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Ion Channel Involvement in the Temperature-Sensitive Response of the Rabbit Corneal Endothelial Cell Resting Membrane Potential

Mitchell A. Watsky[1], **James L. Rae**[2]

[1]Department of Physiology and Biophysics, University of Tennessee, College of Medicine, 894 Union Avenue, Memphis, Tennessee 38163

[2]Departments of Physiology and Ophthalmology, The Mayo Foundation, Rochester, Minnesota 55905

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Abstract. Previous studies have shown that the resting potential (E_m) of the corneal endothelium hyperpolarizes following an increase in temperature above 24°C. Whole-cell studies using the perforated-patch technique were used to compare currents and E_m values from isolated corneal endothelial cells at 24 and 32°C. These studies revealed a small, outwardly rectifying, slowly activating, weakly voltage-dependent current with a reversal potential showing K^+ selectivity (E_{rev} = −80 mV). This current had features similar to the whole-cell current seen following addition of HCO_3^- to these cells. E_m measurements found an average 24 mV hyperpolarization following temperature elevation in NaCl Ringer. Single channel studies found the only change in channel activity following an elevation in temperature to be an increase in the open probability (P_o) of a K^+ channel previously reported in this cell type to be activated by external anions. P_o(−30 mV) at 24 and 32°C equaled 0.003 and 0.06, respectively. Increases in P_o were found at all voltages examined. This increased P_o can account for the magnitude of the hyperpolarization seen in these cells following temperature elevation. Addition of HCO_3^- along with elevated temperature produced a synergistic effect on the increase in P_o along with an increased hyperpolarization of the cell, pointing to separate mechanisms of activation from these two stimuli.

Key words: Corneal endothelium—K^+ channel—Temperature sensitivity—Resting voltage—Patch clamp—Perforated patch

Correspondence to: M.A. Watsky

Introduction

The corneal endothelium acts as both a barrier and a fluid transporter in its role as the primary controller of corneal hydration. As in all fluid transporting cells, the resting voltage (E_m) of the corneal endothelium has a significant influence on the ability of the cell to control its volume and to control the rate of its transport functions. Previous studies have shown that the resting voltage of corneal endothelial cells is temperature sensitive. These studies demonstrated a significant hyperpolarization of these cells at elevated temperatures (Jumblatt, 1981; Watksy & Rae, 1991). Jumblatt recorded an 18.7 mV shift in the E_m of confluent cultured cells tested at 23° and 35°C, while Watsky and Rae recorded a 15 mV hyperpolarization in noncultured dissociated cells tested at 22 and 34°C. The purpose of the present study was to examine the possibility that a change in ion channel activity, presumably through an increase in the open probability (P_o) of one or more K^+ channels, is at least in part responsible for this E_m temperature sensitivity. To date, two K^+ channels have been characterized in corneal endothelial cells. These include an anion-stimulated K^+ channel (Rae, Dewey & Cooper, 1989; Rae et al., 1990*a*) and an "A"-type K^+ channel (Watsky, Cooper & Rae, 1992). In the current study, experiments were carried out using both the whole-cell perforated-patch technique and single channel recordings from noncultured, dissociated rabbit corneal endothelial cells. Results from the whole-cell studies demonstrated the presence of a temperature-sensitive K^+ current that hyperpolarized the cell at elevated temperatures. Single chan-

Table 1. Solutions*

Solution no.	Na^+	K^+	Cl^-	Ca^{2+}	$MeSO_3$	HCO_3^-	Glucose	HEPES	Aspartate (%)	Collagenase (%)	Protease (%)	pH	OSM (mosm/kg)
1	170	5	5				5	5	170	0.125	0.05	7.35	280
2	149	5	159	2.5			5	5				7.35	295
3	149	0	5	2.5	149		5	5				7.35	295
4	20	127	25	2.5	127			5				7.00	283
5		149	5	2.5	149		5	5				7.35	293
6		149	154	2.5			5	5				7.35	288
7	150		5	2.5	125	25	5	5				7.35	290
8		149	5	2.5	124	25	5	5				7.35	290

* Values in mM unless otherwise noted.

nel studies demonstrated that the anion-stimulated K^+ channel has a temperature dependence to its P_o that accounts for the E_m temperature sensitivity in these cells. These results are consistent with a mechanism whereby the temperature-sensitive whole-cell current is at least in part accounted for by the anion-sensitive single channels.

Materials and Methods

Cell Preparation

The procedure for obtaining dissociated rabbit corneal endothelial cells has been described previously (Watsky, Cooper & Rae, 1991). Briefly, rabbits were euthanized with an i.v. injection of pentobarbital into a marginal ear vein. Both eyes were enucleated and their corneas were dissected from the globes. The corneal endothelium was carefully peeled from the stroma along with its basement membrane (Decement's membrane) and pinned to a Sylgard (Dow Corning, Midland, MI) disc. Cells were dissociated from the Decement's membrane by placing the pinned preparation into a commercially available solution (Enzyme-free Dissociation Solution, Specialty Media, Lavallette, NJ) or a sodium aspartate Ringer solution (Table 1, Solution 1) containing collagenase (Sigma 1A) and protease (Sigma type XXIV). After 2 hr, the cells were triturated off the Decement's membrane with a fire-polished transfer pipette, and resuspended in either a NaCl or a $NaMeSO_3$ Ringer solution (Table 1, Solutions 2 and 3, respectively). The $NaMeSO_3$ solution was used for Cl^--free experiments. Cells were then placed into a Plexiglass chamber with a glass coverslip bottom under a custom-constructed Leitz upright microscope. Chamber bath temperature was controlled using a commercially available heater and controller (N.B. Datyner, Stony Brook, NY).

Electrical Recordings

Perforated-patch whole-cell recordings and on-cell patch recordings were used in this study. Whole-cell studies were performed using either an Axopatch 1D or an Axopatch 200 patch-clamp amplifier (Axon Instruments, Foster City, CA). Whole-cell pulse protocols were run using pCLAMP software (Axon Instruments). Records were capacity compensated with the patch-clamp circuitry, filtered at 1 KHz and sampled at 200 μsec/point. Amphotericin (Sigma A-4888) was used in the electrode as the pore-forming agent. Details of this procedure have been reported previously (Rae et al., 1991). Kimble KG-12 glass was used in all perforated-patch experiments.

All single channel experiments were performed in the on-cell configuration. An Axopatch 200 amplifier was used for all single channel experiments. Records were filtered through the amplifier at 50 KHz, and through a separate 8-pole Bessel filter at 10 KHz. Data were recorded on VHS video tape for later analysis. Corning 7052 glass was used in all single channel experiments. Electrodes were fire-polished and coated with Sylgard.

Data analysis was carried out on a modified IBM AT computer using the Axon Instruments pCLAMP package. Data from the VCR were played back through an 8-pole Bessel filter at either 2 or 10 KHz and were read into the computer using pCLAMP's Fetchex program. Data were sampled at either 100 μsec/point or 20 μsec/point for the 2 and 10 KHz records, respectively. Amplitude histograms were obtained from the records filtered at 2 KHz using pCLAMP's Fetchan program, and curve-fitting was done using PSTAT. P_o was calculated from the area under the peaks and number of peaks, constrained to the Binomial distribution (Colquhoun & Hawkes, 1983). Conductance values were determined from records played back through the 10 KHz filter using Fetchan.

Results

Whole-Cell Studies

Currents resulting from a whole-cell perforated-patch experiment with the cell bathed in NaCl Ringer (Table 1, Solution 2) are illustrated in Fig. 1. All whole-cell studies were performed on single, dissociated cells. A K^+-methane sulfonate (KMeS) Ringer solution was used in the electrode (Table 1, Solution 4), and a NaCl Ringer solution (Table 1, Solution 2), was used in the bath. After the access resistance stabilized, an activation protocol was run (Fig. 1*A*, inset). This protocol consisted of 16 voltage pulses, starting from a holding potential (V_h) of −5

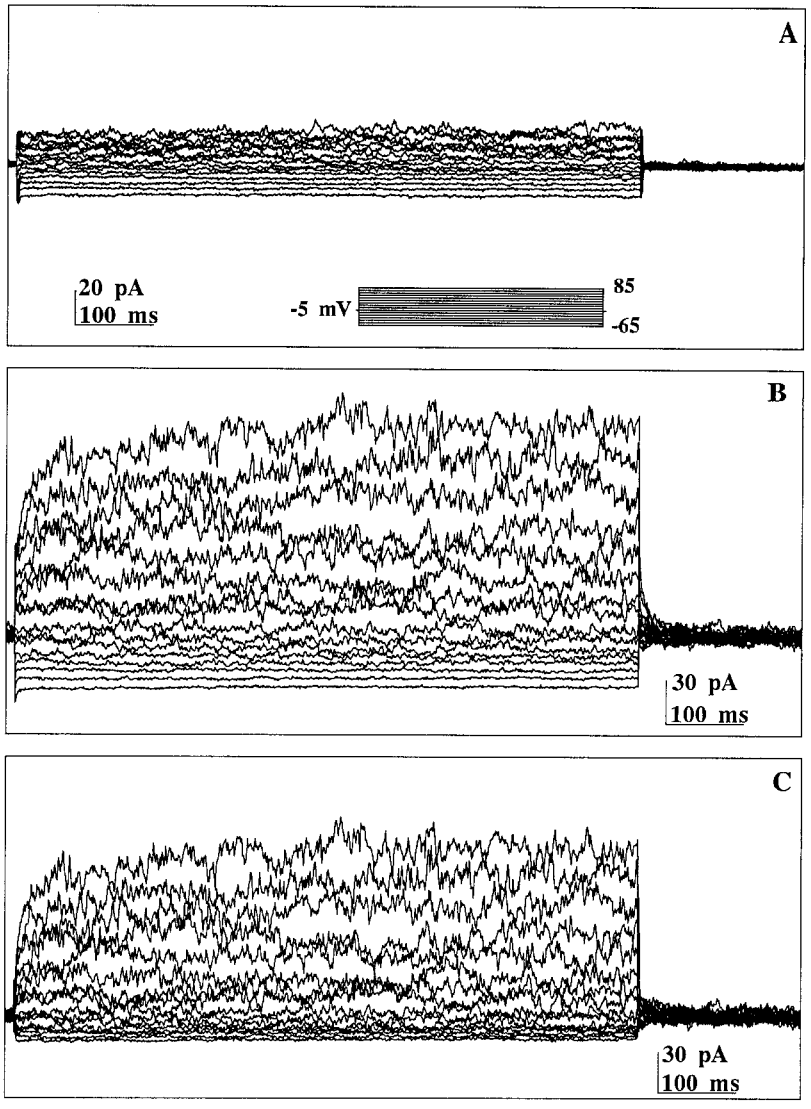

Fig. 1. Effect of temperature elevation on the whole-cell current, as measured with the perforated-patch technique, of a noncultured, dissociated corneal endothelial cell. All records taken with the cell in a NaCl Ringer bath. (*A*) 24°C, inset: pulse protocol. (B) 32°C. (*C*) Temperature-sensitive component obtained by subtracting current traces in *A* from those in *B*. Capacity transients have been edited.

mV, with pulses ranging from −65 to 85 mV in 10 mV steps, followed by a return to V_h. Several experiments were also performed with a holding voltage of −60 mV to test for holding voltage sensitivity. Figure 1*A* shows the current at 24°C, Fig. 1*B* shows the current after elevation of the bath temperature to 32°C, while Fig. 1*C* shows the temperature-sensitive difference current (1*B*–1*A*). Noteworthy features of this current are its small size, outward rectification, slow activation time and lack of inactivation during the time frame of this experiment, and the lack of a tail in NaCl Ringer. The current also shows no holding voltage sensitivity (*data not shown*). This example shows a large temperature-sensitive component (peak current approximately 142 pA). Many of these currents were no larger than 40 pA. This temperature-sensitive current was observed in 33 of 36 cells, and in all bathing solutions tested (*see* Materials and Methods). Figure 2 shows the steady-state current-voltage relationship (*I-V*) from the difference current in Fig. 1*C*. This *I-V* again demonstrates the outward recitification of the current, and shows that it activates around −65 mV.

Selectivity of this current was examined using a tail current protocol on cells bathed in NaCl Ringer. This protocol (Fig. 3, inset) consisted of holding the cells at −15 mV, pulsing to 65 mV for 600 msec, and then stepping from −110 to +10 mV in 15 steps (10 mV increments) for 200 msec per step. The voltage was returned to V_h following each step. Records were corrected for offsets by subtracting the E_{rev} from the cell bathed in potassium solutions from all recorded voltages (Watsky, Cooper & Rae, 1992). Figure 3 shows the *I-V* relationships from a single cell, generated using this protocol. This figure shows the *I-V* curves from tail currents generated at 24°C,

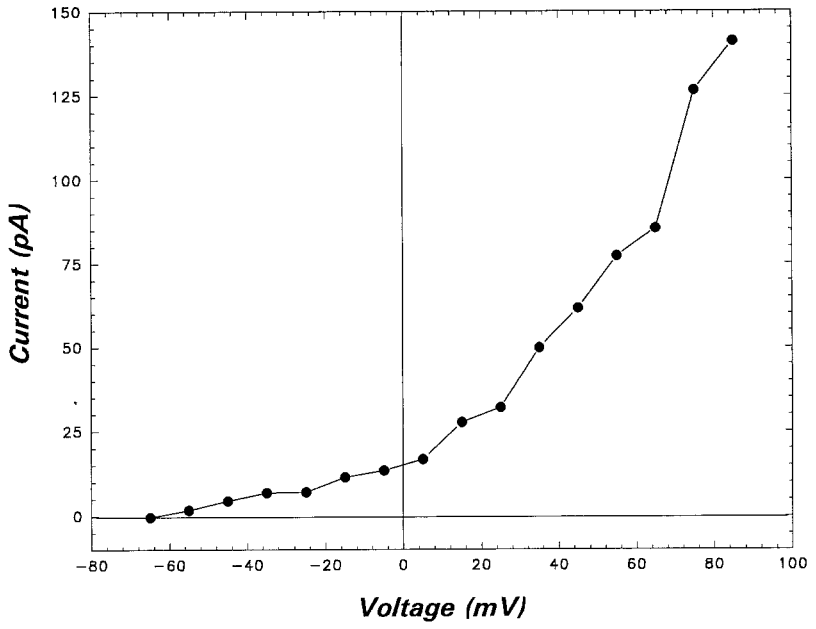

Fig. 2. Steady-state *I-V* relationship of the difference current in Fig. 1*C*, showing outward rectification of the current and current activity at hyperpolarized voltages.

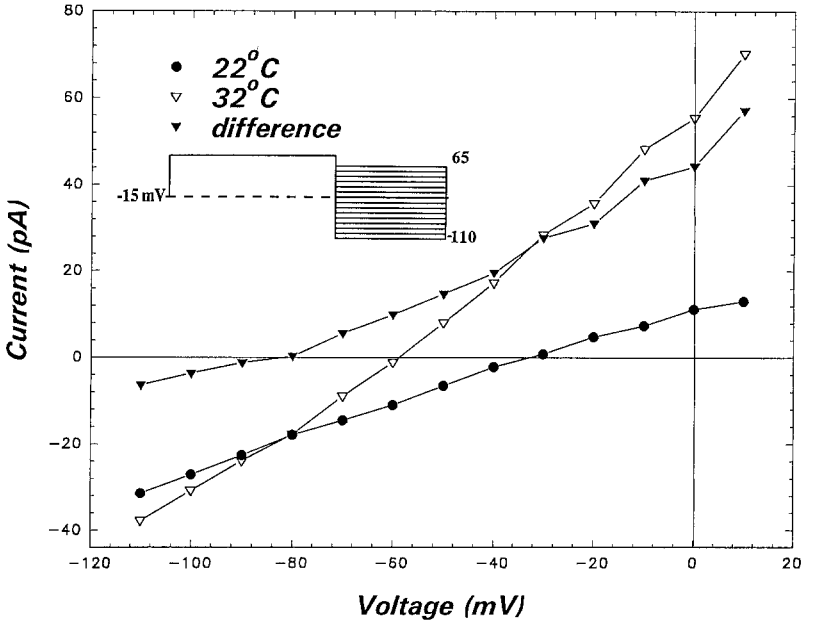

Fig. 3. *I-V* relationships of the peak tail currents and difference current from a cell bathed in NaCl Ringer at 24 and 32°C. Curves show hyperpolarizing shift in E_{rev} following temperature elevation, as well as an E_{rev} = −80 mV for the difference current *I-V*. Inset: pulse protocol for tail current generation.

32°C, and from the difference currents. The curves generated at the two temperatures illustrate an increased conductance and a negative shift in E_{rev} following temperature elevation. The E_{rev} of the difference current in Fig. 3 equaled −80 mV. The expected Nernst potential of a purely K^+-selective current (E_K) under the conditions of this experiment equaled −86 mV. This difference current E_{rev}, coupled with the hyperpolarizing shift seen after elevating the temperature, suggests that this current is carried primarily by K^+. This conclusion is strengthened by the presence of a prominent tail in KCl and KMeS baths (*data not shown*) that was not present in Na^+ bathing solutions (Fig. 1).

Figure 4 illustrates that the temperature-sensitive current was at least partially reversible. This experiment was done in a KCl Ringer bath (Table 1, Solution 6). The temperature-sensitive increase in current was typically seen as soon as the bath temperature stabilized (less than 1 min). In most instances, there was a lag between the return of the bath temperature towards room temperature, and the return of the current towards control amplitudes. In Fig. 4, 9 min elapsed between traces 2 and 3.

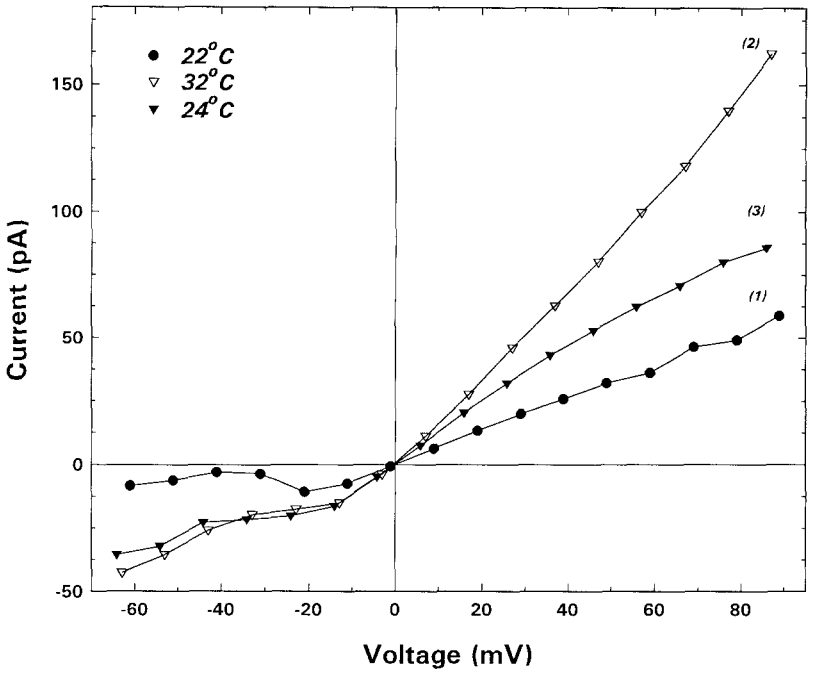

Fig. 4. Steady-state I-V relationship from a pulse protocol similar to that shown in Fig. 1, demonstrating reversibility of the temperature-sensitive current. Numbers at the end of each curve represent the order in which the data were taken.

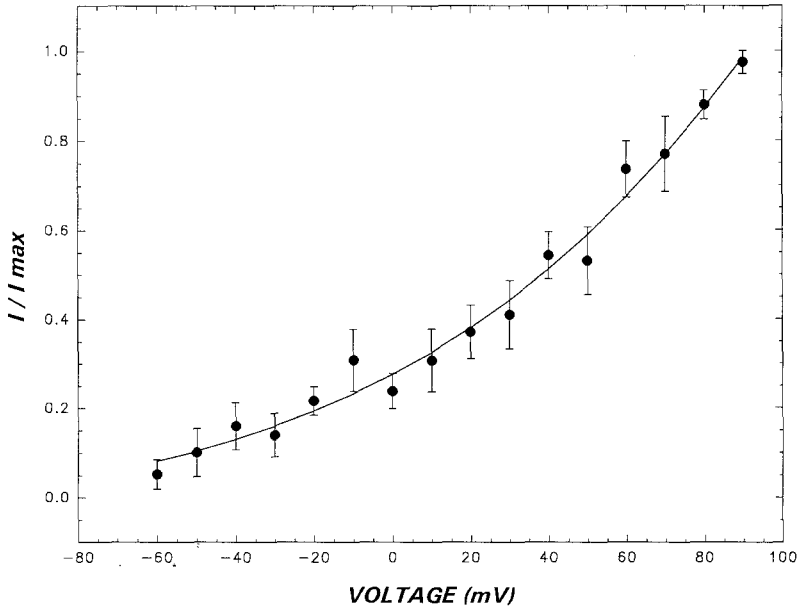

Fig. 5. I/I_{max} *vs.* voltage curve from the tail currents of cells bathed in KCL Ringer generated with a protocol similar to that shown in Fig. 1. Circles represent mean (±SE) values from five cells. The curve represents the fit to a Boltzmann distribution (*see text*). $V_{1/2}$ = 165 mV, and k = 68 mV.

It should be noted that approximately 2 min were required to turn off the heater and change the bath to a room temperature solution, and in the case of the experiment detailed in Fig. 4, the bath did not quite return to its original temperature. Current reversal with cooling was observed in seven of eight cells tested, and could be repeated several times with the same cell.

The I/I_{max} voltage for tail currents is shown in Fig. 5. Tail currents were generated in KCL Ringer using an activation protocol similar to that described earlier, with the final pulse going to −80 mV instead of V_h. I/I_{max} was obtained for each cell (n = 5) from the temperature-sensitive difference currents by dividing the maximum tail current observed at each voltage by the maximum tail current observed over all voltages examined. The data have been normalized between 0 and 1, and fit to a Boltzmann distribution using the equation

$$I = I_{max}/(1 + e^{-(V-V_{1/2}/k)}) \quad (1)$$

Table 2. E_m measurements from temperature-dependence experiments in Na^+ containing baths

Experimental condition		n*	Mean E_m (mV)	SE
NaCl	24°C	21	−17.3	2.0
	32°C	15	−41.7	5.7
	ΔE_m	14	−24.4	4.8
NaMeS	24°C	9	−8.5	2.8
	32°C	7	−11.9	1.4
	ΔE_m	7	−6.2	1.7

* n = number of cells.

where I_{max} = maximum peak current, V = test voltage, $V_{1/2}$ = voltage at which I = 0.5 (I_{max}), and k = slope factor. This fit determined $V_{1/2}$ = 165 mV, and k = 68 mV.

To confirm the temperature sensitivity of the E_m under the specific conditions of this study, E_m measurements were taken both before and after raising the bath temperature during the whole-cell studies. For these measurements, the patch-clamp amplifier was set into the current clamp mode such that the current was clamped to 0 pA, and the E_m was read from the digital display. E_m values were corrected for the junction potential error using the Henderson equation (MacInnes, 1961; Watsky & Rae, 1991). These correction values equaled 8.69 and 8.83 mV for NaCl Ringer at 24 and 32°C, respectively, and 3.68 and 3.74 mV for NaMeS Ringer at the same respective temperatures. E_m measurements from the temperature-dependence experiments in Na^+-containing baths are listed in Table 2. As expected from earlier studies, a significant hyperpolarization occurred following the increase in temperature. In addition, as predicted by the single channel results (*see below*), the E_m and changes in E_m were much lower for cells bathed in a Cl^--free solution.

Single Channel Studies

Single channel experiments were performed on either single cells or on a cell within a clump of 3–6 cells. All experiments used a KMeS solution in the electrode (Table 1, Solution 5). The bathing solution contained either the same electrode solution or a similar solution plus 25 mM HCO_3^- (Table 1, Solution 8). Experiments were started at room temperature with channel activity being recorded at several voltages, and then the temperature was elevated at 32°C, and the procedure repeated. In many experiments, solution changes were performed prior to warming the bath to exclude the possible influence of the actual solution change from the results. In several experiments, HCO_3^- solution was added to the bath either before or after the temperature elevation step. Currents were recorded immediately after these solution changes.

Figure 6 shows a sample single channel record recorded at a pipette voltage of −30 mV, filtered at 2 KHz. Figure 7 shows a sample single channel record from a different cell recorded at a pipette voltage of +30 mV, filtered at 7 KHz. The channel illustrated is a noisy, flickery channel with several subconductance states and an inwardly rectifying conductance, as described previously for the anion-stimulated K^+ channel in this tissue (Rae et al., 1989; Rae et al., 1990*a*). In addition, as described in these previous studies, the channel was rapidly lost after excision of the membrane patch. This channel was found in approximately 65% of the patches examined, with 2–3 channels typically observed per patch. Figure 6 shows the results of first adding HCO_3^- to the bath and then elevating the temperature, while Fig. 7 shows the results of first elevating the temperature and then adding HCO_3^-. Both records illustrate that either temperature elevation or addition of HCO_3^- will increase the P_o of the channel, while addition of both stimuli further increases P_o. Table 3 lists the mean P_o values for the channel following the specific experimental manipulations described, along with the percentage of cells increasing their P_o from the previously applied stimulus. This table quantifies the results illustrated in Figs. 6 and 7. As opposed to the whole-cell currents, elevated temperature stimulation of the single channels was minimally reversible between 5 and 10 min after temperature reversal.

The *I-V* relationship for the single channel currents at 24 and 32°C is illustrated in Fig. 8*A*. This figure shows an increase in channel conductance at elevated temperatures, as well as a small inward rectification of the single channel currents. Figure 8*B* graphs the P_o (±SE) *vs.* voltage for the anion-sensitive K^+ channel at 24 and 32°C. This figure demonstrates that the channel gating has a significant degree of temperature dependence as well as a small degree of voltage dependence. P_o at 32°C was higher than at 24°C for all voltages tested, as well as at the more positive intracellular potentials at both temperatures. This increase in P_o does not appear to be due to a simple shift in the voltage dependence of the channel. To obtain the equivalent of a macroscopic *I-V* from the single channel data ($I = N \cdot i \cdot P_o$), single channel currents (i) from Fig. 8*A* were multiplied by the corresponding P_o values from Fig. 8*B* (N assumed constant), for each voltage (Fig. 8*C*). The resulting curves are similar to those found in symmetric KCL for the whole-cell currents (*compare to* Fig. 4).

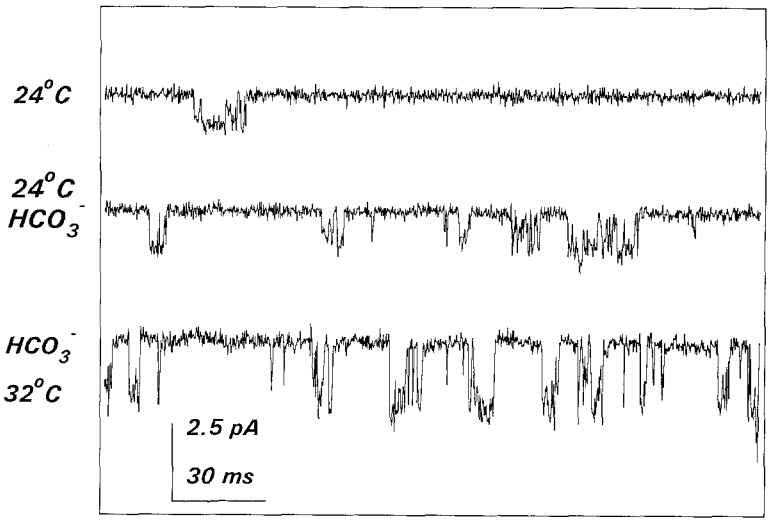

Fig. 6. Single channel records from an on-cell patch of a corneal endothelial cell with a pipette voltage of −30 mV. Top: KMeS Ringer in both the electrode and bath at 24°C. Middle: addition of 25 mM HCO_3^- to the bath. Bottom: Elevation of bath temperature to 32°C in the presence of HCO_3^-. Note the increased channel activity following HCO_3^- addition, and the additional increase in activity after warming. Records filtered at 2 KHz. Lines to the right of the figure indicate closed state.

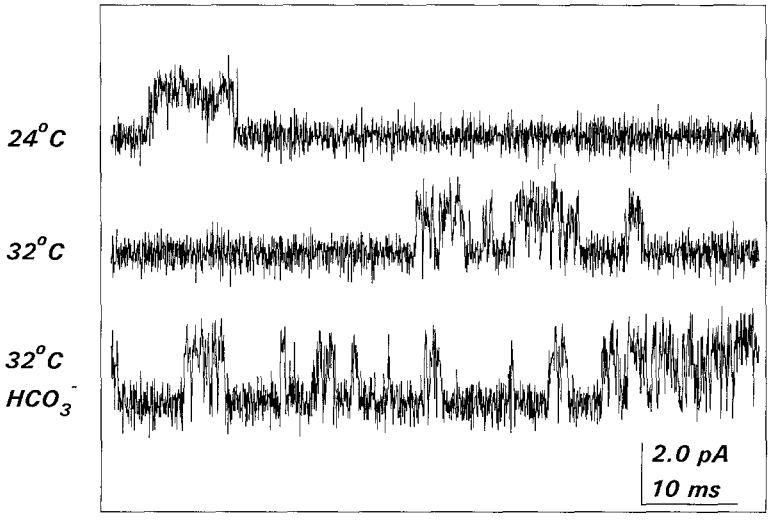

Fig. 7. Single channel records from an on-cell patch of a corneal endothelial cell with a pipette voltage of +30 mV. Top: KMeS Ringer in both the electrode and bath at 24°C. Middle: Elevation of bath temperature to 32°C. Bottom: addition of 25 mM HCO_3^- to the bath at 32°C. Note the increased channel activity after warming, and the additional increase in activity following HCO_3^- addition. Records filtered at 7 KHz. Lines to the right of the figure indicate closed state.

Table 3. Mean P_o values and percentage of cells increasing their P_o after applied stimulus

Experimental condition	+30mV[a]				−30 mV[a]			
	n	P_o	SE	%cells[b]	*n*	P_o	SE	%cells[b]
24°C[c]	13	0.025	0.009		13	0.002	0.001	
24°C + HCO_3^-[d]	8	0.054	0.011	75	7	0.019	0.011	71
32°C	5	0.045	0.016	100	5	0.035	0.027	100
32°C + HCO_3^-	9	0.381	0.092	89	10	0.161	0.053	80

[a] Intracellular potential.
[b] Percent of cells responding with an increase in P_o from previous stimulus.
[c] NaMeS Ringer (Table 1, Solution 3).
[d] NaMeS + 25 mM HCO_3^- Ringer (Table 1, Solution 7).

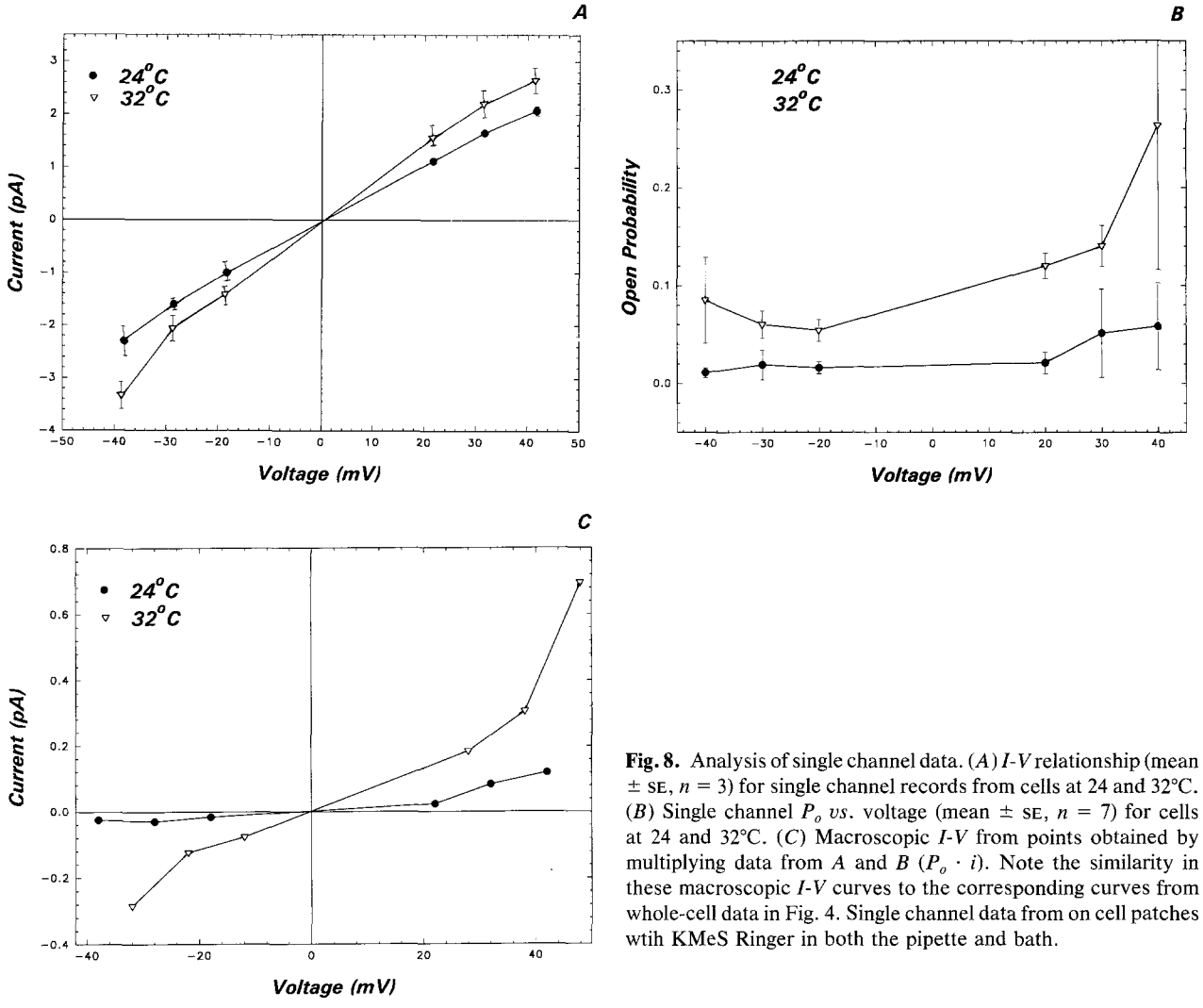

Fig. 8. Analysis of single channel data. (*A*) *I-V* relationship (mean ± SE, $n = 3$) for single channel records from cells at 24 and 32°C. (*B*) Single channel P_o *vs.* voltage (mean ± SE, $n = 7$) for cells at 24 and 32°C. (*C*) Macroscopic *I-V* from points obtained by multiplying data from *A* and *B* ($P_o \cdot i$). Note the similarity in these macroscopic *I-V* curves to the corresponding curves from whole-cell data in Fig. 4. Single channel data from on cell patches wtih KMeS Ringer in both the pipette and bath.

Table 4. Conductance values

Experimental condition	+30 mV[a]			−30 mV[a]		
	n	g(pS)	SE	*n*	g(pS)	SE
24°C[b]	4	57	4	3	74	7
24°C + HCO_3^-[c]	2	58	1	2	66	14
32°C	3	70	5	3	66	11
32°C + HCO_3^-	4	77	11	3	74	10

[a] Intracellular potential.
[b] NaMeS Ringer (Table 1, Solution 3).
[c] NaMeS + 25 mM HCO_3^- Ringer (Table 1, Solution 7).

Table 4 lists the conductance values measured from data such as those in Fig. 7. This table shows minimal influence of HCO_3^- on conductance, while temperature increased conductance primarily in the outward direction (at negative pipette potentials).

Discussion

The data presented in this study point to a significant influence of the anion-stimulated K^+ channel in the temperature sensitivity of the corneal endothelial E_m. The influence of the temperature-dependent increase in P_o of the anion-stimulated K^+ channel on the E_m can be determined using

$$E_m = (G_K/G_K + G_L) \cdot E_K, \quad (2)$$

assuming E_m is determined solely from the potassium conductance (G_K) and the leak conductance (G_L). Using the E_m values from Table 2 for cells in NaCl Ringer and $E_K = -86$ mV, the ratio of $G_{K32C}/G_{K24C} = 3.74$. In addition, from Fig. 8, the P_o values from this channel at a cell voltage of −40 mV equal 0.085 and 0.011 at 32 and 24°C, respectively. This makes for a P_{o32C}/P_{o24C} ratio of 7.73. Since

$$G_K = N \cdot g_K \cdot P_o \quad (3)$$

where g_K = single channel potassium conductance and N is the number of channels (assumed to remain constant), the P_{o32C}/P_{o24C} ratio (7.73) easily accounts for the G_{K32C}/G_{K24C} ratio (3.74) calculated from the E_m values. Of course, the increased activity of the Na^+/K^+ pump at elevated temperatures will further hyperpolarize the E_m. This single channel estimate incorrectly assumes no increase in G_L following temperature elevation. The difference current *I-V* in Fig. 3 shows the temperature-sensitive leak as inward current at potentials negative to E_K. Quantitatively, the temperature-sensitive increase in G_L is required to account for the excess increase in P_o as compared to G_K. Noteworthy is that the P_{o32}/P_{o24} ratio calculated above for the anion-stimulated K^+ channel would also compensate for the G_{K32C}/G_{K24C} ratio calculated from the data of Jumblatt (1981) and Watsky and Rae (1991) (2.02 and 2.48, respectively).

Previously characterized channels in the corneal endothelium include an "A"-type K^+ channel (Watsky et al., 1992), a TTX-insensitive Na^+ channel (Watsky et al., 1991), a nonselective cation (NSC) channel (Rae et al., 1990*b*), and a large conductance anion channel (Rae, Levis & Eisenberg, 1988). None of these channels appeared to contribute to the temperature-sensitive response of whole-cell currents reported in this study. It is particularly unlikely that the "A"-type current is involved because it is essentially inactive at the relatively depolarized holding voltages used throughout most of this study. It is also inactive at the extended time points chosen to collect data during this study. In addition, the "A"-type current is very sensitive to holding voltage whereas the temperature sensitive whole-cell current is not. For single channel studies, the only observable temperature-sensitive response was the increase in P_o of the anion-sensitive K^+ channel.

Although the anion-activated K^+ channel single channel data from this paper are similar to those published earlier (Rae et al., 1989; Rae et al., 1990*a*), no previous attempts have been made to look at the whole-cell current stimulated by addition of HCO_3^- to corneal endothelial cells. Figure 9 shows the whole-cell difference current from a study where the control cell was bathed in NaMeS Ringer following addition of 25 mM HCO_3^- (Table 1, Solutions 3 and 7, respectively) to look at anion stimulation. The current has many similarities to that depicted in Fig. 1 for the temperature sensitive current. These include the small size, outward rectification, slow activation and lack of inactivation during the time course of the pulse protocol, and no holding voltage sensitivity. In addition, it has no tail in Na^+ Ringer solutions, and has an increased conductance and hyperpolarizing shift of its E_{rev} following addition of HCO_3^- to the bath (Fig. 10). The main differences between this current and that stimulated by elevated temperatures were a more depolarized E_{rev} (−50 mV compared to −80 mV) illustrating either more leak or less K^+ selectivity, and a lower frequency of currents that were stimulated following addition of HCO_3^- to a Cl^--free solution (5 of 10 cells). In fact, several cells showed a significant increase in input resistance following addition of HCO_3^-, a situation opposite to the increase in leak seen following temperature elevation. Further studies will be needed to definitively equate the whole-cell currents from these two stimuli.

The data in this study point to a significant contribution of the anion-stimulated K^+ channel in the temperature-sensitive whole-cell K^+ current. The single channel macroscopic *I-V* compares favorably (Fig. 8*C*) to the whole-cell current *I-V* recorded under similar conditions (Fig. 4), as do the single channel P_o *vs.* voltage (Fig. 8*B*) and the whole cell I/I_{max} *vs.* voltage (Fig. 5) curves. In addition, both currents show only a small voltage sensitivity. The whole-cell current in this study was shown to have a $V_{1/2}$ = 165 mV, while Rae et al. (1990*a*) reported a $V_{1/2}$ for the anion-stimulated K^+ channel of 267 mV. The discrepancy between these two values could be due to the comparison of I/I_{max} for whole-cell values compared to the true P_o for single channel data, as well as the much wider range of voltages examined in the Rae et al. paper. Also, there could be an additional voltage-sensitive component to the whole-cell current. Two factors pointing to another component to this current are the differences between the temperature-stimulated and HCO_3^--stimulated whole-cell currents mentioned in the previous paragraph, and the lack of reversibility of the temperature-sensitive K^+ singles compared to the whole-cell current (within the same time frame).

The mechanism of the anion activation of this channel remains unknown. In the study by Rae et al. (1990*a*), attempts that used a variety of stimuli applied to the bath to reactivate channels that inactivated following patch excision were all unsuccessful. These stimuli included changes in pH, as well as addition of GTP-*y*-S, ATP, Mg^{2+}, cAMP and cGMP. In addition, blockers of Na^+/H^+ exchange, Na^+-HCO_3^- cotransport, Na^+/K^+ ATPase, and carbonic anhydrase did not block the stimulatory effect of HCO_3^- on P_o. In the present study, temperature elevation appears to activate the channel via a separate mechanism from that of anion activation. We reach this conclusion due to the synergistic effects on the P_o when both stimuli are applied (Table 3), as well as the rapid time course of temperature-

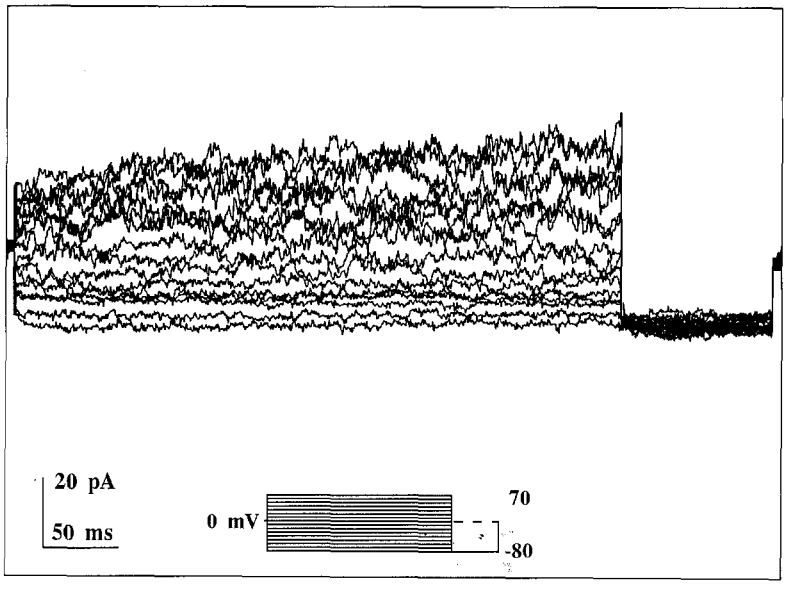

Fig. 9. HCO_3^--stimulated whole-cell current from a noncultured corneal endothelial cell recorded using the perforated-patch technique. Traces are from the difference current of the cell bathed in NaMeS Ringer subtracted from the same cell bathed in NaMeS + 25 mM HCO_3^-.

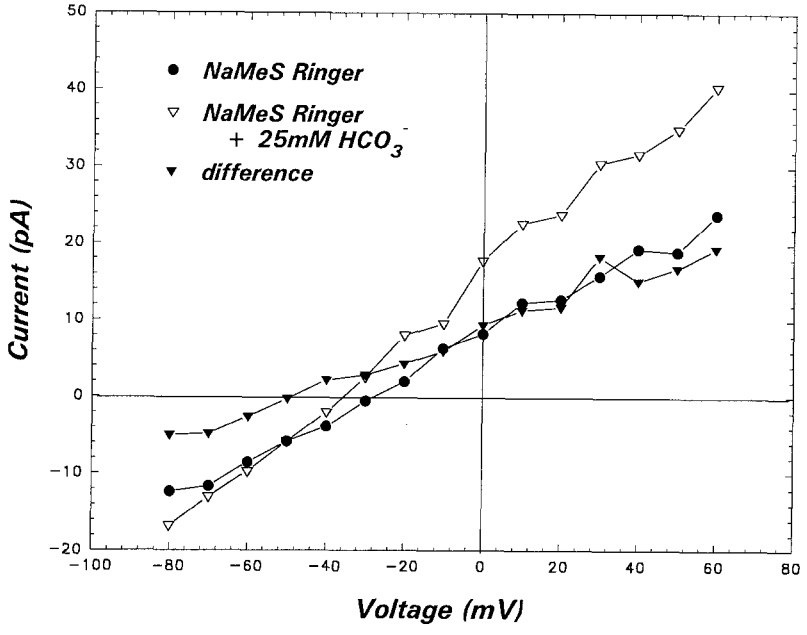

Fig. 10. *I-V* relationships of the steady-state currents and difference current from the cell in Fig. 9 bathed in NaMeS Ringer and NaMeS + HCO_3^-. Curves show hyperpolarizing shift in E_{rev} following addition of HCO_3^-, as well as an $E_{rev} = -54$ mV for the difference current *I-V*.

induced activation compared to HCO_3^- activation. The effects of temperature elevation could be seen prior to the bath reaching the final designated temperature (*data not shown*), where as previously reported (Rae et al., 1990*a*), HCO_3^- activation took approximately 7–8 min to reach its maximal effect. These results point to a possible direct effect of temperature on channel gating as opposed to a second messenger being responsible for the anion effect, but more detailed studies will be needed to confirm this possibility. It is interesting to note that Rae et al. (1989) found that inclusion of 0.1 mM DIDS in the pipette also increased the P_o of this channel in the corneal endothelium above that seen after adding HCO_3^- to the bath. As with the temperature-sensitive increase in channel activity, the DIDS response was also much faster than that seen with HCO_3^- (*unpublished observation*).

The physiological role of the elevated P_o of this channel at elevated temperatures in the corneal endothelium seems clear in terms of E_m maintenance. The average temperature of the cornea is 34.8°C (Mapstone, 1968), while the aqueous humor Cl^- and HCO_3^- concentrations in humans equal 131 and 20 mM (Riley, 1983), respectively. As described in this study, these conditions are favorable for both chan-

nel activation and maintenance of hyperpolarized E_m values, as compared to colder, HCO_3^--free conditions. These hyperpolarized E_m values most closely compare to those measured under similar although not identical conditions in our previous study (Watsky & Rae, 1991).

It is interesting to note that several studies have reported that the corneal endothelium requires the presence of HCO_3^- to properly control the hydration status of the corneal stroma (Hodson, 1971; Dikstein & Maurice, 1972; Dikstein, 1973; Wigham & Hodson, 1981; Liebovitch & Fischbarg, 1982, 1983; Lim, 1982, 1983). Despite work on the exact role HCO_3^- plays in corneal fluid transport, no definitive conclusions have been reached (Doughty, 1989). It seems clear that HCO_3^- is not only important in ion exchange mechanisms located in the corneal endothelial cell membrane, but also serves as a signaling mechanism to control K^+ channel activity and indirectly E_m. Winkler et al. (1992) recently reported that corneal endothelial fluid transport was partially impaired following substitution of Cl^- with NO_3^-, Br^-, or SO_4^{2-}. In the previous study by Rae et al. (1990*a*), it was determined that these same anions were incapable of stimulating this K^+ channel. Although Winkler et al. postulated that the effects of these anion substitutions on fluid transport were linked to the role of Cl^- in the Cl^--HCO_3^- exchanger, they may also be due to the role of Cl^- in stimulating the anion-sensitive K^+ channel and thus maintaining a hyperpolarized E_m.

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