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Inhibition of Ca^{2+}-activated K^+ channels in pig pancreatic acinar cells by Ba^{2+}, Ca^{2+}, quinine and quinidine

N. Iwatsuki * and O.H. Petersen **

M.R.C. Secretory Control Research Group, The Physiological Laboratory, University of Liverpool, P.O. Box 147, Brownlow Hill, Liverpool, L69 3BX (U.K.)

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Patch-clamp whole-cell and single-channel current recordings were made from pig pancreatic acinar cells to test the effects of quinine, quinidine, Ba^{2+} and Ca^{2+}. Voltage-clamp current recordings from single isolated cells showed that high external concentrations of Ba^{2+} or Ca^{2+} (88 mM) abolished the outward K^+ currents normally associated with depolarizing voltage steps. Lower concentrations of Ca^{2+} only had small inhibitory effects whereas 11 mM Ba^{2+} almost blocked the K^+ current. 5.5 mM Ba^{2+} reduced the outward K^+ current to less than 30% of the control value. Both external quinine and quinidine (200–500 μM) markedly reduced whole-cell outward K^+ currents. In single-channel current studies it was shown that external Ba^{2+} (1–5 mM) markedly reduced the probability of opening of high-conductance Ca^{2+} and voltage-activated K^+ channels whereas internal Ba^{2+} ($6 \cdot 10^{-6}$ to $3 \cdot 10^{-5}$ M) caused activation at negative membrane potentials and inhibition at positive potentials. Quinidine (200–400 μM) evoked rapid chopping of single K^+ channel openings acting both from the outside and inside of the membrane and in this way markedly reduced the total current passing through the channels.

Introduction

In epithelial transport studies specific blockers of transport pathways can be helpful. Barium ions (Ba^{2+}) have been used in many studies to reduce K^+ conductance [1–5], but no information has been available about the type of K^+ channel involved and it has not been possible from previous studies to reach conclusions about the mode of action.

In pig pancreatic acinar cells the K^+ conductance of the plasma membrane is entirely accounted for by one type of K^+ channel which has been characterized in considerable detail in recent patch-clamp single-channel and whole-cell current recording studies [6–8]. In the resting pig pancreatic acinar cells there is a relatively small number (< 100) of active K^+ channels with a high unit conductance (maximal conductance about 250 pS) and these channels are voltage-dependent, (depolarization causing opening) and activated by increasing the internal Ca^{2+} concentration [6,7]. Tetraethylammonium inhibits the Ca^{2+}-activated K^+ channels in rat lacrimal acinar cells [9] as well as in the pig pancreatic acinar cells [8]. In the pancreatic cells it was shown that tetraethylammonium is acting specifically from the outside of the plasma membrane to reduce the probability of channel opening as well as to diminish the unit conductance [8].

The purpose of the study reported here was to

* Present address: 1st Department of Physiology, Tohoku University Medical School, Sendai, Japan.
** To whom correspondence should be addressed.

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

investigate the action of Ba^{2+} on the Ca^{2+}-activated K^+ channels in acinar cells. The action of Ba^{2+} has been compared with that of Ca^{2+} itself and in addition the effects of a very different K^+-channel inhibitor quinine and its stereoisomer quinidine, were investigated. Quinidine is known to reduce stimulant-evoked K^+ efflux from salivary glands [10] due to Ca^{2+}-activated K^+ channels [11], but has otherwise not been much used in epithelial transport studies. The data show that Ba^{2+}, Ca^{2+}, quinine and quinidine all inhibit the Ca^{2+}-activated K^+ channels and all act on both sides of the membrane. A preliminary report of some of the results has been made [12].

Methods

All experiments were carried out on isolated pig pancreatic acinar cells or cell clusters obtained from slaughterhouse material as previously described [6–8]. Patch-clamp single-channel and whole-cell current recordings were carried out using the methods described by Neher and collaborators [13,14] and the particular details of our approach have been given in recent publications [6–8]. The 'extracellular' Na^+-rich solution contained (mM): 140 NaCl, 4.7 KCl, 1.2 $CaCl_2$, 1.13 $MgCl_2$, 10 glucose, 10 Hepes (pH 7.2). The 'intracellular' K^+-rich solution contained (mM): 145 KCl, 10 NaCl, 1.13 $MgCl_2$, 10 glucose, 10 Hepes (pH 7.2). No Ca^{2+} was added and EGTA was present (2 to 15 mM). When Ba^{2+} or additional Ca^{2+} was added to the extracellular solution the Na^+ concentration was reduced so as to maintain constant osmolarity. In some of the experiments the free concentration of Ba^{2+} in 'intracellular' media ($[Ba^{2+}]_i$) was determined by the addition of $BaCl_2$ and EGTA buffer mixtures. $[Ba^{2+}]_i$ was calculated using an

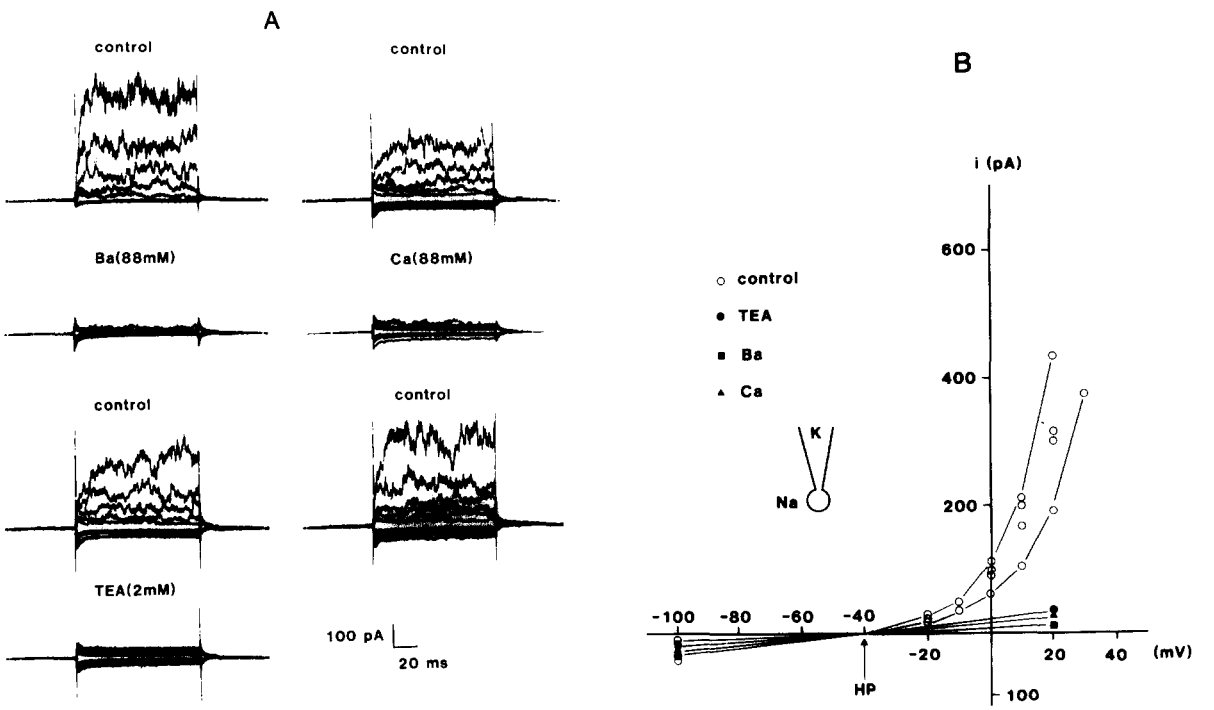
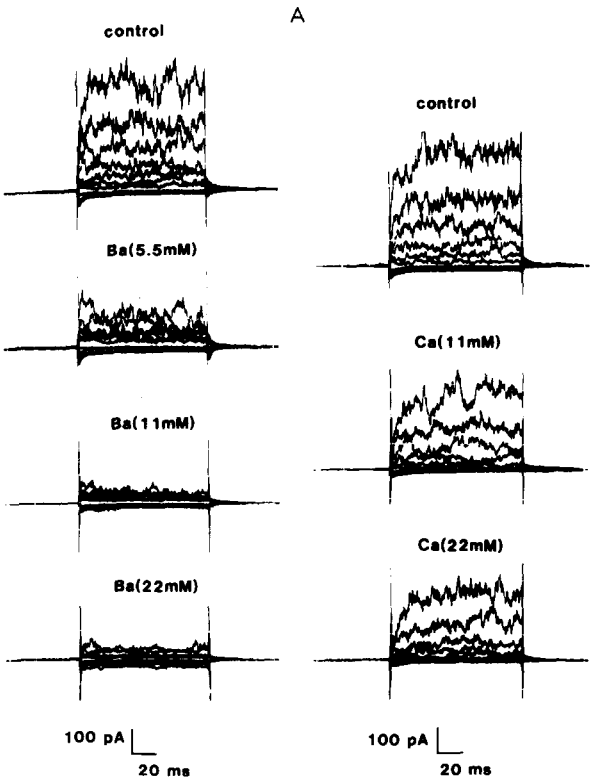


Fig. 1. Voltage-clamp current recordings from a single isolated acinar cell. The holding potential was −40 mV and voltage jumps lasting 100 ms to membrane potentials ranging from −100 to +20 mV were carried out. The consecutive traces in (A) (all obtained on the same cell) show the currents associated with these voltage jumps and the curves displayed in (B) record the relationship between the change in steady-state current and the membrane potential when the potential is changed from −40 mV to higher or lower values. The pipette contained the K^+-rich intracellular solution whereas the Na^+-rich extracellular solution was present in the bath (control). The effect of replacing Na^+ by Ba^{2+} or Ca^{2+} is shown and for comparison the recordings obtained after addition of tetraethylammonium (TEA, 2 mM) to the control solution are also included. Upward deflections represent outward current.



iterative procedure on a BBC (model B) microcomputer using the stability constants for all of the reactions between Ba, Ca, Mg, H and EGTA as previously described for the calculation of $[Ca^{2+}]_i$ [15].

Results

Effects of Ba^{2+} and Ca^{2+}

Fig. 1 shows that Ba^{2+} or Ca^{2+} in as high a concentration as 88 mM in the bath solution, abolishes the large outward whole-cell K^+ currents associated with depolarizing voltage steps. The reversible effect of this high Ba^{2+} or Ca^{2+} concentration is very similar to the previously described action of tetraethylammonium [8] which is also shown in Fig. 1 for comparison. In four separate cells all outward currents were blocked by 88 mM Ba^{2+} and in three different cells 88 mM Ca^{2+} was seen to be as effective as Ba^{2+} in suppressing the K^+ current. When lower concentrations of Ba^{2+} and Ca^{2+} were tried (Fig. 2) it became clear that Ba^{2+} was much more potent than Ca^{2+}. Whereas 22 mM Ca^{2+} only caused a modest reduction in voltage-activated outward K^+ current, 22 mM Ba^{2+} virtually abolished the current and the effect of 11 mM Ba^{2+} was almost the

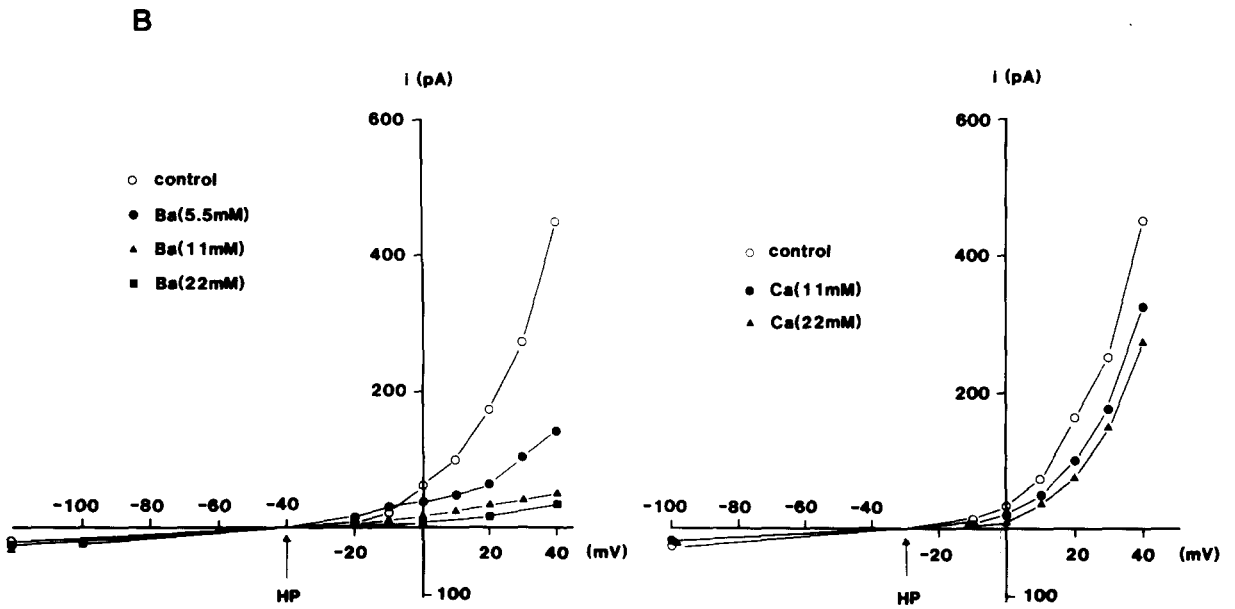


Fig. 2. The effects of external Ba^{2+} (5.5 to 22 mM) and Ca^{2+} (11 and 22 mM) on the whole-cell currents evoked by voltage jumps from the holding potential of −40 mV. Details as in Fig. 1.

same. Even at a concentration of 5.5 mM, Ba^{2+} evoked a very marked reduction in the outward currents (Fig. 2) and 1 mM Ba^{2+} clearly reduced the current in five separate cells. The results shown in Fig. 2 are typical of the 22 experiments of this kind which were carried out.

In single-channel current recording experiments on three excised outside-out membrane patches, Ba^{2+} (0.5 to 5 mM) added to the bath solution evoked the appearance of prolonged periods of channel closure, but had little effect on the amplitude of the currents (Fig. 3). In experiments on excised inside-out membrane patches where Ba^{2+} could be added to the solution in contact with the membrane inside very low concentrations of Ba^{2+} had marked effects. In these experiments solutions highly buffered by EGTA (10–15 mM) were used so that $[Ba^{2+}]_i$ could be precisely defined. No Ca^{2+} was added and $[Ca^{2+}]_i$ was therefore extremely low ($< 10^{-9}$ M). In the absence of Ba^{2+}, with such a very low $[Ca^{2+}]_i$ it was necessary to make the membrane potential positive in order to observe channel opening [8] and very positive potentials (+40 to +60 mV) were normally needed in order to attain an open-state probability (p) above 0.1 (Fig. 4). It has previously been shown that an increase in $[Ca^{2+}]_i$ (for example from 10^{-8} to 10^{-7} M) evokes a marked shift to the left of the curve relating p to membrane potential (i.e. less positive membrane potentials are required in order to attain the same value of p) [6] and Fig. 4 now shows that Ba^{2+} can evoke the same type of effect.

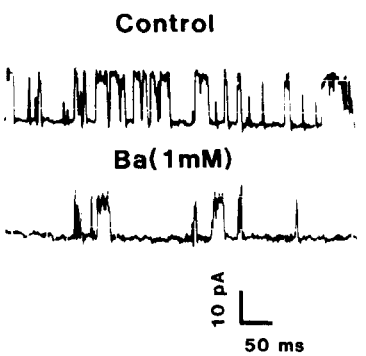


Fig. 3. Single-channel current recording from excised outside-out membrane patch. Membrane potential: +50 mV. The effect of 1 mM Ba^{2+} added to the bath solution (in contact with membrane outside) is shown.

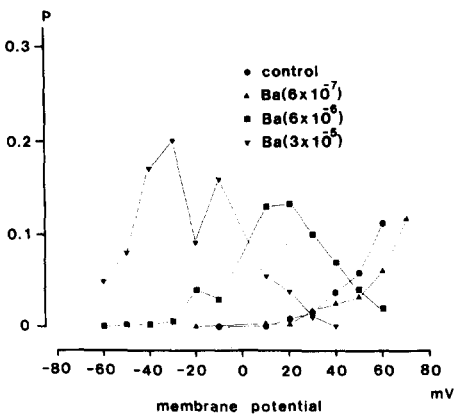


Fig. 4. Summary of results relating channel open-state probability to membrane potential obtained from an excised inside-out membrane patch exposed to K^+-rich intracellular solutions on both sides. The effects of varying $[Ba^{2+}]$ (in mol/l) in the bath solution (in contact with membrane inside) is shown.

The presence of $6 \cdot 10^{-7}$ M Ba^{2+} has very little effect, but at $6 \cdot 10^{-6}$ M it is seen that a p value above 0.1 was attained already at +10 mV whereas in the control situation +60 mV was required in order to obtain such activity. At $[Ba^{2+}]_i = 3 \cdot 10^{-5}$ M channel opening could be readily observed at negative membrane potentials. The maximal value of p that could be attained with Ba^{2+} stimulation, however, was very low (about 0.2) compared with Ca^{2+} activation where values approaching 1 have been reported [6]. Fig. 4 shows that in the presence of $6 \cdot 10^{-6}$ M, Ba^{2+}, reduction of the membrane potential from −40 to −20 mV increases p and reversal of polarity to +20 mV again increases this value, whereas further polarization to +30, +40 and +60 mV evokes a marked decrease in p. This aspect of Ba^{2+} action is illustrated in Fig. 5 with examples of single-channel currents recorded in the presence of $[Ba^{2+}]_i = 6 \cdot 10^{-6}$ M at positive membrane potentials. It is seen that the more positive the potential becomes the greater is the tendency to observe long periods of channel closure. The results shown in Figs. 4 and 5 are typical of 29 experiments on excised inside-out membrane patches.

Effects of quinine and quinidine

Fig. 6 shows that quinidine (500 μM) markedly reduces the whole-cell outward K^+ current associ-

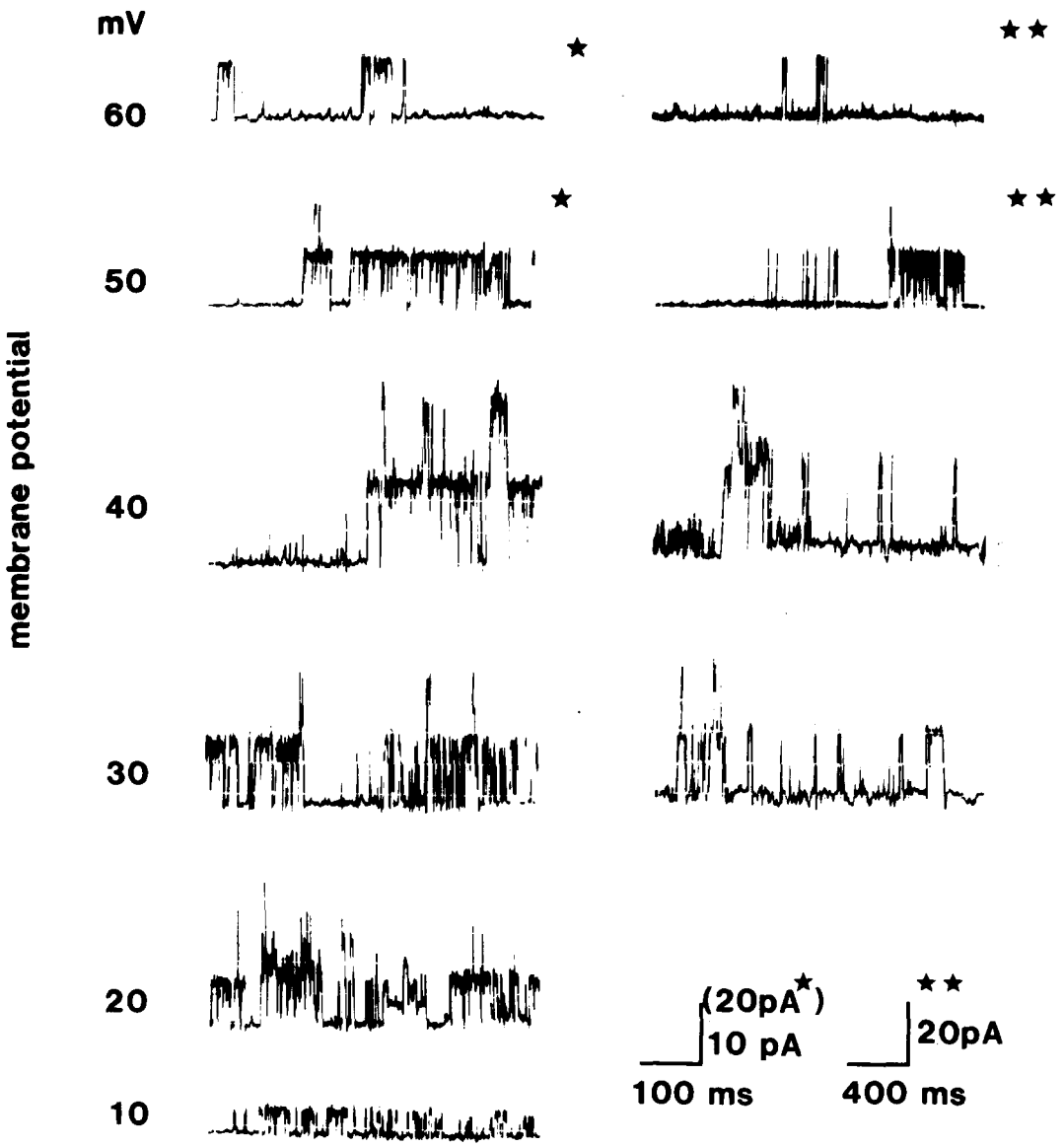


Fig. 5. Single-channel current traces from an excised inside-out membrane patch exposed to K^+-rich solutions on both sides of the membrane. The bath solution (in contact with membrane inside) contained Ba^{2+} ($6 \cdot 10^{-6}$ M). Selections of typical traces at different positive membrane potentials are shown.

ated with depolarizing voltage steps. The outward current evoked by a voltage jump from −40 mV to +30 mV was reduced to about 30% of the control value by this concentration of quinidine. Quinine (200–400 μM) caused very similar effects. Three experiments on three separate cells of this type giving similar results were carried out.

In single-channel current recording experiments on excised membrane patches, quinidine had inhibitory effects when acting on both the inside and outside of the plasma membrane. Fig. 7 shows that 200 μM quinidine added to the outside of the membrane reduced the total K^+ current flowing through the patch membrane by chopping the single-channel events so that what in the control situation appeared as a real single-channel current

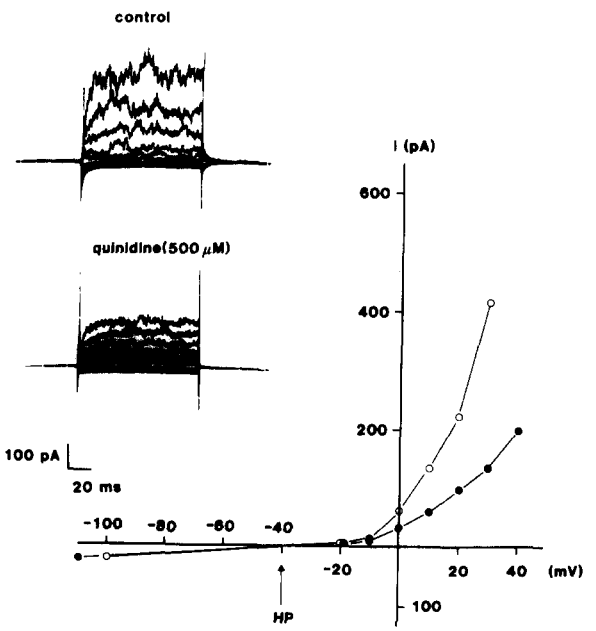


Fig. 6. The effect of external quinidine on the whole-cell currents associated with voltage jumps from a holding potential of −40 mV.

recording was transformed by quinidine into a rapidly fluctuating noise pattern. Fig. 8 shows that very similar effects were evoked by 100 and 400 μM quinidine added to the solution in contact with the inside of the membrane. The effect of 400 μM quinidine was more pronounced than that seen at the 100 μM concentration. Such effects of quinidine (Figs. 7 and 8) were observed in four excised membrane patches. An experiment was also carried out in the cell-attached configuration (i.e. recording from an electrically isolated membrane patch on the intact cell) where quinidine was added to the bath so that it could not directly get in contact with the membrane area from which the recording was made. Also in this case quinidine exerted its inhibitory effect (Fig. 9) indicating that it must be able to penetrate the cell membrane and in this way reach the channel from which recording was made.

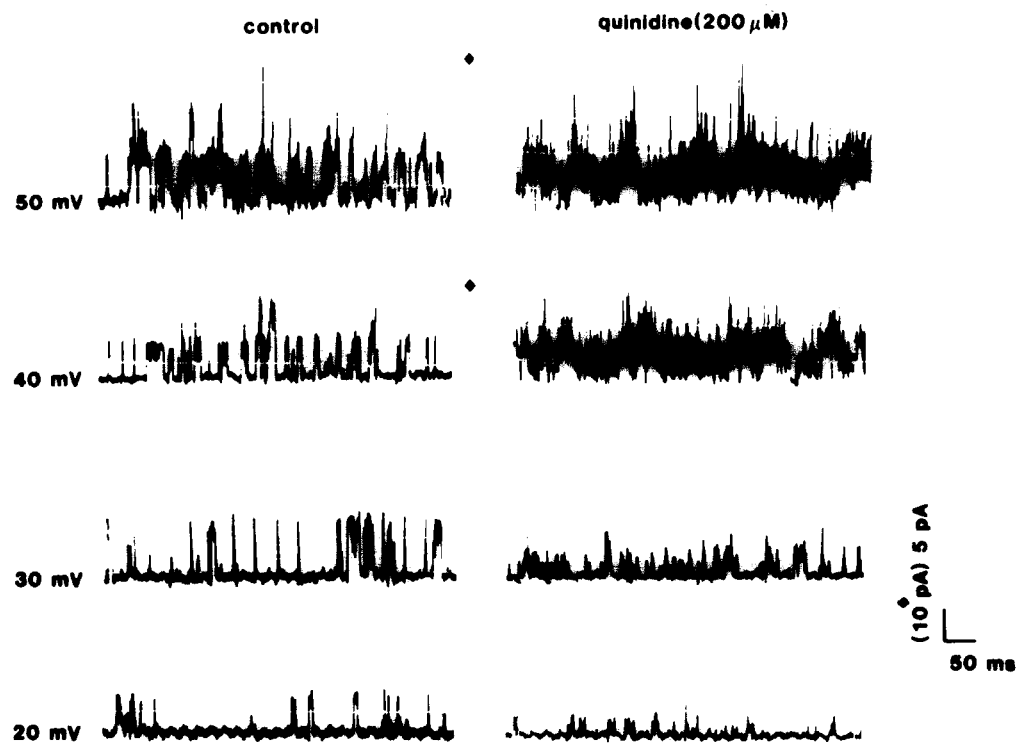


Fig. 7. Single-channel current traces from an excised outside-out membrane patch exposed to K^+-rich intracellular solutions on both sides. The effect of external quinidine (200 μM) is shown.

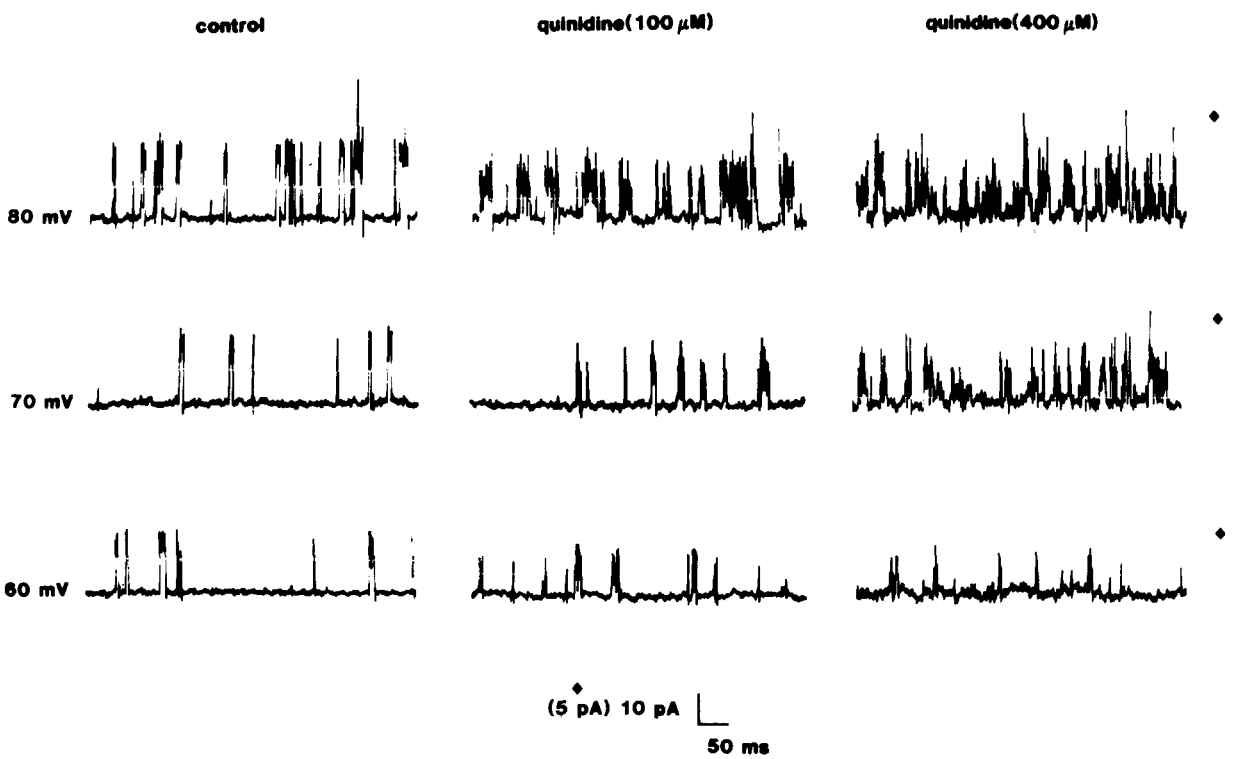


Fig. 8. Single-channel current traces from an excised inside-out membrane patch exposed to K^+-rich intracellular solutions on both sides. The effect of quinidine (100 and 400 μM) added to the bath solution (in contact with membrane inside) is shown.

Discussion

Our results demonstrate that Ba^{2+}, Ca^{2+}, quinidine and quinine can markedly reduce the K^+ conductance of the pig pancreatic acinar plasma membrane by inhibiting the high conductance Ca^{2+}- and voltage-activated K^+ channels that dominate the electrical characteristics of these cells as well as those from the very similar human pancreatic acinar tissue [16].

The inhibitory effect of external Ba^{2+} and Ca^{2+} may at first appear similar to that recently described for tetraethylammonium (Fig. 1) [8], but the mechanisms of action are very different. Tetraethylammonium acts specifically on the outside of the membrane [8] whereas Ba^{2+} has pronounced effects on both sides. Ba^{2+}, acting from the inside of the membrane, can evoke K^+ channel activation (Fig. 4) in a manner qualitatively similar to that previously demonstrated for Ca^{2+} [6,16] but Ba^{2+} is less potent than Ca^{2+} in promoting channel opening. Increasing $[Ca^{2+}]_i$ from below 10^{-9} to 10^{-8} M already causes a clear shift to the left of the curve relating channel open-state probability (p) to membrane potential [16] and a change from 10^{-8} to 10^{-7} M evokes marked activation [6]. $6 \cdot 10^{-7}$ M Ba^{2+} had very little effect on p and only at a concentration of $6 \cdot 10^{-6}$ M was there a marked shift to the left of the curve relating p to membrane potential (Fig. 4). It has previously been shown that internal Ca^{2+} at concentrations of 10^{-7} M and above can evoke prolonged channel closure at very positive membrane potentials [6] and this is now also shown for Ba^{2+} (Figs. 4 and 5). In the case of Ba^{2+} this phenomenon is quantitatively very important and effectively limits the degree of channel opening that can be achieved (Fig. 4). The blocking action of Ba^{2+} from the inside appears to be similar to that evoked from the outside only here much higher concentrations are needed (Fig. 3). The inhibitory effects of Ba^{2+} on Ca^{2+}-activated K^+ channels was previously ob-

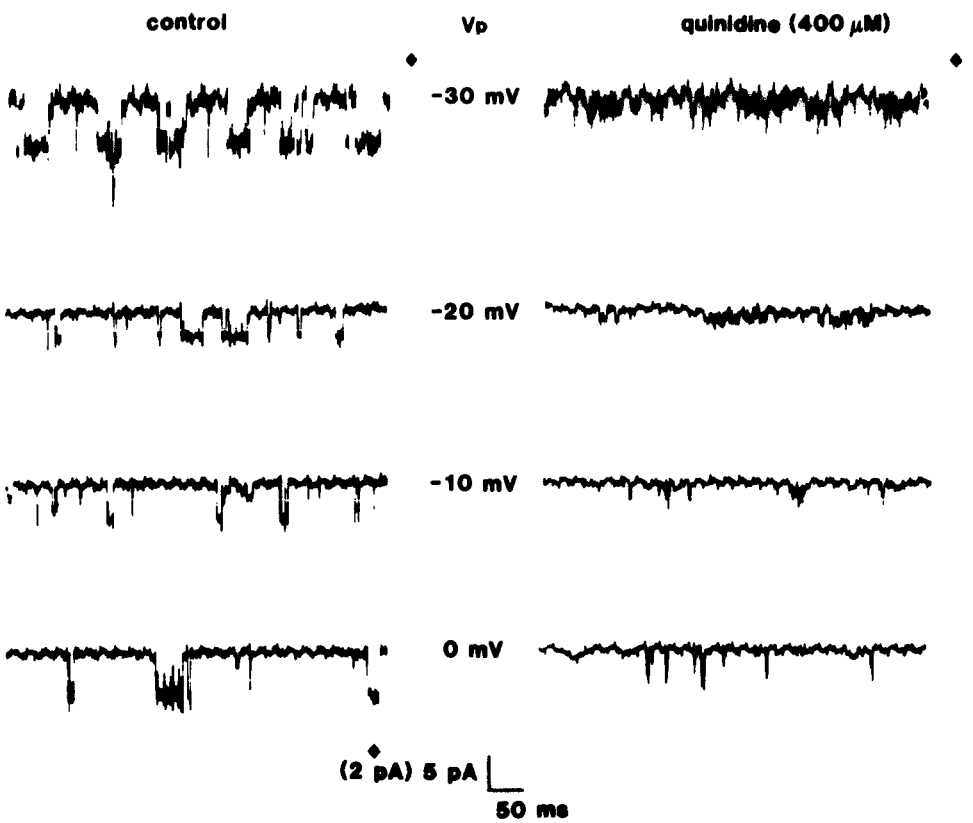


Fig. 9. Single-channel current traces from cell-attached membrane patch. The pipette contained K^+-rich intracellular solution (in contact with membrane outside) and the bath was filled with the Na^+-rich extracellular solution (in contact with cell exterior except for the membrane area covered by pipette tip). Single-channel currents recorded at different pipette voltages (V_p) are shown. (The potential difference across the patch membrane is not known exactly, the intracellular potential (V_m) is about -40 mV and the patch membrane potential can be calculated as the difference between V_m and V_p). The effect of adding quinidine (400 μM) to the external bath solution is shown.

served in a study of channels from rabbit striated muscle incorporated into planar bilayers where it could be shown that micromolar concentrations of Ba^{2+} added to the Ca^{2+}-sensitive side (probably corresponding to the normal inside) blocked the channel whereas millimolar concentrations were needed from from the other side [17]. Ca^{2+} and Ba^{2+} act in a similar manner on the high-conductance K^+ channels, but Ca^{2+} is far more potent than Ba^{2+} as an activator whereas Ba^{2+} is much more effective as a blocker.

Quinine has been regarded as a specific blocker of Ca^{2+}-activated K^+ permeability based on studies in erythrocytes and pancreatic islet cells [18,19], but it has recently been shown that a low concentration (100 μM) completely inhibits a Ca^{2+}-independent inward rectifier K^+ channel in insulin-secreting cells with relatively little effect on the high conductance Ca^{2+}- and voltage-activated K^+ channel [20]. It is also known that the D-stereoisomer to quinine, quinidine, has effects on a number of different ionic currents of molluscan pacemaker neurons [21]. Both quinidine and quinine cause a marked reduction in the overall K^+ conductance in the pig pancreatic acinar cells (Fig. 6). The single-channel current recording experiments show that quinidine acts from both the inside and outside by chopping individual channel opening events (Figs. 7 and 8) and experiments in the cell-attached configuration indicate that quinidine can penetrate through the plasma membrane and in this way reach the isolated patch membrane (Fig.

9). The ability of quinidine to inhibit Ca^{2+}-activated K^+ channels could explain its effect in reducing stimulant-evoked K^+ release from salivary gland cells [10] which have the same type of K^+ channel as the pig pancreatic acinar cells [11].

Ca^{2+}-activated K^+ channels seem to play an important role in regulating fluid secretion [22] and in this context it is interesting that it has recently been shown that Ba^{2+} virtually abolishes acetylcholine-evoked fluid secretion in perfused rat salivary glands [23]. Inhibitors of Ca^{2+}-activated K^+ channels such as Ba^{2+}, quinidine and tetraethylammonium may be useful tools in further studies on the control of transport events.

Acknowledgement

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