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Potassium Channel in Rabbit Corneal Endothelium Activated by External Anions

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Summary. The apical membrane of the rabbit corneal endothelium contains a potassium-selective ionic channel. In patch-clamp recordings, the probability of finding the channel in the open state (P_o) depends on the presence of either HCO_3^- or Cl^- in the bathing medium. In a methane sulfonate-containing bath, P_o is <0.05 at all physiologically relevant transmembrane voltages. With 0 mM $[HCO_3^-]_o$ at +60 mV, P_o was 0.085 and increased to 0.40 when $[HCO_3^-]_o$ was 15 mM. With 4 mM $[Cl^-]_o$ at +60 mV, P_o was 0.083 and with 150 mM Cl^-, P_o increased to 0.36. Low P_o's are also found when propionate, sulphate, bromide, and nitrate are the primary bath anions. The mechanism of action of the anion-stimulated K^+ channel gating is not yet known, but a direct action of pH seems unlikely. The alkalinization of cytoplasm associated with the addition of 10 mM $(NH_4)_2SO_4$ to the bath and the acidification accompanying its removal do not result in channel activation nor does the use of Nigericin to equilibrate intracellular pH with that of the bath over the pH range of 6.8 to 7.8. Channel gating also is not affected by bathing the internal surface of the patch with cAMP, cGMP, GTP-γ-s, Mg^{2+} or ATP. Blockers of Na/H^+ exchange, Na^+-HCO_3^- cotransport, Na^+-K^+ ATPase and carbonic anhydrase do not block the HCO_3^- stimulation of P_o. Several of the properties of the channel could explain some of the previously reported voltage changes that occur in corneal endothelial cells stimulated by extracellular anions.

Key Words cornea · endothelium · potassium channel · HCO_3^- · Cl^- · anions · patch clamp · rabbit

Introduction

Recently we have discovered and partially characterized a potassium-selective ionic channel located in the apical membrane of the rabbit corneal endothelium (Rae, 1985, 1986; Rae, Dewey & Cooper, 1988*a*, 1989; Rae, Levis & Eisenberg, 1988*b*). Using voltage patch-clamp techniques, we have shown that the channel has an inwardly rectifying single channel current-voltage relationship and flickery gating kinetics (Rae et al., 1988*b*). It shows several subconductance levels and blockade by external Cs^+ in the sub-millimolar range (Rae et al., 1989). These block studies suggest that the channel is capable of simultaneous occupancy by more than a single ion.

During early study of the K^+ channels, many of the currents were recorded from cells depolarized in a Na^+-free, K^+ methane sulfonate solution. This was done because the membranes commonly contain three channel types. Two of them, a large conductance anion channel and a Ca^{2+}-activated nonselective cation channel, were gated to such an extent in inside-out patches that the K^+ channels of interest could not be studied effectively in excised patches. So that the channels could be studied in on-cell patches, a K^+ depolarizing solution was used to wash out intracellular Na^+ and to depolarize the cell to near zero mV transmembrane voltage. As we began to use more physiological bathing solutions, i.e., those containing Cl^- and HCO_3^-, we noted that the K^+ channels were much more active.

These results are of interest because the ability of the endothelial cells to remove fluid from the corneal stroma is known to depend on the presence of HCO_3^- in the bathing medium (Dikstein & Maurice, 1972; Dikstein, 1973; Hodson, 1971; Wigham & Hodson, 1981; Liebovitch & Fischbarg, 1982/1983; Lim, 1982/1983). A HCO_3^--stimulated K^+ channel might, therefore, be an integral part of the fluid transport cascade. Also, in models of transcellular solute flux using anion transport and the Na^+ pump, the gating of a K^+ channel is suggested to accompany the increased activity of the Na^+-K^+ ATPase to increase K^+ efflux in proportion to the enhanced K^+ influx inherent in the elevated pumping rates (O'Grady, Palfrey & Field, 1987).

We, therefore, set out to quantify the effect of anions on the open probability of the channel. Here we present the results of those experiments wherein we show that bath anion substitutions produce substantial and repeatable effects on K^+ channel gating.

Materials and Methods

All experiments were done on the aqueous humor-facing apical membrane of the corneal endothelium of 1–2 kg New Zealand white rabbits. The animals were housed in NIH approved housing and handled in accordance with the ARVO resolution on the use of animals in eye research. The animals were sacrificed via a single overdose injection of T-61 euthanasia solution into a marginal ear vein.

Table. Solutions

	Na	K	Cl	Mes	Ca	Glucose	HEPES
Normal Ringer	150	4	158	0	2	5	5
KMeS Ringer	0	150	4	150	2	5	5
NaMeS Ringer	150	4	4	150	2	5	5

All Values are in millimolar. pH = 7.3–7.4.

Tissue Preparation

A button of corneal tissue was obtained by use of an 8 mM trephine applied to the front surface of the cornea in the region of the optic axis in enucleated rabbit globes. The button was transferred to a petri dish containing our normal Ringer's solution so that subsequent manipulations could be done without tissue drying. The endothelium and its basement (Descemet's) membrane were removed from the stroma by gently teasing them loose with two pairs of #5 jeweler's forceps. The removed tissue was pinned around its periphery to a 12-mm Sylgard disk with #000 stainless steel insect pins. The tissue was gently stretched so that no wrinkles or folds existed in the central region.

The Sylgard disk was transferred to a well of the appropriate size in an acrylic plastic chamber bolted to a heavy metal superstructure located under a custom Nikon Labophot upright, compound microscope. The microscope was equipped with 5×, 20×, and 40× extra long working distance metallurgical objectives modified for Hoffman modulation contrast. The objectives had sufficient working distance that a patch electrode fit under them and allowed direct observation of the contact of the electrode tip with the cell. The well was about 50% covered by a piece of glass cut from a standard microscope slide. The electrode contacted a cell located under the glass to allow viewing of the preparation without interference from the meniscus at the outer edge of the pipette. The glass also reduced the loss of CO_2 from the bath when HCO_3^--containing solutions were used.

Patch electrodes were pulled from either Corning #7052 or #7760 glass (Garner Glass, Claremont, CA) by use of a Sutter Instruments (San Francisco, CA) model PC-80 electrode puller. They were coated with Sylgard (Dow Corning, Midland, MI) and firepolished to a distinct endpoint at a magnification of 1500×. The electrodes were attached to a custom polycarbonate electrode holder, which was inserted into a Teflon insulated input connector of a patch-clamp headstage.

The pipettes were pressed against the cell membrane by use of a motorized linear translator (Klinger Scientific, Richmond Hill, NY) while continually recording the current response to a 0.4-mV pulse delivered at 100 Hz. The pipette advance was stopped when the resistance increased by about a factor of two. In many instances, the cell and pipette sealed without the application of suction but in other cases, gentle suction was required.

The cells could not be perfused continually with a HCO_3^--containing Ringer bubbled with CO_2 since constant perfusion increased the background noise. We, therefore, used HCO_3^- solutions that were buffered with 5 mM HEPES buffer and kept in gas-tight syringes, and we simply replaced the bath with these solutions every few minutes. This means that the HCO_3^- concentration, pH, and pCO_2 of the bath were able to change as CO_2 escaped to the room air. The maximum time between bath changes was 8 min. To determine the change in HCO_3^- with time, bath samples acquired in gas-tight syringes at various times after changing the bath were subjected to HCO_3^-, pH, and pCO_2 analysis using an IL Model 813 blood gas analyzer (Instrumentation Laboratory, Lexington, MA). As expected, the bath lost HCO_3^- and CO_2 with time and its pH increased. At 10 min after a bath change to a fresh HCO_3^- Ringer, the bath had lost an average of 5% of its HCO_3^-, 21% of its CO_2 and increased in pH by 0.1 pH unit. These changes were considered to be insignificant given the apparent insensitivity of the channels to intracellular pH, as shown later.

Analysis

The amplitude of the single channel currents was determined by a level crossing optimization algorithm (Sachs, Neil & Bakakarti, 1982), which first determined the current of the baseline and then the current of the open channel. The channel amplitude was taken as the difference between the two levels. Each determination was verified by the operator to be visually correct.

Channel open probabilities were determined from histograms of the raw current records from patches containing only these K^+ channels. The histograms consisted of a Gaussian peak for the baseline, another for one channel open, another for two channels open, etc. The histograms were fitted to the equation for multiple Gaussian distributions using a nonlinear curve-fitting algorithm. The areas under each of the peaks was used along with the number of peaks to calculate the open probability from the Binomial distribution (Colquhoun & Hawkes, 1983).

P_o *vs.* voltage curves were compared to the predictions from the equation for a single Boltzmann distribution (Moczydlowski, 1986)

$$\ln\left[\frac{P_o}{1-P_o}\right] = \frac{Z\delta F}{RT}(V - V_o)$$

where $Z\delta$ = effective gating charge, V_o = voltage where P_o = 0.5, and F, R, T have their usual meanings. The curves and theory were compared by a nonlinear curve-fitting algorithm in which both V_o and $Z\delta$ were allowed to float until the least squares deviation between data and theory was minimized. The values of V_o and $Z\delta$ so obtained were then substituted into the theory equation and a P_o *vs.* V curve was generated. This curve was then overplotted with the actual data points.

Solutions

The solutions used in the pipette or bath are specified in the Table. The DIDS, ouabain, amiloride, acetazolamide, and nigericin were obtained from Sigma. Methane sulfonic acid was from Aldrich. All experiments were done at room temperature (21–22°C).

Results

The effects of $[HCO_3^-]_o$ on the gating of the K^+ channels are demonstrated in Fig. 1. In this figure, we plot individual records of unitary channel currents *vs.* time after the addition of HCO_3^- to the bath at a (−)60 mV transmembrane potential. The cells are initially bathed in 150 mM K^+, 0 mM Na^+

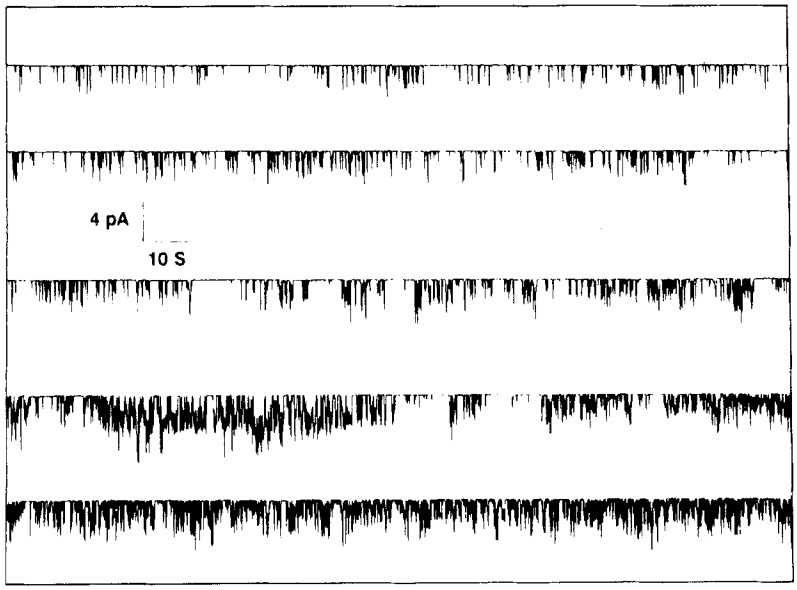
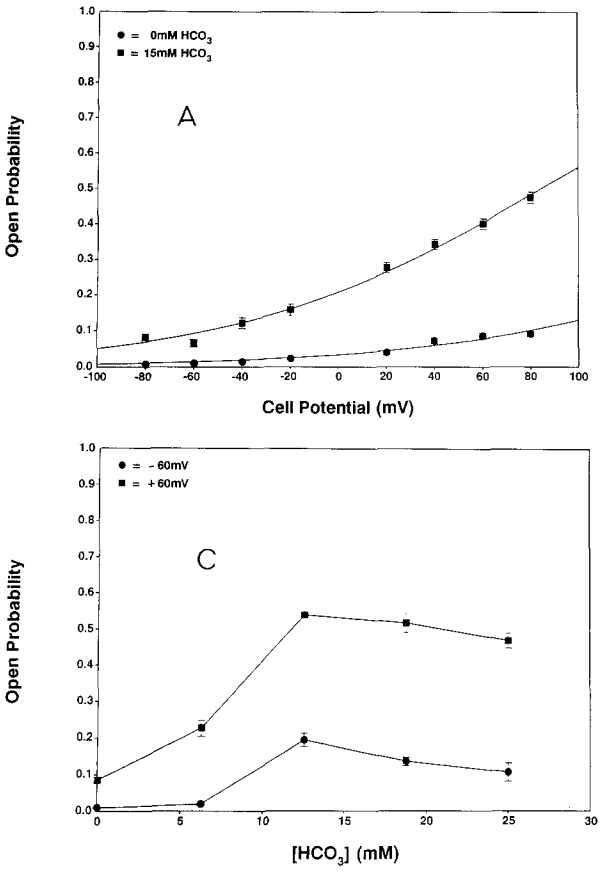


Fig. 1. A representative time course for the stimulation of channel gating by external HCO_3^-. 15 mmol KMeS is replaced by 15 mmol $KHCO_3$ at time zero. Each trace represents about 2 min of recording. Periods of reduced channel activity (double arrows) occur periodically. Bandwidth = 100 Hz, 8 pole Bessel filter. Data is from cell-attached patch at −60 mV. Pipette contains KMeS Ringer

Ringer, which depolarizes them to near zero mV and causes intracellular K^+ levels to approach those in the bath (Rae et al., 1989). External HCO_3^- results in the activation of these K^+ channels by comparison to their activity in a K^+ methane sulfonate-based bathing solution. The time course of the activation produced by the addition of HCO_3^- to the bath was variable, but was always on the order of several minutes. The half time for the bath change was about 20 sec (more rapid superfusion rates led to loss of the gigohm seal). Thus, it took about 2 min for a complete bath change. Typically, activation could be seen at about the time the bath change was completed and increased with additional time to reach a maximum by approximately 7–8 min.

These gating effects can be quantified by comparing plots of the probability of finding the channel open over a wide voltage range for each of the experimental interventions. P_o *vs.* voltage plots were established after waiting a sufficient time for the channels to activate fully for the particular intervention (≈8 min). Figure 2*A* overplots P_o *vs.* voltage curves for 0 and 15 mM $[HCO_3^-]_o$. The effect of external HCO_3^- is to increase P_o at all voltages measured. The stimulatory effect is modest at −80 mV, but becomes much more substantial with depolarization. This stimulation with $[HCO_3^-]_o$ can be modeled as a simple shift in the voltage dependence of gating to more negative voltages. Therefore, each curve is fit to a single Boltzmann distribution (solid lines). Because the voltage dependence is weak, we were unable to apply a voltage that made $P_o = 1.0$. In fact, the maximum P_o here was only about 0.4 at +80 mV. Voltages larger than +80 mV, which did increase P_o further, frequently resulted in "seal spikes" and/or loss of seal and so could not be used routinely. This P_o *vs.* voltage relationship encompassed too small a fraction of the total probability range for one to be sure that a *single* Boltzmann distribution was an appropriate fit to the data. Nevertheless, we proceeded as if it were. The dose-response relationships for HCO_3^- activation of the K^+ channel are shown in Fig. 2*B* wherein P_o *vs.* voltage curves obtained with several different $[HCO_3^-]_o$ are overplotted. The P_o increases as the $[HCO_3^-]_o$ is changed from 0 to 12 mM. Above 12 mM or so, the response shows saturation at depolarizing voltages and less activation over most of the voltage range than that seen with 12 mM. The "dose-response" curve is shown more clearly by plotting P_o *vs.* $[HCO_3^-]_o$ at two transmembrane voltages (Fig. 2*C*).

Figure 3 demonstrates that the channel can also be stimulated by external Cl^-. These plots come from a series of experiments where the channel currents were first recorded in cell-attached patches from cells bathed in KMeS Ringer. Without loss of seal, the bath was changed to NaMeS Ringer and then to NaCl Ringer. In each case, we waited 8–10 min to allow maximum channel activation to be achieved. The two curves in which methane sulfonate was the major bath anion show very similar channel activity. In one, Na^+ was the predominant cation whereas in the other, K^+ was predominate. $[Na^+]_o$ itself, therefore, does not have a substantial activating effect. When, however, the bath contains 150 mM Cl^- (upper curve), the K^+ channels are substantially activated and reach an activity level at depolarizing voltages similar to that produced by 15 mM $[HCO_3^-]_o$. Similar experiments in which bath



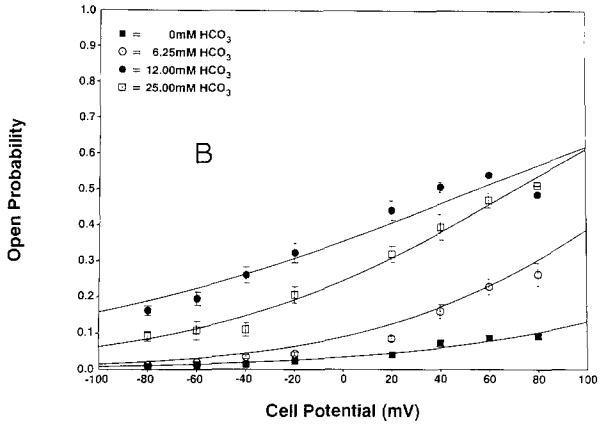


Fig. 2. Data from cell-attached patches with KMeS Ringer in pipette. (*A*) P_o *vs.* transmembrane voltage for HCO_3^--stimulated K^+ channels. Each curve is a Boltzmann distribution which best fits the experimental data points. Each point is the mean and standard error from three membrane patches. The Boltzmann parameters are: bottom trace V_o = 230 mV, $Z\delta$ = −0.37; upper trace V_o = 85 mV, $Z\delta$ = −0.40. (*B*) P_o *vs.* voltage curves for several external concentrations of HCO_3^-. Each data point is the mean and standard error from three membrane patches. Solid lines are fits to a Boltzmann distribution. The Boltzmann parameters are as follows: O HCO_3^-: V_o = 230 mV, $Z\delta$ = −0.37; 6.25 mM HCO_3^-: V_o = 124 mV, $Z\delta$ = −0.47; 12 mM HCO_3^-: V_o = 55 mV, $Z\delta$ = −0.27; 25 mM HCO_3^-: V_o = 75 mV, $Z\delta$ = −0.40. (*C*) P_o *vs.* external HCO_3^- concentration at ±60 mV transmembrane potential

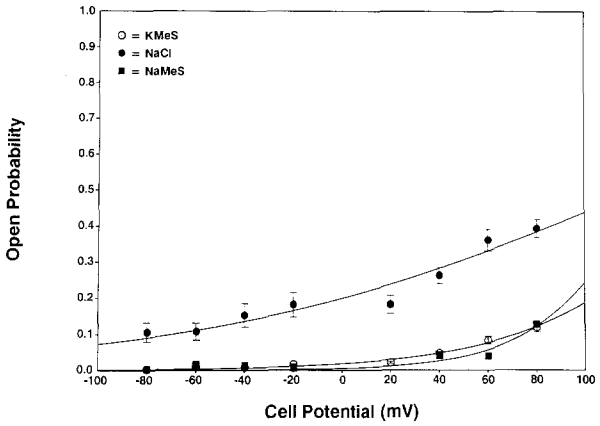


Fig. 3. P_o *vs.* voltage curves for external Cl^- stimulation of K^+ channel gating from cell-attached patches with KMeS Ringer in pipette. Each data point is mean and standard error from three membrane patches. Solid lines are fits to a Boltzmann distribution. The Boltzmann parameters are (bottom trace) V_o = 158 mV, $Z\delta$ = −0.37; (middle trace) V_o = 127, $Z\delta$ = −1.06; (upper trace) V_o = 122, $Z\delta$ = −0.29

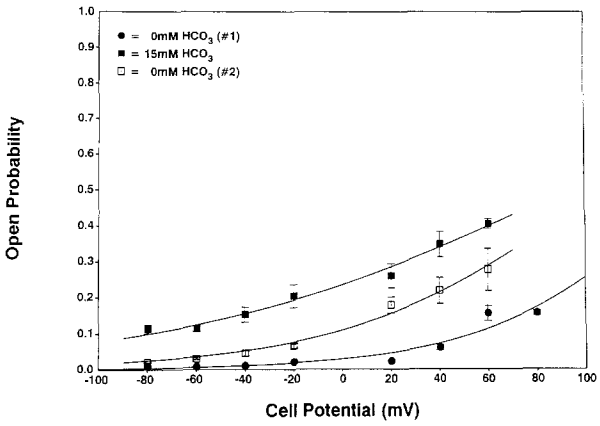


Fig. 4. P_o *vs.* voltage curves before, during, and after stimulation by external HCO_3^- in cell-attached patches with KMeS Ringer in pipette. The 15 mM HCO_3^- curve (upper trace) was determined 8–10 min after the addition of HCO_3^-. The middle trace was determined 20 min after the removal of HCO_3^-. Each data point is the mean and standard error from three membrane patches. The solid lines are fits to a single Boltzmann distribution. The Boltzmann parameters are (upper trace) V_o = 92 mV, $Z\delta$ = −0.33; (middle trace) V_o = 105 mV, $Z\delta$ = −0.51; (bottom trace) V_o = 143 mV, $Z\delta$ = −0.62

methane sulfonate was replaced by NO_3^-, SO_4^-, Br^-, or propionate produced no obvious activation. Because of the suspected role for external HCO_3^- but not Cl^- in fluid transport by endothelium, subsequent experiments were aimed at quantifying the HCO_3^- effect.

The HCO_3^- stimulation of P_o can at least, in part, be reversed. In Fig. 4, we overplot P_o *vs.* voltage before HCO_3^- stimulation, during stimulation and after washing the HCO_3^- from the bath with a

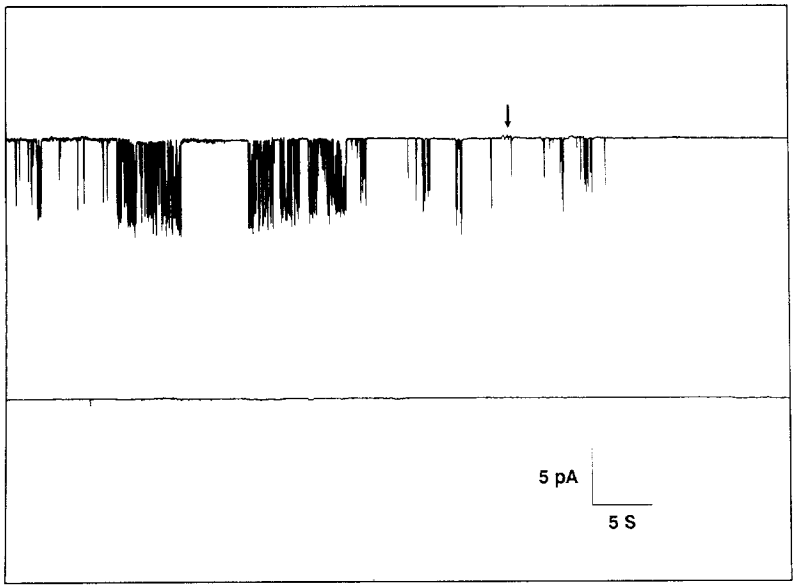


Fig. 5. Time course for the loss of channel activity following excision of a cell-attached patch into the 15 mM HCO_3^--containing solution. The arrow marks the point where the patch was excised. Pipette contains KMeS Ringer

methane sulfonate Ringer. The P_o *vs.* voltage curve does not return to prestimulation levels by 20 min following removal of HCO_3^-. By Boltzmann analysis, the voltage dependence remains shifted by about −40 mV at this time. We were unable to hold patches long enough to study recovery at longer times.

When membrane patches at the peak of stimulation were excised into the high K^+ and HCO_3^- containing bath, the K^+ channel activity decreased within a few seconds to a very low level. A record from an experiment in which only these K^+ channels were present is shown in Fig. 5. The activity decay rate was quite variable. Activity most often fell quickly, but in some instances it continued for several minutes and in a few patches activity actually increased. We have not yet quantified this time course because of its variability. For now, we simply note that the decay time is usually very fast by comparison to both the on-cell activation by $[HCO_3^-]_o$ and the on-cell loss of activity following $[HCO_3^-]_o$ removal.

We attempted to reactivate the channels in excised patches by several means. From excised patches which had previously shown substantial $[HCO_3^-]_o$ activation on-cell, we were unable to reactivate the channels by changing the bath pH over a 6.6 to 7.8 range. Internal pH was therefore inadequate by itself to stimulate channel gating. We also added a mixture of compounds including 1 μM GTP-γ-S, 5 mM ATP, 2 mM Mg^{2+}, 100 μM cAMP and 100 μM cGMP to the bath. These compounds produced no obvious activation of the K^+ channels either individually or when presented as a mixture. Pinacidil, a K^+ channel-opening drug (Cook, 1988), when applied at 10 μM either from the inside or outside of the patch did not stimulate channel gating nor did it activate channels in cell-attached patches when added to the bath.

In cell-attached patches, we tried several additional interventions to activate the channels in a HCO_3^- and Cl^- free bathing medium. In these experiments, KMeS Ringer served as the bathing medium. Adenosine and glutathione, when added to the bath at concentrations known to enhance endothelial fluid transport (Anderson, Fischbarg & Spector, 1974; Dikstein, 1973; Fischbarg, Lim & Bourguet, 1977), had no noticeable effect on gating of the K^+ channels. $(NH_4)_2SO_4$ at 10 mM also had no effect over a 30 min time period, either when added to or when removed from the bath. NH_4^+ salts at this concentration are known in many cells to produce biphasic intracellular pH changes over this time period (Boron & de Weer, 1976; Roos & Boron, 1981). The cells become alkaline when the NH_4^+ salts are added and acidic when they are removed. Such pH changes have been reported from NH_4^+ salts used with *bovine* corneal endothelial cells under somewhat different conditions (Jentsch et al., 1988). The addition of Nigericin to the high K^+ bath also had no effect on channel gating even when the bath pH (and thus the intracellular pH) was changed over the range of pH 6.8 to 7.8.

We considered the possibility that the stimulation of the K^+ channel gating might be due to cell volume changes resulting from bath changes to anions having different reflection coefficients. To test that possibility, we determined if the K^+ channels could be activated by suction. They could not. The channels were also not activated in cell-attached patches following changes in bath osmolality to as much as 50% above and below the physiological level of 300 mOsm/liter. Such interventions produced substantial cell morphology changes easily

seen through the microscope, but had no obvious effect on K^+ channel gating.

Discussion

The K^+ channel studied here has been shown to exist in the endothelium of several other species including human (Rae et al., 1989). It appears to be a transporting mechanism which, at least to date, is unique to the corneal endothelium. Its activation by external anions in rat corneas has also been verified (J.L. Rae and J. Dewey, *unpublished observations*).

P_o of the channel is increased in the presence of HCO_3^- or Cl^- in the bathing medium, but Br^-, SO_4^{2-}, NO_3^- and propionate have no obvious effect. The activating effects of external Cl^- and HCO_3^- on this channel appear to involve some intracellular change. Cl^- and HCO_3^- in the pipette filling solution do not produce channel activation. Either Cl^- or HCO_3^- must be presented to the whole cell monolayer via the bathing solution for their activating effects to be seen. Excision of the patch into a HCO_3^--containing bathing solution usually results in rapid loss of channel activity, but removal of bath Cl^- or HCO_3^- while recording from a cell attached patch results in a very slow loss of activity over a time period of tens of minutes. These results in composite point to some change inside the cell being responsible for the activation. We have been unsuccessful to date in identifying a factor which when added to the bath activates the channels in excised patches and so the activation mechanism remains unspecified at present. In cells bathed in a NaCl Ringer with 25 mM $NaHCO_3^-$, the open probability of the channel was 0.1 ± 0.02 ($n = 3$, mean ± SE) at -60 mV resting potential. This should be similar to its physiological activity at room temperature.

A reasonable first guess at the activation mechanism would have been that CO_2 from the HCO_3^--containing bathing solution crosses the membrane and produces intracellular acidification to activate the channel through some pH-sensitive mechanism. Excising patches into a bath whose pH varies over a range of 6.8 to 7.8 shows that direct pH changes at the interior of the membrane do not produce channel activation. Likewise, the addition of 10 mM $(NH_4)_2SO_4$ to the bath and its removal had no obvious effect on channel currents recorded in cell-attached patches. This $(NH_4)_2SO_4$ treatment should produce an alkalinization of cell cytoplasm as it is added to the bath followed by an acidification when it is removed as shown in bovine corneal endothelium and elsewhere (Boron & de Weer, 1976; Roos & Boron, 1981; Jentsch et al., 1984*b*, 1988). Our failure to see an effect of $(NH_4)_2SO_4$ suggests that intracellular pH is not directly involved. Nigericin, a K^+/H^+ exchanger, is used in other cells to equilibrate the intracellular pH with that of the bath. Jentsch and coworkers (1988) used it routinely to calibrate their BCECF measurement of intracellular pH from cultured bovine corneal endothelial cells and, therefore, demonstrated that it works in at least some kinds of corneal endothelial cells. With Nigericin, we saw no appreciable activation of the K^+ channel when the bath pH was changed from pH 6.8–7.8, again suggesting that an intracellular pH change is not directly involved in the gating increase.

We have chosen to think about the P_o *vs.* voltage curves as if a simple Boltzmann distribution applies. The Boltzmann distribution is meant to describe the relative population of two states separated by an energy difference. In this sense, it is appropriate to use the analysis to describe the fraction of time the channels are closed *vs.* the fraction of time they are open since the closed and open states can be modeled as different conformations of the channel protein separated by an energy difference. This analysis says little about the process or processes which might change the equilibrium occupancy of the two states but does give a formal construct in which to estimate the energy differences involved in the transitions. Based on this analysis the HCO_3^- activation is explainable by a process that shifts the voltage dependence of K^+ channel gating some 200 mV in the minus direction as $[HCO_3^-]_o$ is changed from 0 to 15 mM. Clearly, epithelial cells which do not, in general, experience large resting voltage changes would have little ability to turn these channels on in the absence of external HCO_3^-. This suggests that, in many instances, the transmembrane voltage per se may have little relevance to channel gating. It seems more likely that channel gating in epithelia will often depend on intracellular processes that shift the voltage dependence of gating into the rather small range of voltages these cells likely experience in vivo.

While our experiments were not designed to test the models of fluid transport put forth by Fischbarg et al. (1985), Jentsch, Keller, and Wiederholt (1985*a*), Widerholt, Jentsch and Keller (1985), and Lyslo et al. (1985), the results in retrospect may prove useful in explaining some of the phenomena seen and predicted by these authors. These authors propose a similar transport scheme in which Na^+ entry is via an amiloride blockable Na^+/H^+ exchanger in the basolateral membrane (*see also* Middlefart & Ratkje, 1985). The exchanged H^+ reacts with extracellular HCO_3^- to pro-

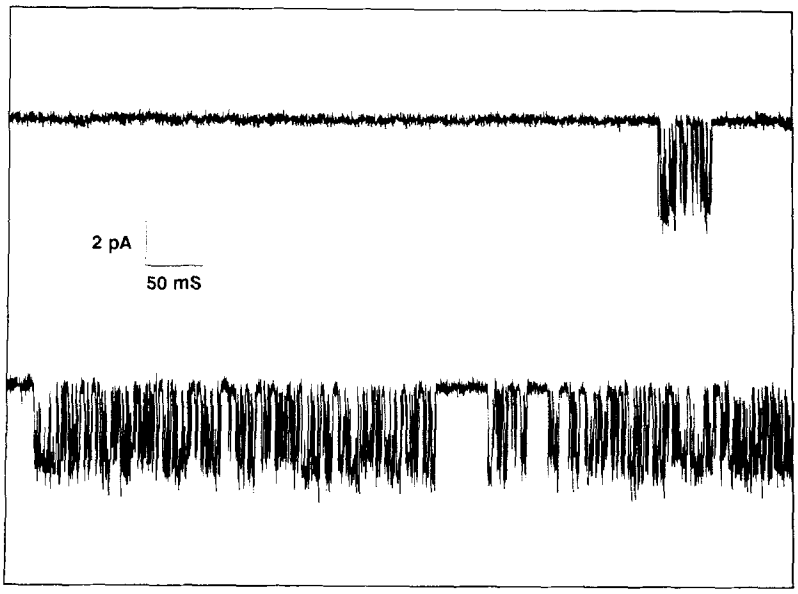


Fig. 6. Records of channel currents from cell-attached patches at −60 mV in the presence of external Ba^{2+}. The pipette contains 1 mM $BaCl_2$ in KMeS Ringer for both traces. The upper trace is for KMeS Ringer in the bath. The bottom trace is obtained 5 min following the substitution of 15 mM $KHCO_3$ for 15 mM KMeS in the bath

duce CO_2 which enters the cell directly through the membrane lipid. This CO_2 reacts with H_2O in the cell to produce HCO_3^- via a carbonic anhydrase-dependent reaction. Na^+ and HCO_3^- exit the cell through an electrogenic Na^+-HCO_3^- cotransporter in the apical membrane. In the model, an electrogenic Na^+ pump is placed in the lateral membranes where it has been found in this tissue by several investigators using morphological staining techniques (Kaye & Tice, 1966; Tervo & Palkama, 1975; Leuenberger & Novikoff, 1984). Jentsch et al. (1984*a*,*b*, 1985*a*–*c*, 1988) and Wiederholt et al. (1985) have provided considerable information, both from intracellular pH measurements and from microelectrode measurements in cultured bovine endothelium, which supports the proposed model. Key to those experiments was the finding that the normally steady membrane voltage transiently depolarized when HCO_3^- was removed from the bath and transiently hyperpolarized when it was returned. They attributed the majority of the effect to a Na^+-HCO_3^- electrogenic cotransport mechanism. The K^+ channel we report here would, however, be a candidate to subserve at least part of that response since its gating changes produced by HCO_3^- addition and withdrawal would be expected to change the cell voltage in the same direction as they report. They originally considered this possibility, but concluded that a K^+ channel was not involved in the phasic voltage change with HCO_3^- removal and readmission because the change continued unabated in the presence of external Ba^{2+}. We, however, found that Ba^{2+} was not an affective blocker of the rabbit endothelial K^+ channel. Figure 6 shows time records of unitary channel currents from a cell-attached patch with 1 mM $BaCl_2$ included in the pipette-filling solution in the presence and absence of external HCO_3^-. Ba^{2+} fails to block the channels, and the HCO_3^--stimulated increase in open probability goes on in the presence of Ba^{2+}. This K^+ channel cannot, however, explain the majority of their results. Jentsch and coworkers (1984*b*) noted that the phasic voltage response was too complex to be dependent only on their proposed Na^+-HCO_3^- cotransporter and hypothesized that there must exist a parallel SITS and DIDS insensitive cotransporter which they could not at that time identify. If bovine cells were to contain the K^+ channels reported here as have all other corneal endothelial cells we have tested (Rae et al., 1989), such channels could explain part of the phasic voltage response with HCO_3^- changes, particularly that not blocked by DIDS since these K^+ channels are activated by external DIDS.

We attempted to discover whether any of the transport processes in the above model were linked in any direct way to the gating of the K^+ channel. We therefore tested the ability of extracellular HCO_3^- to stimulate the K^+ channel in cell-attached patches in the presence and absence of inhibitors of these other transport processes. Amiloride (1 mM), a blocker of Na^+/H^+ exchange, acetazolamide (10 μM), a blocker of carbonic anhydrase, ouabain (0.1 mM), a Na^+-pump blocker, and DIDS (0.1 mM) were unable to block the HCO_3^--dependent gating increase.

Stimulation of K^+ channel gating by external HCO_3^- has also been proposed by Stoddard and

Reuss (1988) in *Necturus* gallbladder. In their studies, they found that HCO_3^- induced changes in membrane resistance which they attributed to an increase in conductance to both K^+ and Cl^-. They also did not discover the detailed mechanism by which the HCO_3^- works.

At present, therefore, we do not know the specific pathway whereby external HCO_3^- interacts with K^+ channel gating. Future work must delineate the molecular basis for this interesting channel control mechanism.

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