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Ca^{2+}-Activated K^+ Channels in the Apical Membrane of *Necturus* Choroid Plexus

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Summary. The properties of Ca^{2+}-activated K^+ channels in the apical membrane of the *Necturus* choroid plexus were studied using single-channel recording techniques in the cell-attached and excised-patch configurations. Channels with large unitary conductances clustered around 150 and 220 pS were most commonly observed. These channels exhibited a high selectivity for K^+ over Na^+ and K^+ over Cs^+. They were blocked by high cytoplasmic Na^+ concentrations (110 mM). Channel activity increased with depolarizing membrane potentials, and with increasing cytoplasmic Ca^{2+} concentrations. Increasing Ca^{2+} from 5 to 500 nM, increased open probability by an order of magnitude, without changing single-channel conductance. Open probability increased up to 10-fold with a 20-mV depolarization when Ca^{2+} was 500 nM. Lowering intracellular pH one unit, decreased open probability by more than two orders of magnitude, but pH did not affect single-channel conductance. Cytoplasmic Ba^{2+} reduced both channel-open probability and conductance. The sites for the action of Ba^{2+} are located at a distance more than halfway through the applied electric field from the inside of the membrane. Values of 0.013 and 117 mM were calculated as the apparent Ba^{2+} dissociation constants (K_D(0 mV)) for the effects on probability and conductance, respectively. TEA^+ (tetraethylammonium) reduced single-channel current. Applied to the cytoplasmic side, it acted on a site 20% of the distance through the membrane, with a K_D(0 mV) = 5.6 mM. A second site, with a higher affinity, K_D(0 mV) = 0.23 mM, may account for the near total block of channel conductance by 2 mM TEA^+ applied to the outside of the membrane. It is concluded that the channels in *Necturus* choroid plexus exhibit many of the properties of "maxi" Ca^{2+}-activated K^+ channels found in other tissues.

Key Words choroid plexus · brush border membrane · Ca^{2+}-activated K^+ channels · calcium · barium · TEA · intracellular pH · cerebrospinal fluid secretion

Introduction

The choroid plexus secretes cerebrospinal fluid (CSF) into the ventricles of the brain from the blood. The primary driving force for fluid secretion is active Na^+ transport by Na^+/K^+ pumps, located in the apical membrane of the choroidal epithelium (Quinton, Wright & Tormey, 1973; Masuzawa et al., 1984; Ernst, Palacios & Siegel, 1986). Na^+ transport is followed by the movement of HCO_3^-, Cl^- and water into the CSF, and is accompanied by a small net absorption of K^+ from the CSF to blood. In addition to containing the Na^+/K^+ pumps, the apical membrane is also thought to contain conductive pathways for K^+ (Zeuthen & Wright, 1981), HCO_3^- (Saito & Wright, 1984) and Cl^- (Saito & Wright, 1987; Zeuthen, 1987).

A previous study of K^+ transport showed that over 90% of the K^+ conductance of the cell is also found in the apical membrane (Zeuthen & Wright, 1981). It was concluded that most of the K^+ entering the cell via the Na^+/K^+ pump leaves across the same membrane. This reflux of K^+ across the apical membrane explains why there is only a small net movement of K^+ from CSF to blood.

We have applied the patch-clamp technique (Sakmann & Neher, 1983) to study the nature and regulation of the ion-conductive pathways in the apical membrane. This paper provides an account of the channels found predominantly in the apical membrane of *Necturus* choroid plexus. These channels are activated by membrane depolarization and intracellular Ca^{2+}, highly selective for K^+ over Na^+ and Cs^+, and are very sensitive to intracellular pH. We have also further characterized the channels using the blockers TEA^+ and Ba^{2+}. In contrast to previous studies in most other epithelia, where the channels were found in the basolateral membrane, the Ca^{2+}-activated K^+ channels in the present study are in the apical membrane. In the accompanying paper (Loo, Brown & Wright, 1988) the contribution of the currents through these channels is assessed in whole-cell recordings of the epithelial cells from *Necturus* choroid plexus.

A preliminary account of part of this work has

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already been presented (Brown et al., 1986). Since the completion of the present study, an independent study has appeared (Christensen & Zeuthen, 1987) and their observations agree with some of the findings of this study.

Materials and Methods

The choroid plexus was removed from the third ventricle of *Necturus maculosa* (purchased from C.D. Sullivan, Nashville, TN, and Nasco, Fort Atkinson, WI). The tissue was pinned to the bottom of a small (0.5 ml) transparent chamber and viewed under a Nikon inverted microscope (400×) with Hoffman-modulated optics.

A $NaHCO_3$ Ringer solution was used to bathe the tissue. The solution contained (mM): Na^+, 110; K^+, 2; Ca^{2+}, 1; Mg^{2+}, 1; Cl^-, 91; HCO_3^-, 25 and was buffered with 10 mM HEPES/NaOH at pH 7.3 (ungassed). A KCl Ringer, containing (mM): K^+, 110; Na^+, 1; Mg^{2+}, 1; Cl^-, 113 and buffered with 10 mM HEPES/KOH at pH 7.3, was used as a pipette solution and as a bath solution for excised patches. Ca^{2+} activity in the pipette solution was maintained at 5 nM, while that of the bath solution was varied according to its use: 5 nM, 500 nM and 75 μM. Activities in the sub-micromolar range were produced using Ca^{2+}-EGTA buffers at ratios calculated using the stability constants of Martell and Smith (1974). The calculations assumed 96% purity of the EGTA from Sigma (Miller & Smith, 1984; Findlay, Dunne & Petersen, 1985), and took into account the pH and Mg^{2+} concentrations. At pH 7.3, 1 mM $CaCl_2$ and 10 mM EGTA were added to obtain 5 nM Ca^{2+}, while 1.16 mM $CaCl_2$ and 1.34 mM EGTA were added to obtain 500 nM Ca^{2+}. In one series of experiments were Ca^{2+} was maintained at 500 nM and the pH varied, the following $CaCl_2$ and EGTA ratio concentrations were used: at pH 6.8, $CaCl_2$ = 0.63 mM and EGTA = 1.34 mM; at pH 7.8, $CaCl_2$ = 1.27 mM and EGTA = 1.34 mM. The 75 μM activity was produced by the addition of 0.2 mM $CaCl_2$ to the 500-nM solution at pH. 7.3. The pipette solution was filtered through a 0.22-μm filter before use. All experiments were performed at room temperature (20–23°C) between November and April.

Data Acquisition

Single-channel currents from cell-attached or excised-membrane patches were monitored using a patch-clamp amplifier as described by Horn, Vandenberg and Lange (1984). The head stage was designed around a dual-junction field-effect transistor Siliconix U430 (Santa Clara, CA). The patch-amplifier gave an output of 10 mV for each pA of current passing through a channel. Fire-polished electrodes with tip resistances of 2–4 MΩ (~1.5 μm i.d.) were made from 100-μl Blue Tip hematocrit capillary (VWR, San Francisco, CA). Electrical seal resistances between the apical membrane and patch pipette of 10–30 GΩ were commonplace. Some seals formed spontaneously on contact of the glass with the cell, but light suction was generally needed. No enzymatic treatment of the tissue was required.

Channel activity was studied by holding the patches (cell-attached, inside-out, or outside-out) at different constant DC pipette potentials. The observed current records were stored using a TEAC R-200 analog tape recorder (Montebello, CA) for subsequent analysis.

Data Analysis

Data were analyzed using an IBM personal computer (Model XT) interfaced to the Tecmar Labmaster data acquisition system (Cleveland, OH) and the computer program "pclamp" (Axon Instruments, Burlingame, CA). The currents were filtered by a 8-pole lowpass Bessel filter (Model LP902, Frequency Devices, Haverhill, MA) with the corner frequency set either at 1 or 3 kHz, and then digitized once every 200 μsec. The digitized data were stored in 100 continuous records each consisting of 512 data points (i.e., a total of 10.24 sec of recorded data).

For analysis of single-channel events, digitized current records were displayed on a graphics monitor. We modified the "pclamp" program so that we could set the baseline and the threshold levels for channel events. This modification allowed us to determine the times for channel openings at more than one current level. The threshold was set at 50% of a full opening, and an event only considered acceptable if the current remained above this value for longer than 600 μsec (3 digitized points). The exclusion of such short openings (or "flickers") simplified analysis, yet was of little consequence to our estimates of channel-open probability (e.g., in an experiment where comprehensive analysis included such short openings, they made up 13% of the total number of events, but accounted for less than 1% of the total open time).

The "pclamp" program determined the open time of each event and the closed duration between events. Single-channel currents for individual openings were also calculated, as the mean of all the digitized data points above the threshold level for each event. Except where stated, 20 sec of recorded data were analyzed by computer in each situation. For probabilities less than 10^{-4}, up to 5 min of data were analyzed.

The open probability (P_o) of a single channel was defined as the fraction of the total time the channel was open. In multichannel patches, P_o for each channel was estimated using Eq. (1)

$$P_o = \frac{(T_1 + T_2 + \cdots + T_N)}{N\,T_{\text{tot}}}. \tag{1}$$

Where N = number of channels in the patch, T_{tot} was the total time analyzed, and T_1 is the time that one or more channels are open, T_2 is the time two or more channels are open and so on. N was found empirically by counting the maximum number of current steps observed under conditions (high Ca^{2+} and depolarizing potentials) at which the channels show their greatest activity. T_1, T_2, . . . T_N were estimated by setting the baseline and current thresholds at progressively higher levels from 1 to N. It should be noted that this method is analogous to that used by Reuter et al. (1982) and Gallacher and Morris (1986).

Statistics

All statistics are expressed as a mean ± SEM of n observations except where stated. Straight-line current-voltage (I-V) relationships were fitted by linear regression analysis.

Results

Conductance and Selectivity

The choroid plexus possesses a ciliated epithelium, and yet despite the constant movement of the cilia,

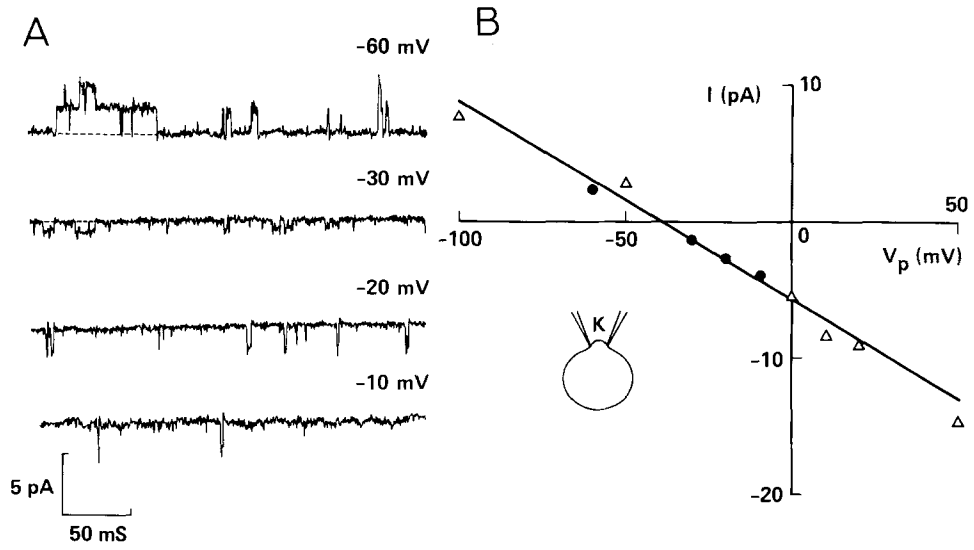

Fig. 1. Single-channel currents for two K^+ channels in a cell-attached patch. (*A*) Shows single-channel records (filtered at 1 kHz) in a patch held at 4 pipette potentials: −60, −30, −20, and −10 mV. In this and subsequent figures outward current (from the cell to the pipette) is positive, giving an upward deflection. The baseline level is shown by the broken line. The pipette contained 110 mM KCl Ringer (5 nM Ca^{2+}) and the tissue bathed in the $NaHCO_3$ Ringer. (*B*) Mean currents, for as many as 300 openings, are plotted as a function of V_p. The circles represent currents at V_p's illustrated in *A*; the triangles show currents at other V_p's. The line has been fitted by linear regression analysis and has a slope of 149 ± 8 pS and a reversal potential of −41 ± 9 mV

seal resistances of over 10 GΩ could be obtained in up to 70% of the pipettes used. On achieving a seal, two types of channels were observed: i) large conductance (150–220 pS) channels; and ii) a smaller (80 pS) conductance channel with a rectifying *I-V* relationship in fewer than 20% of the patches. It is the large conductance channel that is the subject of this paper.

Single-channel current records from a cell-attached patch containing two of the large conductance channels are shown in Fig. 1*A*. The patch was held at a series of negative pipette potentials. Throughout this paper potentials are expressed as the pipette potential (V_p). In cell-attached or inside-out patches, making the pipette potential more negative is equivalent to depolarizing the membrane potential of the patch. Channel openings appeared as discrete current steps.

Depolarization of the patch in Fig. 1*A* caused: (i) changes in the size and direction of the individual current steps, and (ii) increased frequency and the apparent length of channel openings. At $V_p = -10$ mV, there were two brief openings of the channel, apparent as discrete negative current steps equivalent to the movement of cations out of the pipette and into the cell. Depolarization to $V_p = -30$ mV decreased current size and at $V_p = -60$ mV the current had reversed direction. At this potential, two distinct levels in current amplitude were observed. We assumed that the second higher level was due to the simultaneous opening of the two channels in the patch. An alternative explanation was that the patch contained a single channel with different conductive states. However, this seems unlikely as the steps in current amplitude were of the same size (e.g., in Fig. 1*A*, at $V_p = -60$ mV, both steps were of 2.4 pA), and different numbers of current levels were observed from patch to patch (in the present study the numbers of current levels varied between 0 and 5 with a mean of 2).

Mean currents were calculated from as many as 900 events, depending upon the P_o and open durations of the channel. However, at hyperpolarizing potentials or in low Ca^{2+} concentrations, as few as 10 events were observed in 5 min of recorded data. Currents for the channels shown in Fig. 1*A* are plotted as a function of V_p in Fig. 1*B*. A straight line was fitted through these values with a slope of 149 ± 8 pS. In different preparations, these channels appeared to have conductances with means clustered at either 152 ± 15 ($n = 5$) or 218 ± 8 pS ($n = 7$). Representatives of these two classes of channels were never seen in a single patch or even in patches from the same animal. Conductance aside, the channels seemed similar in other respects. In cell-attached patches, for instance, both channels had a P_o of about $4.7 \pm 0.3 \times 10^{-4}$ at rest ($n = 4$, $V_p = 0$). Current reversal was also observed at similar potentials and was −39 ± 1 mV ($n = 3$) and −37 ± 6 mV ($n = 6$) for the 220- and 150-pS channels, respectively.

The value for the reversal potential was similar

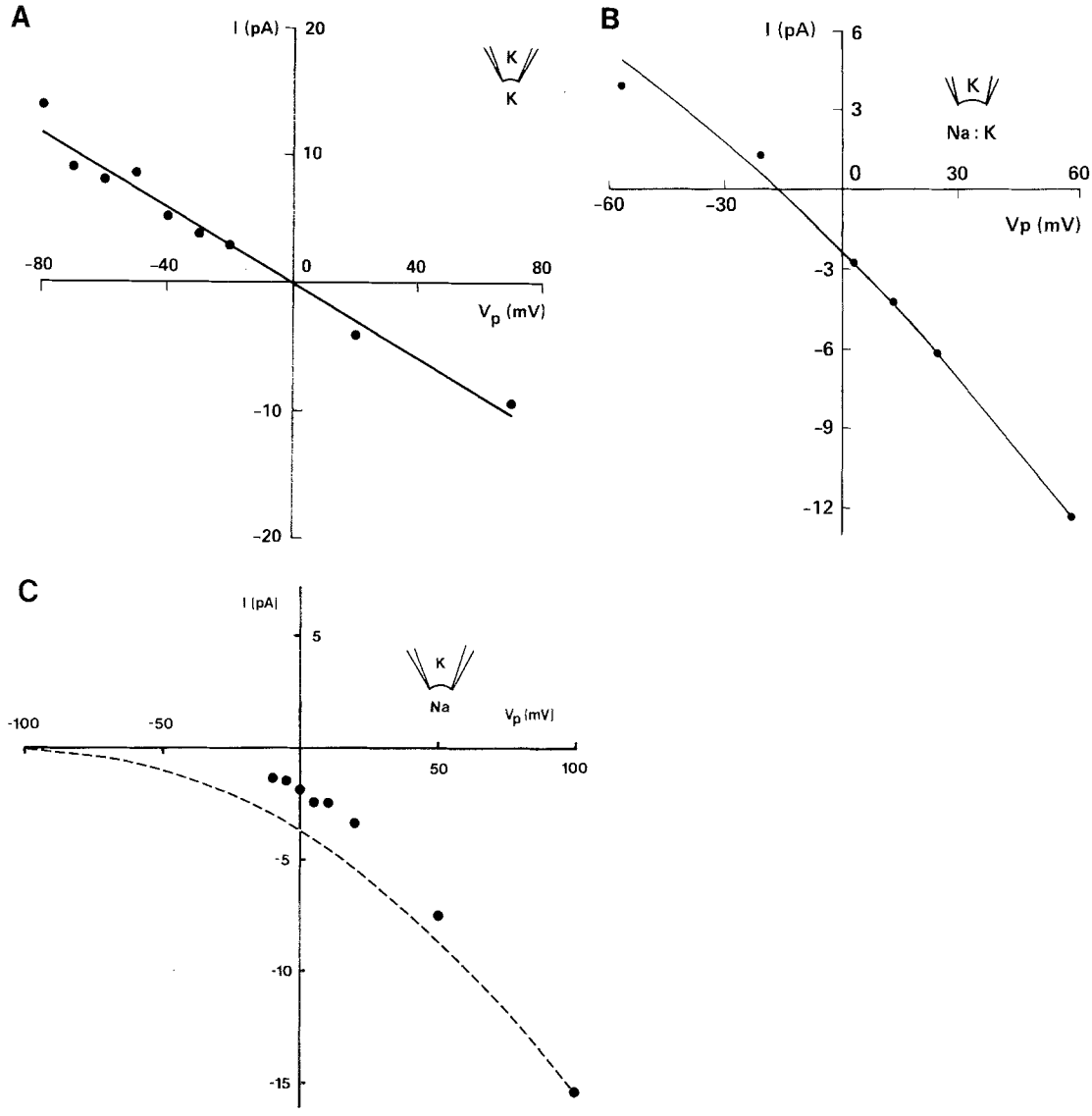

Fig. 2. (*A*) Current-voltage relationships for 150-pS channels in an excised patch. Both sides of the patch were bathed by the 110 mM KCl Ringer solution with 5 mM and 500 nM Ca^{2+} in the pipette and bath, respectively. The line fitted by regression analysis has a slope of 148 ± 9 pS and current reversal at 0.4 ± 8.5 mV. (*B*) *I-V* relationship for two 220-pS channels in a patch with a 110 mM KCl pipette and equal (55 mM) concentrations of Na^+ and K^+ in the bath (an equal mixture of $NaHCO_3$ Ringer and 110 mM KCl Ringer). The line shows the least squares fit to the equation $I = I_K + I_{Na}$ where $I_K = -[P_K \cdot F^2V_p/RT] \cdot [K_o - K_i \cdot \exp(-V_pF/RT)]/[1 - \exp(-V_pF/RT)]$, $I_{Na} = -[P_{Na} \cdot F^2V_p/RT] \cdot [Na_o - Na_i \cdot \exp(-V_pF/RT)]/[1 - \exp(-V_pF/RT)]$, and K_i, Na_i, K_o and Na_o are the K^+ and Na^+ concentrations in the bath and pipette solutions. P_K and P_{Na} are the single-channel K^+ and Na^+ permeabilities. V_p is the pipette potential and F, R and T have their usual meanings. Estimated values are $P_K = 4.6 \times 10^{-13}$ cm³ · sec⁻¹ per channel and $P_{Na} = 1.1 \times 10^{-16}$ cm³ · sec⁻¹ per channel, giving $P_K/P_{Na} = 4260$. The reversal potentials for K^+ and Na^+ were −17.5 mV and +101.2 mV, respectively, and the curve gives a reversal potential of −17.5 mV. The channels were assumed to be impermeable to Cl^- in the calculation, because the reversal potential was far removed from that for Cl^- (+2.5 mV). (*C*) Results are from the same patch as in *A*, but the bath solution was $NaHCO_3$ Ringer, with 110 mM KCl in the pipette. Observed currents are shown by the circles, and the broken line shows the *I-V* relationship for K^+ predicted by the constant field equation $I = -[P_K \cdot F^2V_p/RT] \cdot [K_o - K_i \cdot \exp(-V_pF/RT)]/[1 - \exp(-V_pF/RT)]$ using $P_K = 3.5 \times 10^{-13}$ cm³ · sec⁻¹ per channel calculated from the limiting conductance. The symbols are as defined in *B*

to the membrane potential of these epithelial cells, which was −43 ± 5 mV (n = 3) as measured with microelectrodes (Y. Saito and E.M. Wright, *unpublished data*). Assuming the intracellular K^+ activity was about 91 mM (Zeuthen, 1981), which is roughly equivalent to that in the pipette, current reversal at this potential provided preliminary evidence that the channels are selective for K^+.

Single-channel conductance was not affected by the excision of a patch into the inside-out config-

uration in a 110-mM KCl bathing solution. Figure 2*A* shows a current-voltage (*I-V*) relationship for channels in an inside-out patch held in 110-mM KCl solutions. Over the range of potentials studied (V_p = −80 to 80 mV), this relationship was linear and gave a channel conductance of 148 ± 9 pS, with a reversal potential of 0 mV. Similar results were observed in 12 other patches. The linearity of the *I-V* relationship and the reversal at 0 mV suggest that the channels are selective for either K^+ or Cl^- (or both).

The selectivity was studied using inside-out patches with 55 mM K^+ and 55 mM Na^+ in the bath solution (Fig. 2*B*). The current now reversed at −17 mV, which is close to the expected reversal potential for K^+ (V_K = −17.5 mV). The line shows the least squares fit to the constant field equation (Goldman, 1943; Hodgkin & Katz, 1949), assuming the channels were impermeable to Cl^-. This line predicts a current reversal at −17.5 mV so that the channels are virtually impermeable to Na^+. In three experiments, the reversal potential was 16.8 ± 1.0 mV, so that P_K/P_{Na} = 122.

In two further experiments, an attempt was made to measure Na^+ currents through the channels. For these experiments, all the KCl in the pipette solution was replaced by NaCl (110 mM Na^+). In the cell-attached configuration, K^+ (outward) currents could clearly be observed. After excision into the inside-out configuration in a bath solution containing $NaHCO_3$ Ringer (110 mM Na^+), the single-channel steps were not observed. But when the bath solution was exchanged for 110 mM KCl, the single-channel steps were present. Thus, it can be again concluded that the channels are virtually impermeable to Na^+.

Figure 2*C* shows single-channel currents (●) from an excised inside-out patch measured when the bath solution was $NaHCO_3$ Ringer (110 mM Na^+). Current reversal was not observed in this, or any other of four experiments where single-channel currents were measured under these conditions (K^+ reversal potential is at V_p = −101 mV). The line shows the K^+ currents predicted by the constant field equation. The observed currents were clearly less than the predicted values, and at V_p = 0, they were only half the predicted value. The results show that the constant field equation did not hold under these circumstances and suggest that at high cytoplasmic concentrations, Na^+ can block these channels. In chromaffin cells, Yellen (1984) also reported that Na^+ blocks Ca^{2+}-activated K^+ channels.

The selectivity of the channels to Cs^+, another alkali/metal ion, was also tested. Figure 3 shows *I-V* relationships obtained for channels in an inside-out patch with: (i) 110 mM K^+ (○); (ii) 55 mM K^+ and 55 mM Cs^+ (□); and (iii) 22 mM K^+ and 88 mM Cs^+ (●) in the bath solution. The curves were predicted by the constant field equation, assuming the channels are permeable only to K^+. In each case, there is good agreement between the measured currents and these theoretical curves, suggesting that the channels are virtually impermeable to Cs^+. In three inside-out patches, where the bath solution contained 55 mM Cs^+ and 55 mM K^+, a reversal potential of 16.7 ± 1.0 mV was observed. A value of P_K/P_{Cs} = 134 was calculated for these experiments.

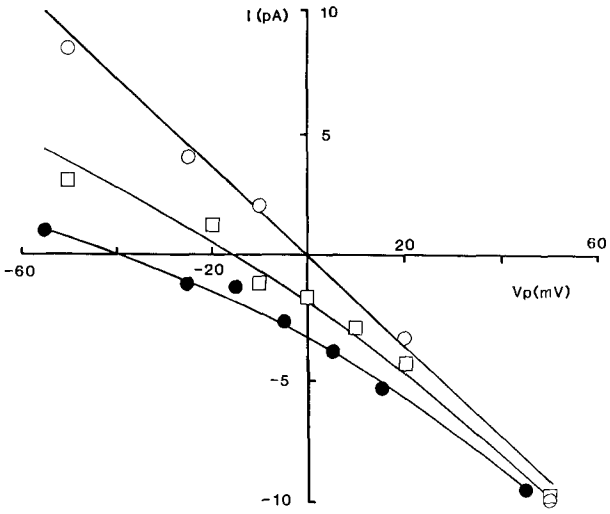

Fig. 3. Channel selectivity for K^+ against Cs^+. Currents were measured from a single inside-out patch with: (○) 110 mM K^+, (□) 55 mM K^+ and 55 mM Cs^+ and (●) 22 mM K^+ and 88 mM Cs^+ in the bath solution. The solutions were made by replacing equal amounts of KCl with CsCl; all other constituents remained the same. In each case, the lines drawn are the theoretical potassium currents predicted by the constant field equation (*see* Fig. 2*C*) assuming zero permeability to Cs^+, and using a value of P_K = 4.38×10^{-13} cm^3 · sec^{-1} (corresponding to a 184-pS channel). 1 mM CsCl was added to the pipette solution to give a finite Cs^+ concentration for the permeability calculations

Channel Regulation

The effects of Ca^{2+} on channel activity were studied on inside-out patches held at depolarizing potentials. Figure 4*A* shows a period of channel openings in a patch containing two channels; held at V_p = −55 mV with 500 mM Ca^{2+} in the bath solution. The frequent channel openings are revealed as upward deflections on the current trace. The record shows only 125 msec of data, but to estimate P_o, 60 sec of recorded data were analyzed. In this case, the channels had a P_o of 0.031, while a mean value of 0.031 ± 0.010 was obtained for channels in four patches held under similar conditions (V_p = −55 mV). A P_o of 0.013 ± 0.010 (n = 4) was measured at −20 mV.

Reducing bath Ca^{2+} to 5 nM (Fig. 4*B*) caused a

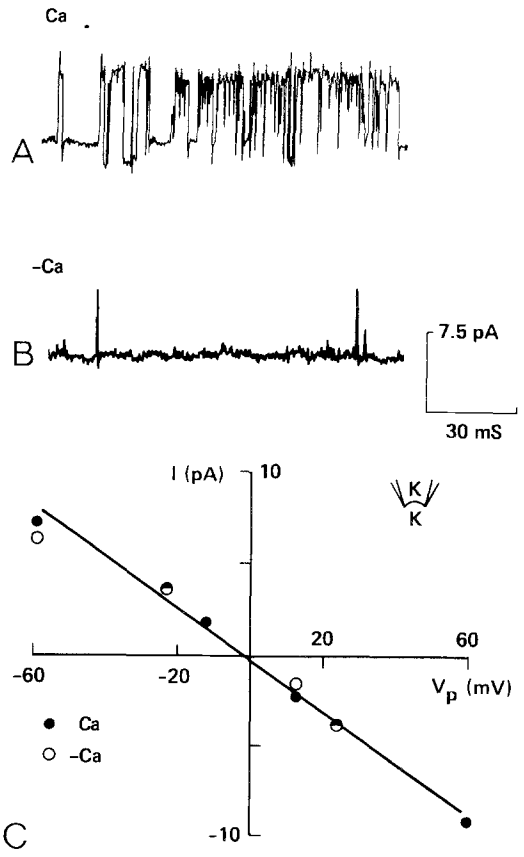

Fig. 4. Ca^{2+} dependence of K^+ channel activity. *A* and *B* are single-channel records (filtered 3 kHz) for two channels in an inside-out patch (110 mM KCl/110 mM KCl Ringer), $V_p = -55$ mV. Bath Ca^{2+} was (*A*) 500 nM and (*B*) 5 nM. (*C*) *I-V* relationship for single-channel currents at 500 nM (●) and 5 nM (○) bath Ca^{2+}. The line fitted through all the points gives a conductance of 132 ± 5 pS

marked decrease in channel activity. In Figure 4*B*, the reduction was to $P_o = 0.0017$, and in four experiments to means of 0.0011 ± 0.0001 and 0.0007 ± 0.0001 at −55 and −20 mV, respectively. The reduction in P_o at $V_p = -55$ mV of 19- to 28-fold for this change in Ca^{2+} was significant ($P < 0.05$) by the unpaired *t* test. Altering the Ca^{2+} activity at the internal aspect of the membrane affected only P_o, and not channel conductance as is illustrated in Fig. 4*C*. Single-channel currents measured in the presence of 500 nM (●) and 5 nM Ca^{2+} (○) are plotted against V_p. The currents did not appear to be different with all the points falling on or close to the same line (132 ± 5 pS). Depolarization of the membrane potential of cell-attached patches invariably caused an increase in channel activity (e.g., Fig. 1*A*). Results from three experiments showed that the P_o of channels in cell-attached patches increased from $4.7 \pm 0.3 \times 10^{-4}$ at $V_p = 0$ mV to $8.6 \pm 0.4 \times 10^{-4}$ and $43.0 \pm 2.1 \times 10^{-4}$ at $V_p = -20$ and −50 mV, respectively. The effects of potential on P_o were studied in more detail with inside-out patches with 500 nM Ca^{2+} in the bath solution (Fig. 5*A*). As the patch potential was depolarized (V_p negative), the number of openings increased. A detailed analysis of up to 5 min of recorded data allowed the P_o for channels in two patches to be estimated at a number of potentials (Fig. 5*B*). P_o was markedly increased by depolarization of patch-membrane potential, and the relationship was most conveniently plotted on a semilog scale. The line through the points in Fig. 5*B*, was fitted by eye. It appears to be asymptotic towards constant opening of the channel ($P_o = 1$) and a 22.5-mV depolarization from the resting potential ($V_p = 0$) results in a 10-fold increase in P_o. Increases in channel activity with depolarization were observed in all inside-out patches bathed in 500 nM Ca^{2+} examined in this study ($n = 11$).

Table. Effect of pH on K channel open probability

V_p(mV)	P_o		
	pH 6.8	pH 7.3	pH 7.8
−12	0.0001 ±0.0001	0.0009 ±0.0002	0.0513[a] ±0.0221
−24	0.0003 ±0.0001	0.0033 ±0.0009	0.0998 ±0.0363
−55	0.0020 ±0.0004	0.0225 ±0.0040	0.3540[a] ±0.0563

Single-channel open probability (P_o, mean ± SEM) is listed for channels in four inside-out patches held in symmetrical (pipette and bath) 110 mM KCl Ringer solution. Bath pH was varied over a range of 1 unit while bath Ca^{+2} activity was maintained at 500 nM by making the appropriate changes to $CaCl_2$ and EGTA concentrations. Single-channel conductances were 215 ± 13 pS, 218 ± 10 pS and 218 ± 15 pS at pH 7.8, 7.3 and 6.8, respectively.

[a] Values are significantly different ($P > 0.100$) from control (pH 7.3) by paired *t* test.

At 75 μM Ca^{2+}, P_o was about two orders of magnitude greater than at 500 nM Ca^{2+} (Fig. 6 (○) *vs.* Fig. 5*B*). The effects of membrane potential were less marked at 75 μM Ca^{2+} over this range of potentials. In fact, P_o began to decrease at large depolarizing (−55 mV) potentials. This decrease in P_o was due to the channels becoming totally inactive for periods of up to several seconds (e.g., at $V_p = -55$ mV, 28% of the closed time was spent in periods of longer than 1 sec). Such long periods of inactivity did not occur in 500 nM Ca^{2+} at this holding potential.

One other factor that influenced P_o was the pH at the cytoplasmic face of the membrane. Values for P_o of channels in four inside-out patches held at pH 6.8, 7.3 and 7.8 are shown in the Table. From a

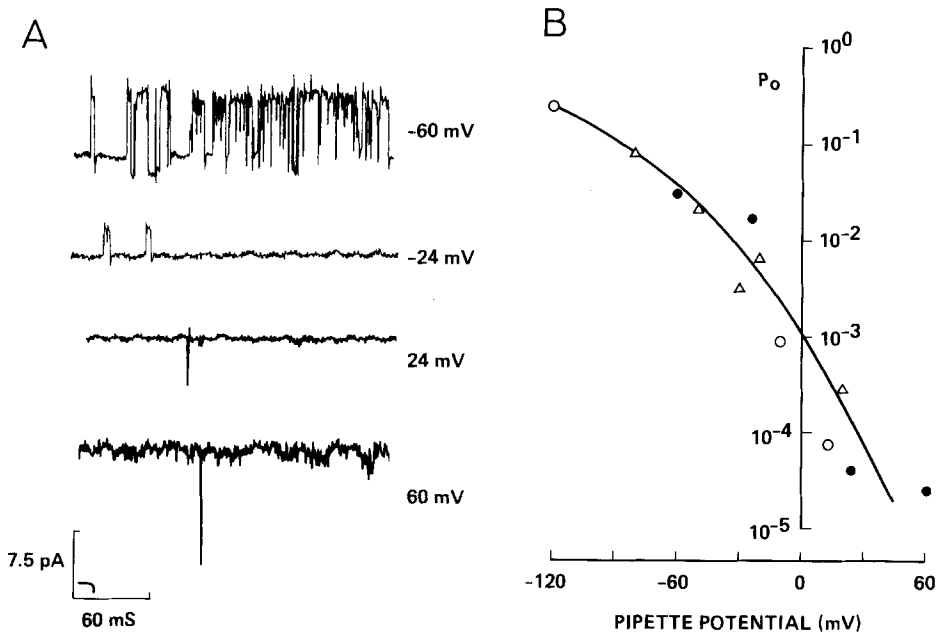

Fig. 5. Channel-open probability (P_o) as a function of pipette potential. (*A*) Single-channel records (filtered 3 kHz) for an inside-out patch containing two channels in symmetrical KCl 110 mM solutions with 500 nM Ca^{2+} in the bath solution. V_p was varied between −60 and 60 mV. (*B*) Open probabilities at these (●) and other potentials (○) plotted against V_p, were calculated using Eq. (1). Values for channels in another patch held under identical conditions are also shown (△). The line was fitted by eye and indicates that there is good agreement for the values obtained from both patches

control pH of 7.3, raising pH to 7.8 increased the P_o of the channels, while acidification to 6.8 reduced P_o. A unitary change in pH caused P_o to vary by about two orders of magnitude. At all pH values, depolarization of the patch caused an increase in P_o. At pH 7.8, the voltage activation was less marked (~sevenfold for a 43-mV depolarization to ~24-fold at pH 7.3). This behavior was somewhat reminiscent to that seen at high (75 μM) calcium activities. Single-channel conductance was not affected by changes in cytoplasmic pH.

K Channel Block

The channel blockers TEA^+ and Ba^{2+} were used in a further characterization of the channels. The action of the blockers on both P_o and single-channel conductance were studied. To increase the number of channel openings and thus allow a study of Ba^{2+} block over a wide range of potentials, the bath Ca^{2+} activity was 75 μM. Addition of 5 mM Ba^{2+} to the bath solution (●) reduced P_o as compared to the control values (○) at all potentials (Fig. 6). The block was voltage dependent, so that the reduction of P_o was more pronounced at depolarizing potentials (e.g., Ba^{2+} reduced P_o 880-fold at V_p = −55 mV, but only 11-fold at V_p = +55 mV). Ba^{2+} appeared to cause long periods where no channel

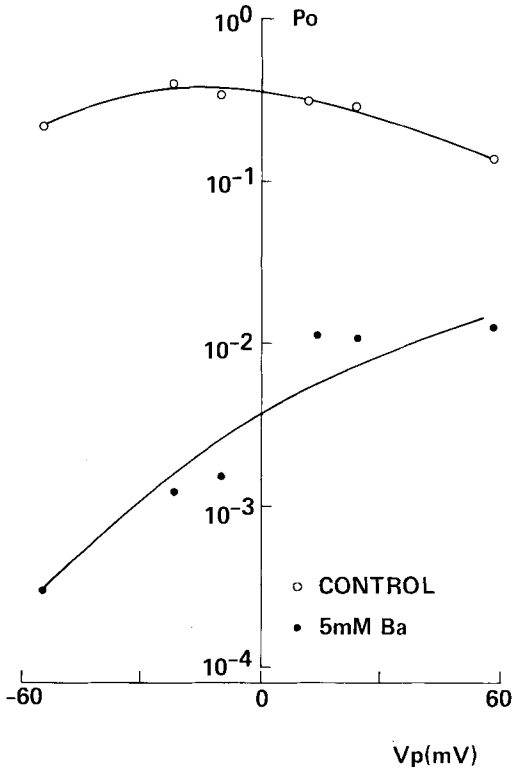

Fig. 6. Voltage-dependent block of K^+ channel-open probability by Ba^{2+}. P_o for two channels in an inside-out patch are shown in the presence (●) and absence (○) of 5 mM Ba^{2+} as a function of potential. Bath Ca^{2+} activity was 75 μM

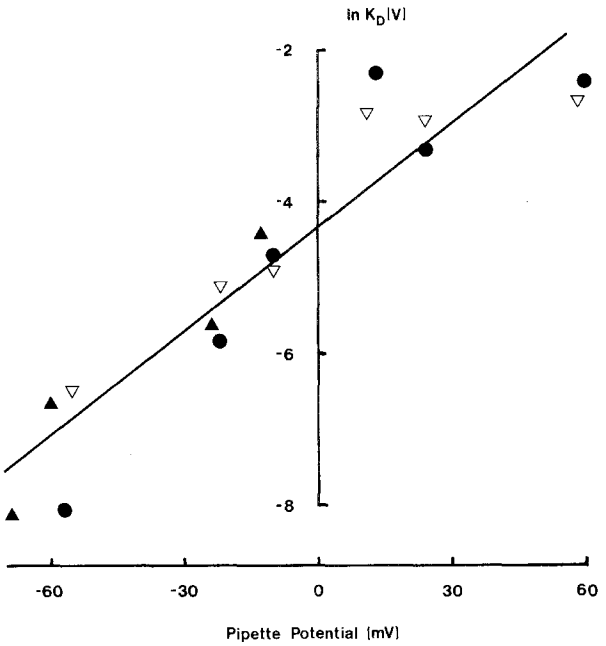

Fig. 7. Voltage dependence of K_D. Values for $K_D(V)$ calculated using Eq. (2) from the data shown in Fig. 6 (●) and two other experiments (▲, △) were plotted against V_p. The line has been fitted through the combined data using Eq. (4), so its slope = $-zdF/RT$. The value of d = slope · RT/zF and was 0.58. K_D(0 mV) was calculated from the y-intercept and was 0.013 mM. The Ca^{2+} activity in the control was 75 μM, but the addition of 5 mM Ba^{2+} will shift the Ca^{2+}:EGTA equilibrium increasing Ca^{2+} by up to 150 μM. The calculated K_D(0 mV) may be a slight underestimate, assuming that the Ca^{2+} tends to increase P_o.

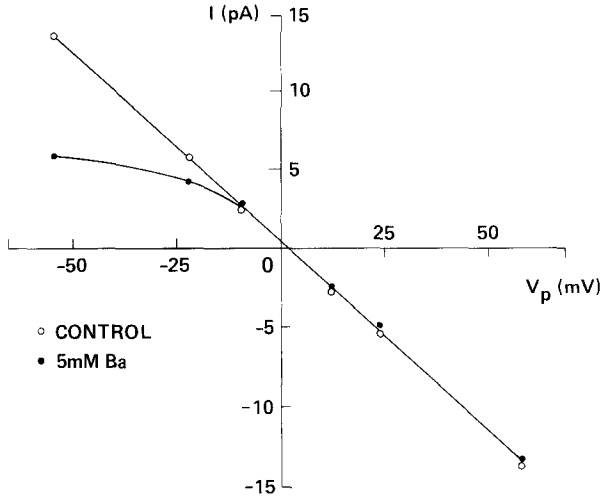

Fig. 8. Effect of internal Ba^{2+} on the single-channel I-V relationship. Currents were from the same patch as Fig. 6, as shown in the absence (○) and presence (●) of 5 mM Ba^{2+}. The straight line was fitted through the control values (slope = 237 ± 10 pS). The curve through the points measured in the presence of Ba^{2+} were fitted by eye

openings were observed. At V_p = −55 mV, over 99% of time that both channels were closed was spent in inactive periods of longer than 1 sec, compared to 28% of the closed time in control (75 μM Ca^{2+}) conditions. Long periods of channel quiescence have been observed with Ba^{2+} in other tissues (Vergara & Latorre, 1983; Benham et al., 1985), and they are considered to be due to inactivation caused by the binding of Ba^{2+} to the channel.

The action of Ba^{2+} can be further described, if a simple three-state model is adopted for channel behavior. Two of the states are the open and closed channel, while the third is a blocked state caused by the binding of a single Ba^{2+} ion to a site on the channel. The dissociation constant, K_D, for Ba^{2+} binding to the open channel is equal to the ratio (k_{-1}/k_1) of the "Off-" (k_{-1}) to the "On-" (k_1) constants for Ba^{2+} binding (as is shown in the simplified model for channel activity, Eq. (2)).

Closed Open Blocked

$$C \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} O\,Ba^{2+}. \qquad (2)$$

These rate constants can be determined for a single channel from the mean open, closed and blocked times in the presence of Ba^{2+}. In a multichannel patch, the mean closed times of a single channel cannot be easily obtained. However, the kinetics of the blockage can be studied using the following relationship derived by Benham et al. (1985)

$$K_D(V) = \frac{[\mathrm{Ba}] \cdot P_{\mathrm{Ba}} \cdot P_o}{P_o - P_{\mathrm{Ba}}}. \qquad (3)$$

Where $K_D(V)$ is the voltage-dependent dissociation constant for Ba^{2+} binding, P_{Ba} and P_o are the open probabilities in the presence and absence of Ba^{2+} (at a concentration [Ba]). Equation (3) is derived from the kinetic model shown in Eq. (2) and assumes that the rate constants α and β are not affected by Ba^{2+}.

By plotting the estimated values for ln $K_D(V)$ against potential (Fig. 7), we can show that $K_D(V)$ is dependent on potential as in Eq. (4)

$$K_D(V) = K_D(0\ \mathrm{mV}) \exp(-zdFV/RT). \qquad (4)$$

Where K_D(0 mV) is the dissociation constant at 0 mV, z is the valence of the blocking ion, and R, T and F have their usual meanings. d is defined as the fractional distance across the electrical field at which the blocking site is located. Data from three experiments are plotted in Fig. 7, and from the slope of the line through the combined data, d = 0.57 ± 0.06. K_D(0 mV) was obtained from the y-

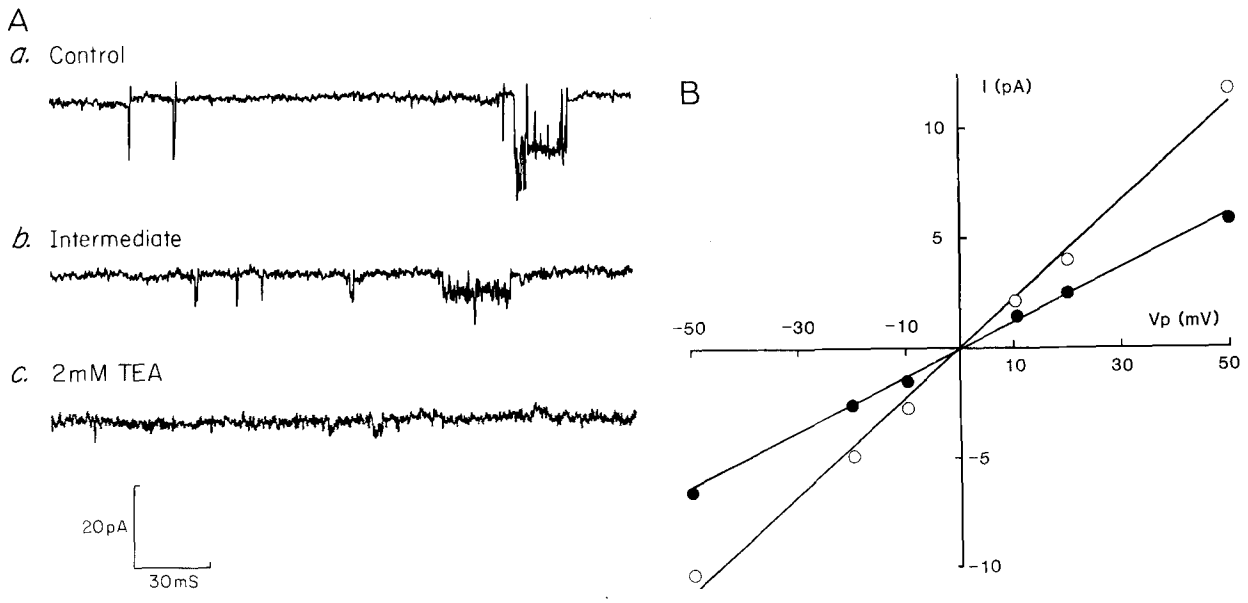

Fig. 9. TEA^+ block of K^+ currents in an outside-out patch. (*A*) The currents recorded: (*a*) under control conditions (*c*) in the presence of 2 mM bath TEA^+, and (*b*) during the perfusion of 2 mM TEA^+ into the bath are shown, at V_p = 108 mV. (*B*) Shows single-channel currents measured in two outside-out patches in the absence (○) and presence (●) of 0.2 mM TEA^+. The lines through the points are fitted by linear regression giving conductances of 220 ± 10 pS and 124 ± 4 pS in the absence and presence of TEA^+, respectively. The pipette and bath contained 110 mM KCl Ringer with 500 nM Ca^{2+}

intercept of the line and was 0.013 mM. The value for d suggested that the blocking site for Ba^{2+} lies halfway through the electric field from the inside of the membrane. Because a large portion of the electric field is sensed, this could account for the strong voltage dependence of the Ba^{2+} block.

It also appeared that Ba^{2+} reduced single-channel conductance (Fig. 8). The *I-V* relationship in control condition was linear (○); slope = 237 ± 10 pS. In the presence of 5 mM Ba^{2+} (●), the relationship deviated from linearity. At positive V_p's, the currents measured were similar to control, but at negative V_p's the single-channel currents were reduced. This reduction in current was more pronounced at larger depolarizing potentials. Thus, Ba^{2+} exerted a voltage-dependent block of channel conductance. Values for K_D(0 mV) and d were calculated from the differences between the conductances measured in the presence and absence of Ba^{2+} at V_p = −60 to 60 mV (*see* Blatz & Magleby, 1984). The value obtained for d (0.58 ± 0.11, n = 4) was not very different from that for P_o. This suggests that the blocking sites for the conductance and P_o effects are close to each other. The K_D(0 mV) of 117 ± 23 mM (n = 4), however, was 10^4 times greater than that for P_o.

In contrast to Ba^{2+}, TEA^+ produced a marked reduction in single-channel current at low (2 mM) concentrations. Figure 9*A* shows the effect of perfusing 2 mM TEA^+ into the bath solution of an outside-out patch (external TEA^+). The currents measured at V_p = 108 mV are shown under (a) control conditions, (b) during the perfusion, and (c) on completion of the perfusion. Single-channel currents of 15.7 pA observed under control conditions were virtually abolished over the course of the TEA^+ perfusion.

Figure 9*B* shows the effect of 0.2 mM external TEA^+ on the *I-V* relationships of channels in two outside-out patches. The data are pooled and show that both in the absence (○) and presence (●) of 2 mM TEA^+ the currents appeared to be a linear function of V_p (i.e., the external TEA^+ block shows little voltage dependence). The mean control conductance was 220 pS and in the presence of 0.2 mM TEA^+, it was 124 pS. A K_D(0 mV) of 0.23 mM was calculated from these values.

The addition of 0.2 mM TEA^+ to the bath solution of the two outside-out patches reduced P_o to about half its control value. The reduction in P_o did not seem to be voltage dependent, e.g., in two experiments at V_p = 50 it was reduced to 27% (0.0041 to 0.0011); at V_p = 10 mV it was reduced to 51% (0.0096 to 0.0049); at V_p = −50 mV it was reduced to 43% (0.0044 to 0.0019). This reduction of P_o was similar to that reported by Iwatsuki and Petersen (1985) from their experiments on channels in pancreatic acinar cells.

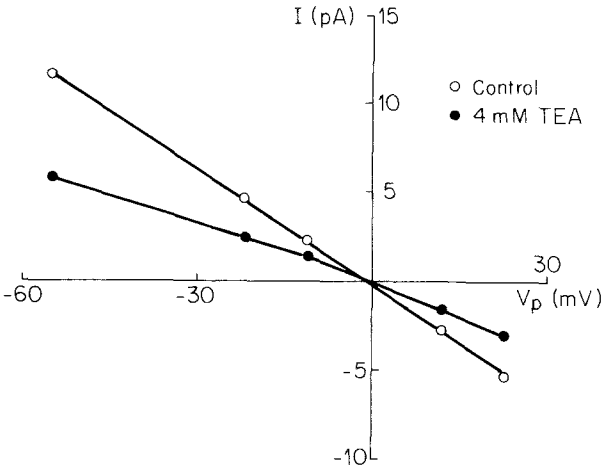

Fig. 10. Single-channel *I-V* relationship with 4 mM TEA^+ in the bath solution of an inside-out (110 mM KCl/110 mM KCl) patch (●). The line through these points was fitted by eye as the currents at negative V_p's deviate slightly from linearity. Currents measured under control conditions (○) are also shown, and the straight line fitted through them, by regression analysis, gives a conductance of 212 ± 4 pS. Bath Ca^{2+} activity was 500 nM

A higher concentration of TEA^+ was required to reduce single-channel currents when the ion was applied to the cytoplasmic face of an inside-out patch. At least 10 mM internal TEA^+ was required to reduce single-channel currents to a level at which they were indistinguishable from baseline noise (*data not shown*). Figure 10 shows the results from one of three similar experiments in which it was possible to measure single-channel currents at a number of potentials in the presence and absence of 4 mM TEA^+. A straight line was fitted to the control points (○) giving a conductance of 212 ± 4 pS. In the presence of 4 mM TEA^+ (●), currents were reduced by ~50%. The currents were actually reduced to a greater degree at depolarizing potentials; at a V_p of 23 mV the current was 58% of the control compared to only 49% at −55 mV. This deviation from linearity indicated a weak voltage dependence of the block, and from the results in this experiment $d = 0.2$. This suggests that there is a binding site for TEA^+ located about 20% of the way through the applied electric field from the inside. The value for the $K_D(0\text{ mV})$ for TEA^+ binding to this site was calculated to be 5.6 mM.

Discussion

Voltage-dependent, Ca^{2+}-activated K^+ channels have been found in the apical membrane of the choroid plexus. These channels can be classified as "maxi" Ca^{2+}-activated K^+ channels according to the criteria of Latorre and Miller (1983). In this discussion, we shall compare the properties of these channels with Ca^{2+}-activated K^+ channels found in other tissues. The possible importance of the channels to the choroid plexus will be discussed in detail in the following paper (Loo et al., 1988).

Although the channels have conductances around two distinctive means (150 and 220 pS), they seemed similar in other respects. The channels are highly selective to K^+ and are virtually impermeable to Na^+ and Cs^+. The high selectivity of Ca^{2+}-activated K^+ channels despite their high conductances has been reported elsewhere (Latorre & Miller, 1983; Blatz & Magleby, 1983).

Channel activity was affected by at least three factors: (i) membrane potential, (ii) intracellular pH, and (iii) intracellular Ca^{2+}. A 22.5-mV depolarization of the patch-membrane potential can cause up to a 10-fold increase in P_o (Fig. 5, Ca^{2+} = 500 nM), while a unit change in pH affects P_o by up to two orders of magnitude (Table). Increasing Ca^{2+} from 5 to 500 nM (Fig. 4) and then to 75 μM (Fig. 6) increased P_o at each potential examined. The voltage dependence of channel opening, measured over a similar range of potentials, was less marked at the higher Ca^{2+} activity (Fig. 5 *vs.* Fig. 6). Thus the effects of Ca^{2+} and potential on P_o, appear as though they may be related. Such interdependency may be explained by the model for Ca^{2+}-activated K^+ channels proposed by Moczydlowski and Latorre (1983). This predicts that there are two Ca^{2+} binding sites per channel that affect its opening. Changes in potential are thought to facilitate Ca^{2+} binding to these sites. Increasing Ca^{2+} activity acts to shift the sigmoidal curves relating V_p to P_o, so that the channel is open more frequently at the same potential (Wong, Lecar & Alder, 1982; Moczydlowski & Latorre, 1983). By fitting our data to a sigmoidal dependence of open probability (P_o) on membrane potential, we have found that a 10-fold increase in Ca^{2+} activity would result in a 85-mV shift, in the hyperpolarizing direction, of the potential at which the channel should be open for 50% of the time. This change is similar to the 75-mV shift per 10-fold increase in Ca^{2+} measured in anterior pituitary cells by Wong et al. (1982).

The effect of pH upon P_o has not been addressed in detail here, but it seems likely that it too may be related to changes in Ca^{2+} binding. Increasing H^+ activity seems to have an opposite effect to Ca^{2+}. Based on our present results, we estimate that a 10-fold increase in H^+ activity causes an 75-mV shift, in the depolarizing direction, of the potential giving P_o = 0.5. This is in the range (38 to 115 mV) of the shift reported for channels in pancreatic β-cells (Cook, Ikeuchi & Fujimoto, 1984). Thus, a

10-fold increase in Ca^{2+} seems to have an equivalent effect to a 10-fold decrease in H^+ activity, over a similar concentration range (10^{-5} to 10^{-8} M). From such observations, it can be tentatively concluded that protons may be competing with Ca^{2+} ions for the same binding sites on the channels. A similar conclusion was reached by Christensen and Zeuthen (1987). However, Christensen and Zeuthen did not observe an increase in channel activity on increasing pH from 7.4 to 8.4. This may well be because of the much higher (10 μM as opposed to 500 nM) Ca^{2+} concentrations used in their experiments, which may have been sufficient to saturate (or nearly saturate) the binding sites even at pH 7.4.

The kinetics of Ca^{2+} binding are also affected by a number of factors, including the lipid environment of the channels (Moczydlowski & Latorre, 1983), giving channels different sensitivities to activation by Ca^{2+}. Such differences have been used to explain variations in P_o for channels in various tissues from a number of species (Latorre & Miller, 1983; Benham et al., 1985). A low sensitivity to Ca^{2+} may also help explain why the channels from choroid plexus show a relatively low P_o ($P_o = 10^{-4}$ at 0.5 μM Ca^{2+}), when compared to values measured for channels from rat muscle ($P_o = 0.6$ at 1 μM Ca^{2+}, Barrett, Magleby & Pallotta, 1982), or mouse lacrimal gland ($P_o = 1.0$ at 0.1 μM Ca^{2+}, Findlay, 1984) at a similar potential ($V_p = -40$ mV) and pH (7.2–7.3).

At high (75 μM) Ca^{2+} concentrations, long periods of channel inactivity were observed, which caused P_o to decrease slightly at large depolarizing potentials (−55 mV, Fig. 6). These long closings were reminiscent of those seen with Ba^{2+} (*see below*). Where periods of channel inactivity at high Ca^{2+} have been reported elsewhere (Vergara & Latorre, 1983; Findlay et al., 1985), they were considered the result of a Ca^{2+} block of the channels. A Ca^{2+} block of the channels in choroid plexus may explain why experimentally P_o's of greater than 0.5 were rarely measured.

The experiments using Ba^{2+} and TEA^+ show that the channels may be blocked in two ways: through a reduction of P_o or through a reduction of conductance. The way in which the action of a blocker is manifest depends upon the time for which the blocker is bound to the channel (Hille, 1984). Ba^{2+} is generally considered to be a "slow" blocker affecting P_o only. Here we have shown that Ba^{2+} applied to the inside of the membrane primarily causes a decrease in P_o (Fig. 6), but it also appears to reduce the single-channel conductance (Fig. 8), which is characteristic of a "fast" blocker. In both cases, the block was voltage dependent and was more effective at depolarizing potentials. It has previously been reported that Ba^{2+} reduces the P_o of Ca^{2+}-activated K^+ channels in a voltage-dependent manner (Vergara & Latorre, 1983; Benham et al., 1985). The voltage dependence can be accounted for because the binding site for Ba^{2+} is more than halfway through the applied electric field, in this case at a functional distance of 0.57 from the inside. This value for d and the estimated $K_D(0\text{ mV})$ = 0.013 mM are quite similar to the values measured for channels from rat muscle $d = 0.8$ and $K_D(0\text{ mV})$ = 0.036 mM (Vergara & Latorre, 1983), and for channels in guinea pig smooth muscle $d = 0.96$ and $K_D(0\text{ mV}) = 0.073$ mM (Benham et al., 1985).

It was calculated that the apparent $K_D(0\text{ mV})$ for the Ba^{2+} block of conductance was 117 mM and $d = 0.58$. It is unlikely that there are two sites for Ba^{2+} binding, both at a similar location within the channel; one affecting conductance and the other P_o. The reduced conductance at high Ba^{2+} concentrations may be caused by the rapid occupation or reoccupation of a single site at a rate high enough to prevent the full channel current ever being resolved. Whatever the mechanism, the effect of Ba^{2+} on conductance when applied internally to Ca^{2+}-activated K^+ channels has not previously been reported. However, McCann and Welsh (1986), recently reported that external Ba^{2+} blocks the conductance of K^+ channels from tracheal smooth muscle in a voltage-dependent manner.

TEA^+ on the contrary is known mainly to affect single-channel conductance. When applied to the cytoplasmic face of a patch, 4 mM TEA^+ reduces single-channel currents by about 50% ($K_D(0\text{ mV})$ = 5.6 mM). The block showed only slight voltage dependence ($d = 0.20$). The channels from choroid plexus seemed quite sensitive to internal TEA^+ compared to other tissues. Values for $K_D(0\text{ mV})$ in the range 35–60 mM have been reported for channels from rat muscle (Blatz & Magleby, 1983; Vergara, Moczydlowski & Lattorre, 1984). Values for d (0.26 to 0.34) similar to that for choroid plexus were also reported (Blatz & Magleby, 1983; Vergara et al., 1984).

When TEA^+ was applied to the outside of the patch (Fig. 9), it reduced single-channel currents with a $K_D(0\text{ mV}) = 0.23$ mM. This apparent higher affinity of TEA^+ binding from the outside, compared to that from the inside, has been reported in other studies. $K_D(0\text{ mV})$ values of ~0.3 and 0.29 mM were reported for channels for rat muscle (Blatz & Magleby, 1983; Vergara et al., 1984). The different affinities of TEA^+ binding at opposite sides of the membrane suggest that the channels possess two binding sites for TEA^+. Further evidence for this is provided by the fact that external TEA^+ block is not voltage dependent (*see also* Blatz &

Magleby, 1983; Vergara et al., 1984). It can be concluded that a high affinity site is found at the external opening of the channel, while a lower affinity site is located at or near the internal side (Vergara et al., 1984).

In general the binding kinetics of TEA^+ and Ba^{2+} to channels from choroid plexus seem to agree quite well with those observed for channels from mammalian muscle. Therefore, it is intriguing to speculate that the Ca^{2+}-activated K^+ channels described here from *Necturus* choroid plexus, share a common molecular structure with these channels from other tissues. The blockers we have studied will be valuable tools in the study of the contribution of the K^+ channels to the whole-cell conductance in this epithelium (Loo et al., 1988).

In most epithelia studied until now, the majority of Ca^{2+}-activated K^+ channels have been observed in the basolateral membrane of the cell (Findlay, 1984; Petersen & Maruyama, 1984; Sepulveda & Mason, 1985). It is also well established that the sodium/potassium pumps are located in the basolateral membrane of most epithelia (Stirling, 1972; Lamb, Ogden & Simmons, 1981). Although the roles of the Ca^{2+}-activated K^+ channels are still unclear, it has frequently, if tentatively, been proposed that their activity is in some way linked to that of the sodium pumps.

We now report that Ca^{2+}-activated K^+ channels are a predominant feature of the apical membrane of choroid plexus: a membrane already known to be highly conductive to K^+ (Zeuthen & Wright, 1981), and to contain the sodium/potassium pumps (Quinton et al., 1973; Masuzawa et al., 1984; Ernst et al., 1986). It is tempting to suggest that the channels may contribute a large proportion of the conductance of the apical membrane, and so be ultimately responsible for the efflux of the K^+ pumped into the cell by the sodium pumps. The contribution of the channels to the permeability of the apical membrane can be estimated. Since the patch area is 1.8 $(\mu m)^2$ (diameter 1.5 μm) and on the average there are two channels per patch, channels with conductances of 152 or 218 pS would contribute a P_K of 4.0 to 5.8 $\times$ 10^{-5} cm $\cdot$ sec^{-1} if they were totally open. This is in good agreement with the value of 2.2 $\times$ 10^{-5} cm $\cdot$ sec^{-1} estimated by Christensen and Zeuthen (1987) in patch-clamp studies for channels in the choroid plexus of the third ventricle, again assuming that the channels are fully open. But since the probability (P_o) of a channel opening ($\sim 10^{-4}$) is extremely low under normal resting conditions, the contribution of these channels to P_K will be at least two orders of magnitude less than this value. Using microelectrodes, Zeuthen et al. (1987) obtained a value for P_K of 2.1 to 2.4 $\times$ 10^{-5} cm $\cdot$ sec^{-1}. This is close to the estimate of the contribution from Ca^{2+}-activated K^+ channels if they were always open. If this estimate of P_K using microelectrodes is accurate, then the Ca^{2+}-activated K^+ channels would make a very small contribution to the apical membrane resting K^+ conductance. However, there is an uncertainty in the estimates of P_K from both single-channel and microelectrode studies as the role of microvilli has not been taken into account. The surface area of the apical membrane may be increased up to 20-fold due to microvilli. To what degree this will underestimate P_K from the patch-clamp measurements cannot be assessed at present. Likewise, the estimates of P_K in the microelectrode studies of Zeuthen et al. (1987) may be an overestimate as no correction has been made for the increase in surface area due to microvilli. To resolve this uncertainty, in whole-cell recordings we have determined the cell capacitance, which indicates membrane area, and have measured the Ca^{2+}-activated K^+ currents (Loo et al., 1988). It will be seen that these channels make a small contribution of $\sim$10% to the total resting conductance of the cell, but their relative contribution increases drastically to as high as 40% with depolarization of the apical membrane, as is observed when the choroid plexus is stimulated to secrete CSF by cAMP.

In conclusion, the present study shows that there are several factors, Ca^{2+}, intracellular pH and membrane potential, that control channel activity and hence the K^+ conductance of the apical membrane. It is probable that these factors, through their action on the K^+ channels, have an important bearing on the transepithelial K^+ transport.

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