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Action of Tetraethylammonium on Calcium-Activated Potassium Channels in Pig Pancreatic Acinar Cells Studied by Patch-Clamp Single-Channel and Whole-Cell Current Recording

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Summary. The effects of tetraethylammonium ions on currents through high-conductance voltage- and Ca^{2+}-activated K^+ channels have been studied with the help of patch-clamp single-channel and whole-cell current recording on pig pancreatic acinar cells. In excised outside-out membrane patches TEA (1 to 2 mM) added to the bath solution virtually abolishes unitary current activity except at very positive membrane potentials when unitary currents corresponding to a markedly reduced conductance are observed. TEA in a lower concentration (0.2 mM) markedly reduces the open-state probability and causes some reduction of the single-channel conductance. In inside-out membrane patches bath application of TEA in concentrations up to 2 mM has no effect on single-channel currents. At a higher concentration (10 mM) slight reductions in single-channel conductance occur. In whole-cell current recording experiments TEA (1 to 2 mM) added to the bath solution completely suppresses the outward currents associated with depolarizing voltage jumps to membrane potentials of 0 mV and blocks the major part (70 to 90%) of the outward currents even at very positive membrane potentials (30 to 40 mV). In contrast TEA (2 mM) added to the cell interior (pipette solution) has no effect on the outward K^+ current. Our results demonstrate that TEA in low concentrations (1 to 2 mM) acts specifically on the outside of the plasma membrane to block current through the high-conductance Ca^{2+}- and voltage-activated K^+ channels.

Key Words pancreatic acinar cell · patch clamp · Ca^{2+}-activated K^+ channel · TEA action · block of K^+ channel

Introduction

Tetraethylammonium ions (TEA) are widely used as blockers of many different types of K^+ currents (Stanfield, 1983), but the Ca^{2+}-activated K^+ current may be particularly sensitive to external TEA (Hermann & Gorman, 1981). After the introduction of the improved patch-clamp technique for single-channel current recording (Hamill et al., 1981) the action of TEA on single high-conductance Ca^{2+}-activated K^+ channels has been investigated in several preparations. In clonal anterior pituitary cells, TEA was reported to block single-channel currents from the inside of the cell membrane (Wong et al., 1982) whereas in the rat lacrimal acinar cells blocking from the outside was observed in both single-channel and whole-cell current recordings (Trautmann & Marty, 1984). Ca^{2+}-dependent K^+ channels from skeletal muscle transverse tubule membranes reconstituted in planar lipid bilayers were blocked by TEA added to the *trans*, but not the *cis* side whereas Ca^{2+} activation only occurred from the *cis* side. This would seem to indicate that TEA in this case acted from the side of the channel normally found at the outside of the native membrane (Latorre et al., 1982).

In view of the uncertainty about the site of action of TEA on the high-conductance Ca^{2+}-activated K^+ channel in its normal membrane environment we have for the first time carried out a systematic investigation exploiting all the known patch-clamp recording conformations. We have chosen to carry out this study on pig pancreatic acinar cells as the large voltage- and Ca^{2+}-activated K^+ channel totally dominates the electrical properties of these cells (Maruyama et al., 1983; Maruyama & Petersen, 1984). We have found that TEA in relatively low concentrations (1 to 2 mM) almost completely blocks single-channel currents from outside-out membrane patches when added to the bath solution whereas in the case of inside-out patches TEA in a higher concentration (10 mM) only causes a very modest reduction in conductance. In whole-cell current recording experiments TEA (1 to 2 mM) when present inside the cell has no effect on the voltage-activated outward currents whereas these are markedly suppressed when the same concentration of the drug is present outside the isolated cell in the bath solution.

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Materials and Methods

Pig pancreata were obtained at the local abattoir and transported to the laboratory kept in physiological saline solution. Tissue fragments (about 50 mg) were injected with physiological saline containing pure collagenase (Worthington, Lorne Diagnostics, Reading, U.K.) (100 units of activity per ml) and incubated with the same type of collagenase solution at 37°C for 30 min (once or twice). At the end of this period there were large undigested lumps, clusters containing from a few up to 20 to 30 acinar cells as well as isolated single acinar cells. The cells were rinsed with collagenase-free physiological saline solution for 15 min to 1 hr before use.

Single-channel currents were recorded using the patch-clamp methods previously described (Hamill et al., 1981; Sakmann & Neher, 1983). We used the extracellular patch-clamp system L/M-EPC-5 (List-electronic, Darmstadt, FRG) and pulled our pipettes from Cee Bee (Copenhagen, Denmark) glass capillaries using the David Kopf (Tajunga, California) microelectrode puller (Model 700 C). Our pipettes when filled with standard extracellular or intracellular physiological saline solutions had resistances of 3 to 10 MΩ. The recording pipette was connected to the amplifier head stage of the patch-clamp system which was mounted on a Narishige (Tokyo, Japan) hydraulic micromanipulator. The acinar cell clusters were placed in a 2-ml chamber on the stage of an inverted microscope. The data were recorded on tape (Racal 4DS, Southampton, U.K.) and when replayed filtered as required using a Kemo (Beckenham, U.K.) filter type VBF/3 before being displayed on a storage oscilloscope screen from which photographs were taken with a Polaroid® camera. 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 (ethylene glycol tetraacetic acid; Sigma, Poole, U.K.) was present (2 to 4 mM).

The patch pipette was normally filled with intracellular K^+-rich solution while the bath fluid was the extracellular Na^+-rich solution. After recording in the cell-attached conformation the electrically isolated patch membrane was either excised into the inside-out conformation after changing the bath solution to the intracellular K^+-rich fluid or the patch membrane was broken in order to allow whole-cell current recording as described in detail by Hamill et al. (1981). Starting from the whole-cell current recording configuration excision produced an outside-out membrane patch as also previously discussed by Hamill et al. (1981).

Analysis of the single-channel data to determine the average open-state probability was carried out using 10 or 20 sec continuous tape records for each situation at a given membrane potential. Data were digitized at 2 kHz and analyzed by a BBC (Acorn Computers, Cambridge, U.K.) (model B) microcomputer. Multiple channel open states were handled by first determining the proportion of time spent above a preselected threshold current for each channel current level and then averaging the results to obtain the mean open-state probability.

TEA was added to the bath solution in the concentrations stated in the text either as TEA-Cl (Sigma, Poole, U.K.) or TEA-Br (Fluka, Switzerland). The bromide salt was used as it is known that commercial samples of TEA-Cl contain significant amounts of triethylammonium whereas this is not the case for TEA-Br (Hermann & Gorman, 1981; Zucker, 1981). In our experiments there were no differences between the effects of the two TEA salts.

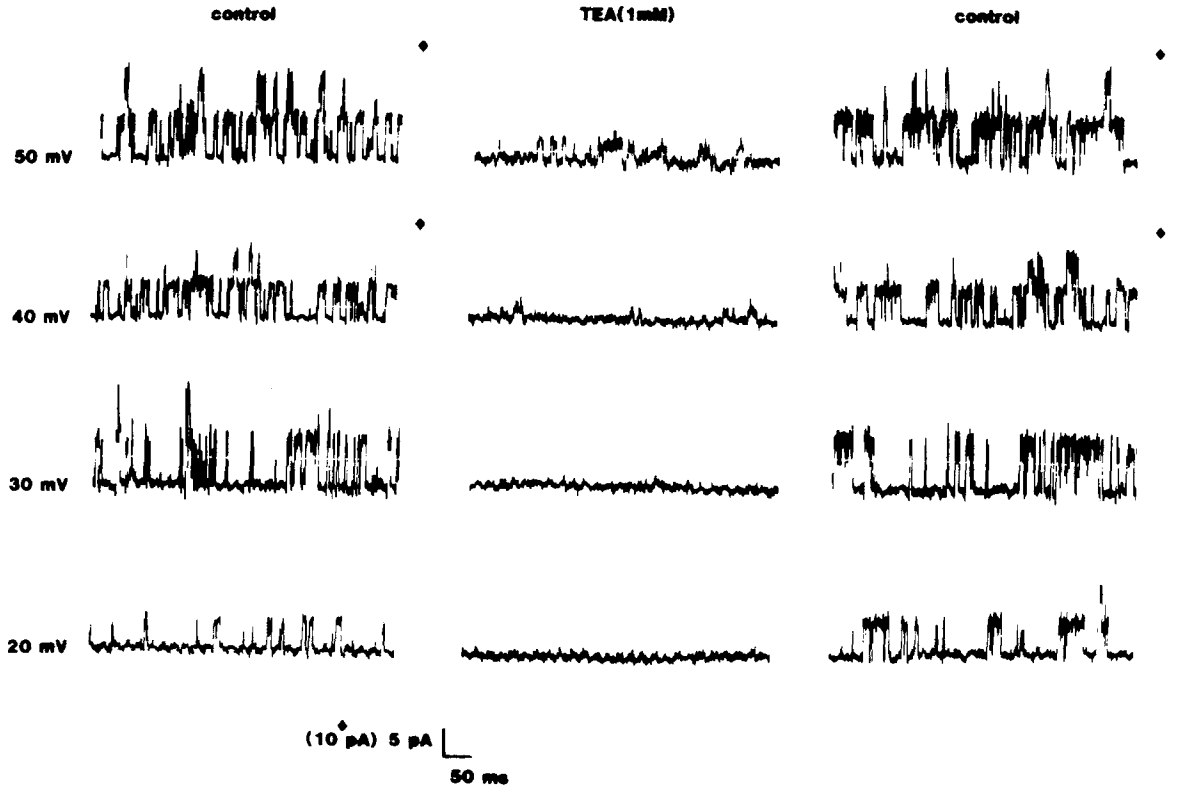


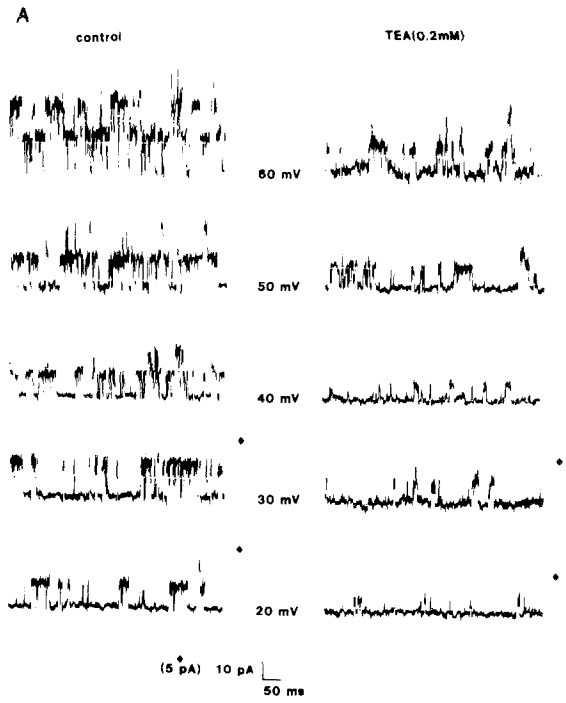
Fig. 1. Single-channel currents recorded from an excised outside-out membrane patch before, during the presence of TEA in the bath, and after return to the control situation. K^+-rich intracellular solutions were used in both the pipette and the bath

Results

Single-Channel Currents from Outside-Out Membrane Patches

Figure 1 shows an example of the action of TEA (1 mM) when added to the bath solution (in contact with membrane outside). At the positive membrane potentials of 20 and 30 mV the unitary activity has been abolished by TEA, but when the membrane is polarized to 40 and 50 mV, current steps with a much smaller amplitude than in the control situation are observed corresponding to a single-channel conductance of about 60 pS. Before or after the period of TEA exposure the single-channel conductance was about 300 pS. The smaller current steps seen in the presence of TEA could either represent partially blocked Ca^{2+}- and voltage-activated K^+ channels or another voltage-gated K^+ channel unmasked when TEA completely blocks the Ca^{2+}-activated channel. The first possibility is the most likely as there are no 60-pS steps in the control recordings and is also consistent with the TEA-evoked reduction in conductance seen in the study of Vergara et al. (1984). In all the four outside-out membrane patches in which the effects of TEA were studied, TEA (1 to 2 mM) abolished all unitary activity up to membrane potentials of +20 or +30 mV whereas at very positive membrane potentials (+40 to +60 mV) much reduced single-channel currents were observed.

Figure 2*A* shows an example of the effect of TEA in a relatively low concentration (0.2 mM) on the single-channel K^+ currents. The main effect of TEA at all the membrane potentials studied is a marked reduction in the average open-state probability whereas there is only a small reduction in the single-channel conductance to about 67% of the control value (Figs. 2*B* and *C*).



Single-Channel Currents from Inside-Out Membrane Patches

In five inside-out membrane patches TEA (0.2 to 2 mM) applied to the bath had no effect on the amplitude of the single-channel currents and also no effect on the open-state probability. In two patches

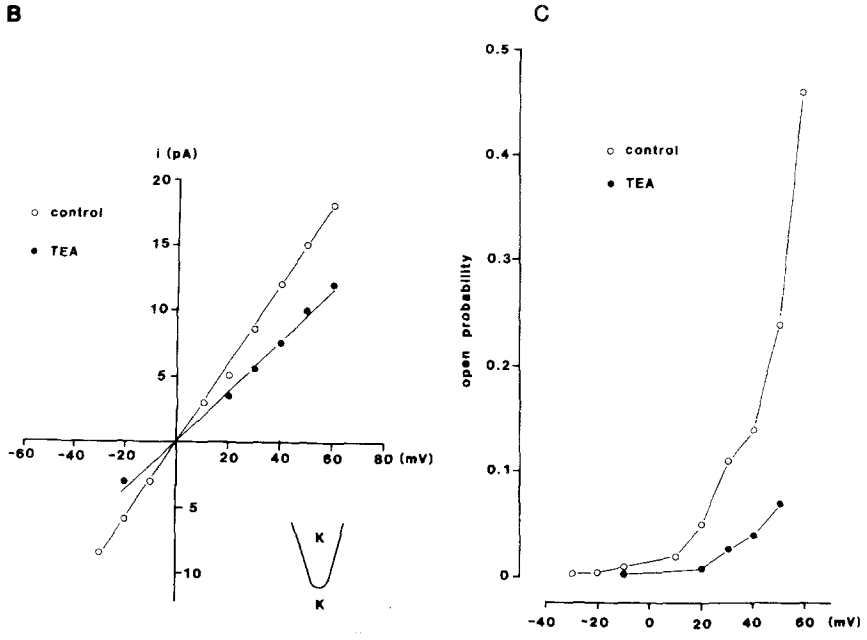


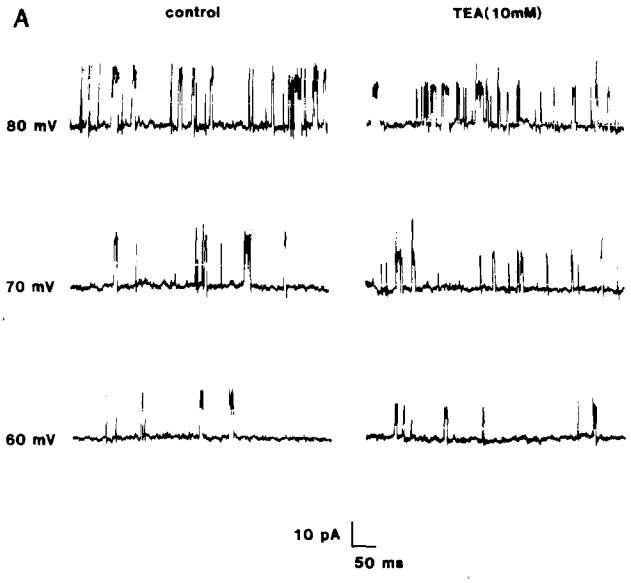
Fig. 2. Effect of bath application of TEA (0.2 mM) on single-channel currents recorded from an excised outside-out membrane patch. Symmetrical K^+-rich solutions were used. (*A*) Typical traces obtained at different membrane potentials before and after addition of TEA to the bath. (*B*) Single-channel *i/V* relationships in control and TEA-containing bath solution. (*C*) Relationship between open-state probability and membrane potential during the control period and during the action of TEA

the effects of TEA in a higher concentration (10 mM) were investigated and traces from one of these experiments are shown in Fig. 3*A*. TEA (10 mM) evoked a small reduction in the amplitude of the single-channel current to about 75% of the control value, but had little effect on the open-state probability (Fig. 3*B* and *C*). Similar effects were obtained in another inside-out membrane patch.

Voltage-Clamp Experiments on Single Isolated Cells (Whole-Cell Current Recording)

In all these experiments the holding potential was −40 mV and the effects of de- or hyperpolarizing voltage jumps on the transmembrane current flow were investigated. Figure 4 shows typical examples of the current recordings obtained from a single isolated cell. Depolarizing voltage jumps were associated with large outward currents whereas hyperpolarizing jumps only evoked very small inward currents. TEA in a low concentration (0.1 mM) slightly reduced the magnitude of the outward currents evoked by depolarization, but higher concentrations of the drug (0.5 and 1 mM) were associated with much more pronounced inhibitory effects which were largely reversible. The outward currents evoked by voltage jumps to 0 mV were virtually completely suppressed by 1 or 2 mM TEA in each of the eight different single cells investigated if the linear leakage current was subtracted from the total current. Larger depolarizing voltage jumps elicited some outward current (in addition to the linear leakage current) in the presence of 1 mM TEA, but these currents were in all the experiments only a small fraction of those measured in the control situation following the period of TEA exposure. Typically the outward current in the presence of 1 mM TEA associated with a voltage jump from −40 to +20 mV (leakage current subtracted) was only about 5 to 15% of the control value, whereas for a voltage jump to +40 mV the corresponding value was about 10 to 30%.

Inclusion of TEA (2 mM) in the pipette solution (in contact with the cell interior) did not markedly influence the whole-cell current-voltage relationship (Fig. 5). Subsequent addition of TEA (2 mM) to the bath solution, however, reduced the magnitude of the outward currents associated with depolarizing voltage jumps very severely as described above and this effect was reversible (Fig. 5).



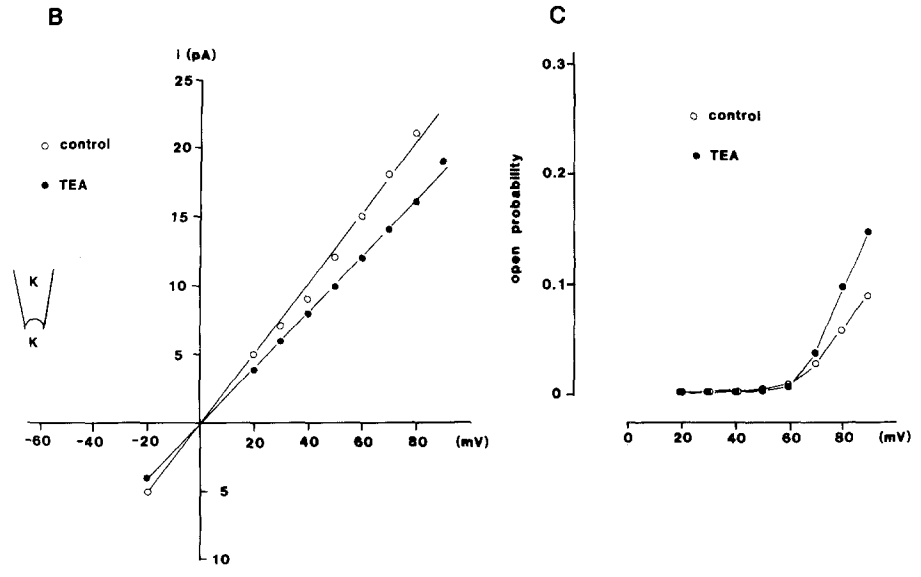


Fig. 3. Effect of TEA (10 mM) added to the bath solution on single-channel currents recorded from an excised inside-out membrane patch. Symmetrical K^+-rich intracellular solutions were used. (*A*) Typical traces obtained before and after addition of TEA to the bath. (*B*) Single-channel *i/V* relationship during control periods and during the action of TEA. (*C*) Open-state probability as function of membrane potential before and after TEA application

Discussion

The major finding of this report is that TEA in a relatively low concentration (1 to 2 mM) acts specifically from the outside of the plasma membrane to block current through high-conductance Ca^{2+}- and voltage-activated K^+ channels. TEA in this low concentration (1 to 2 mM) has no effect when added to a solution exclusively in contact with the inside of the plasma membrane, but in a higher concentration (10 mM) TEA slightly reduces the single-channel conductance. TEA effects from the membrane inside are therefore very minor compared to the pronounced blocking action of TEA from the membrane outside.

There is a reasonable degree of correspondence between our single-channel and whole-cell current data as 1 to 2 mM TEA abolished unitary activity in outside-out membrane patches at membrane potentials up to 20 or 30 mV (Fig. 1) and blocks 80 to 90% of the total outward membrane current in single cells evoked by depolarizing voltage jumps to the same membrane potentials (Figs. 4 and 5). Further polarization to more positive membrane potentials in the presence of 1 mM TEA shows much reduced currents in both the single-channel (Fig. 1) and whole-cell recordings as compared to the control situation (Figs. 4 and 5).

TEA is known to affect several types of K^+ currents and can in many cases act from both sides of the membrane (Latorre & Miller, 1983). Our results confirm the conclusion drawn from experiments on Ca^{2+}- and voltage-activated K^+ channels reconstituted in planar lipid bilayers that TEA blocks best from the noncalcium-sensitive side of the channel corresponding to the normal outside of a cell membrane. Our study demonstrates directly that the Ca^{2+}- and voltage-activated high-conductance K^+ channel when present in its native plasma membrane can be readily blocked by TEA acting from the outside of the cell membrane but is much less sensitive to the drug when it is added to the membrane inside.

The effect of TEA on the conductance of the Ca^{2+}- and voltage-activated K^+ channel in the pig pancreatic acinar cells is very similar to that described by Vergara et al. (1984) for the rat muscle channel incorporated into planar lipid bilayers. Thus the single-channel current steps observed in the presence of 1 mM TEA in contact with the membrane outside in our excised outside-out patches correspond to a conductance which is only about 20% of the control value (Fig. 1) and in Vergara and co-workers' (1984) study it is shown that 1 mM TEA acting from the *trans* side reduces the single-channel conductance to about 25% of the control value. 0.2 mM TEA from the outside reduced the single-channel conductance to about 67% of the control value in our excised outside-out patches (Fig. 2) and the same concentration of TEA from the *trans* side in Vergara and co-workers' (1984) study reduced the conductance to about 60% of the control value. Ten mM TEA added to the solution in contact with the inside of our inside-out membrane patches reduced the single-channel conductance to about 75% of the control value (Fig. 3) and in the experiments

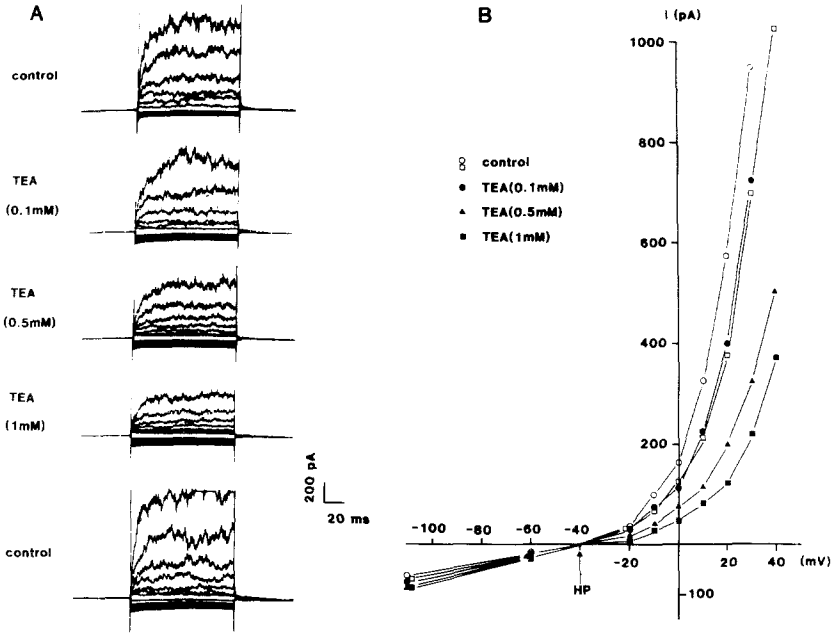


Fig. 4. Effects of extracellular TEA on whole-cell currents associated with depolarizing or hyperpolarizing voltage steps from a holding potential of −40 mV. (*A*) Traces of transmembrane current from a single isolated acinar cell. The pipette contained the K^+-rich intracellular solution whereas the Na^+-rich extracellular solution was present in the bath. Voltage steps lasting 100 msec of ±20 to 70 (80) mV were applied and the resulting currents recorded before TEA application, during the presence of 0.1, 0.5 and 1 mM TEA in the bath and after return to the control solution. (*B*) Relationship between the change in steady-state current and the membrane potential when the potential is changed from the holding potential of −40 mV to higher or lower values in the presence or absence of TEA

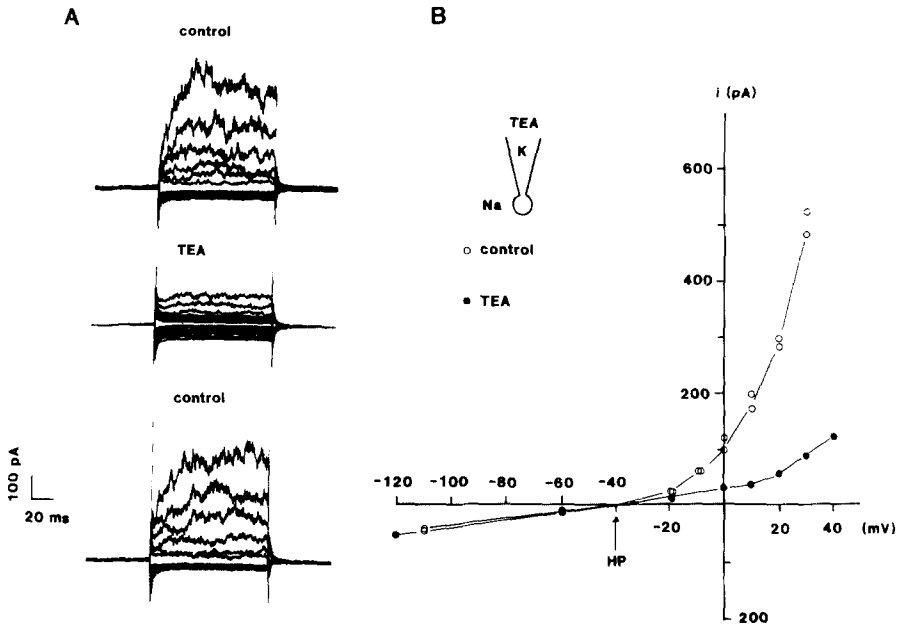


Fig. 5. Effects of intracellular and extracellular TEA on whole-cell currents evoked by depolarizing or hyperpolarizing voltage steps from a holding potential of −40 mV. Pipette contained intracellular K^+-rich solution to which 2 mM TEA had been added. Bath was filled with extracellular Na^+-rich solution. (*A*) Traces of whole-cell currents before, during the presence of TEA (2 mM) in the bath, and after return to the control situation. Voltage steps of ±20 to 70 (80) mV were applied. (*B*) Whole-cell current-voltage relationship in the control situations before and after TEA application and in the presence of 2 mM TEA in the bath

of Vergara et al. (1984) this concentration of TEA added to the *cis* side reduced the conductance to about 70% of the control value.

Our single-channel data indicates that TEA acting on the membrane outside in addition to reducing the single-channel conductance also markedly reduces the average open-state probability (Figs. 1 and 2). Further kinetic studies are needed to clarify the mechanism of action.

In previous reports on the K^+ conductance in pig pancreatic acinar cells (Maruyama et al., 1983; Maruyama & Petersen, 1984) and indeed also in other exocrine acinar cells (Petersen & Maruyama, 1984), we have argued that the membrane electrical properties are totally dominated by the large Ca^{2+}-activated K^+ channel. The marked blocking action of TEA observed in both single-channel and whole-cell current recordings and the reasonable correspondence between the two sets of results confirms this conclusion. The major part of the whole-cell outward K^+ current can clearly be accounted for by a relatively small number (50 to 100) of high-conductance K^+ channels (Maruyama et al., 1983; Trautmann & Marty, 1984).

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