical activity in pancreatic B-cells. *Biochem. Biophys. Res. Commun.* **156:**769–775

Henquin, J.C., Meissner, H.P. 1984. Significance of ionic fluxes and changes of membrane potential for stimulus-secretion coupling in pancreatic B-cells. *Experientia* **40:**1043–1052

Henquin, J.C., Nenquin, M., Schmeer, W. 1990. The role of Ca-activated K (K.Ca) channels in glucose-induced electrical activity in B-cells. *Diabetes* **39(Suppl. 1):**139*a*

Hermann, A., Gorman, A.L.F. 1984. Action of quinidine on ionic currents of molluscan pacemaker neurons. *J. Gen. Physiol.* **83:**919–940

Hille, B. 1984. Ionic Channels of Excitable Membranes. pp. 272–302. Sinauer Associates, Sunderland (MA)

Iwatsuki, N., Petersen, O.H. 1985. Inhibition of Ca-activated K channels in pig pancreatic acinar cells by Ba, Ca, quinine and quinidine. *Biochim. Biophys. Acta* **819:**249–257

Kakei, M., Noma, A. 1984. Adenosine-5-triphosphate-sensitive single potassium channels in the atrioventricular node cell of the rabbit heart. *J. Physiol.* **352:**265–284

Mancilla, E., Rojas, E. 1990. Quinine blocks the high conductance, calcium-activated potassium channel in rat pancreatic β-cells. *FEBS Lett.* **260:**105–108

Meissner, P., Henquin, J.C., Preissler, M. 1978. Potassium dependence of the membrane potential of pancreatic B-cells. *FEBS Lett.* **94:**87–89

Moczydlowski, E., Lucchesi, K., Ravindrau, A. 1988. An emerging pharmacology of peptide toxins targeted against potassium channels. *J. Membrane Biol.* **105:**95–111

Noma, A. 1983. ATP-regulated K^+ channels in cardiac muscle. *Nature* **305:**147–148

Ohno-Shosaku, T., Zünkler, B.J., Trübe, G. 1987. Dual effects of ATP on K currents of mouse pancreatic B-cells. *Pfluegers Arch.* **408:**133–138

Petersen, O.H., Findlay, I. 1987. Electrophysiology of the pancreas. *Physiol. Rev.* **67:**1054–1116

Ribalet, B., Biegelman, P.M. 1980. Calcium action potentials and potassium permeability activation in pancreatic B-cells. *Am. J. Physiol.* **239:**C124–C133

Ribalet, B., Ciani, S., Eddlestone, G.T. 1989. ATP mediates both activation and inhibition of K(ATP) channel activity via cAMP-dependent protein kinase in insulin-secreting cell lines. *J. Gen. Physiol.* **94:**693–717

Rorsman, P., Trübe, G. 1986. Calcium and delayed potassium currents in mouse pancreatic beta-cells under voltage-clamp conditions. *J. Physiol.* **374:**531–550

Santerre, R.F., Cook, R.A., Crisel, R.M.S.D., Sharp, J.D., Schmidt, R.J., Williams, D.C., Willson, C.P. 1981. Insulin synthesis in a clonal cell line of Simian virus 40-transformed hamster pancreatic B-cells. *Proc. Natl. Acad. Sci. USA* **78:**4339–4343

Satin, L.S., Cook, D.L. 1988. Evidence for two Ca currents in insulin-secreting cells. *Pfluegers Arch.* **411:**401–409

Satin, L.S., Cook, D.L. 1989. Calcium current inactivation in insulin-secreting cells is mediated by calcium influx and membrane depolarization. *Pfluegers Arch.* **414:**1–10

Satin, L.S., Hopkins, W.F., Fatherazi, S., Cook, D.L. 1989. Expression of a rapid, low-voltage threshold K current in insulin-secreting cells is dependent on intracellular calcium buffering. *J. Membrane Biol.* **112:**213–222

Sturgess, N.C., Ashford, M.L.J., Cook, D.L., Hales, C.N. 1985. The sulphonylurea receptor may be an ATP-sensitive K channel. *Lancet* **2:**474–475

Trübe, G., Hescheler, J. 1984. Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pfluegers Arch.* **401:**178–184

Trübe, G., Rorsman, P., Ohno-Shosaku, T. 1986. Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic beta-cells. *Pfluegers Arch.* **407:**493–499

Valdeolmillos, M., Santos, R.M., Contreras, D., Soria, B., Rosario, L.M. 1989. Glucose-induced oscillations of intracellular Ca^{2+} concentration resembling bursting electrical activity in single mouse islets of Langerhans. *FEBS Lett.* **259:**19–23

Wong, B.S. 1981. Quinidine interaction with *Myxicola* giant axons. *Mol. Pharmacol.* **20:**98–106

Wong, B.S. 1989. Quinidine block of calcium-activated potassium channels in dissociated gasteric smooth muscle cells. *Pfluegers Arch.* **414:**416–422

Zünkler, B.J., Trübe, G., Ohno-Shosaku, T. 1988. Forskolin-induced block of delayed rectifying K^+ channels in pancreatic beta-cells is not mediated by cAMP. *Pfluegers Arch.* **411:**613–619

Received 30 March 1990; revised 10 September 1990