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A Nonselective High Conductance Channel in Bovine Pigmented Ciliary Epithelial Cells

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Abstract. An ion channel activated by hyperpolarization was identified in excised patches of bovine pigmented ciliary epithelial cells using the single channel patch clamp technique. In symmetrical NaGluconate, the channel had a maximum conductance of 285 pS. The channel was characterized by frequent flickery transitions between the fully open and closed levels. The channel did not discriminate very clearly between anions and cations; when the cytoplasmic face of excised patches was bathed in a dilute NaCl solution, the channel had a chloride-to-sodium permeability ratio (P_{Cl}/P_{Na}) of 1.3. However, the channel showed a small anion selectivity (P_{Cl}/P_{Na} = 3.7) when bathed in a concentrated NaCl solution. Gd^{3+} blocked the channel reversibly. Channel kinetics were characterised by slow (≈ min) voltage-dependent activation and inactivation rate constants. The channel was most active in the range −60 to −140 mV and showed a peak at −120 mV. A similar time- and voltage-dependent activation was also observed in cell-attached recordings. In conclusion, hyperpolarization of pigmented ciliary epithelial cell membrane patches activated a large conductance, nonselective ion channel. This combination of nonselectivity and hyperpolarizing activation is consistent with the involvement of this channel in ion loading from the blood into pigmented ciliary epithelial cells—the first phase in the secretion of aqueous humor.

Key words: Ciliary epithelium — Anion channel — Patch clamp — Activation kinetics — Gadolinium

Introduction

The ciliary epithelium contributes to the secretion of aqueous humor. The ionic mechanisms underlying this secretion are expected to be complex, for the epithelium is a bilayer of pigmented cells coupled to nonpigmented cells by a series of gap junctions [18]. The cells act as a syncytium and are thought to cooperate in the production of aqueous humor, but it is presently unclear how the activities of the two cell layers are coordinated.

As the pigmented cells lie closest to the blood, they are thought to be primarily responsible for loading the ions to be secreted. Recent findings from this laboratory suggest that this loading phase is not continuous but undergoes temporal fluctuations driven by oscillations in membrane potential. Whole-cell current clamp recordings have shown that when pigmented ciliary epithelial cells are injected with a hyperpolarizing current, the membrane potential oscillates from about −70 to −130 mV [24]. The effect of the constantly applied hyperpolarizing current on a decreasing membrane conductance was to drive the cell membrane potential negative until a threshold was reached where a repolarizing current activated. Fluctuations in the internal calcium concentration were not responsible for these oscillations. Instead, the hyperpolarizing phase of these oscillations was shown to be caused by the inactivation of the inward rectifier potassium current [23]. The repolarizing phase of these oscillations was characterized by a large, inward current that activated between −110 to −130 mV and inactivated −60 to −75 mV. This whole-cell current reversed close to 0 mV and was distinguished by its conspicuous levels of noise.

In this study, the single channel patch-clamp technique was used to search for a channel with unusual kinetic attributes that could underlie the repolarizing phase of the oscillations. This paper represents an attempt to analyze the properties of the high conductance, nonselective ion channel with rapid current fluctuations that has often been referred to in passing [6, 17, 20], and anecdotally but which, with rare exceptions [2, 26] has not been the subject of extensive study.

Portions of this work have previously been presented inabstract form [13, 14].

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Materials and Methods

Cell Preparation

Ciliary epithelial tips were removed from the ciliary body of bovine eyes using a method described previously [9]. The tissue was incubated at 37°C for 25 mins in a solution containing 0.25% trypsin (Sigma, UK) and 0.02% EDTA. The tissue was triturated in a solution of E199 + 10% foetal calf serum, spun at 800 RPM and washed twice before being plated on 13 mm glass coverslips. Cells were kept overnight in E199 + 10% serum and recordings were performed 15–24 hr after the dissociation.

Electrophysiological Procedures

Unless otherwise noted, single channel recordings were made from excised inside-out patches. Electrodes were pulled on a two-stage puller (Sutter P30) and typically gave a resistance of 5–10 MΩ when filled with the pipette filling solutions. Recordings were performed in chamber connected to a Ag/AgCl-pellet via a 3 M KCl / 2% agar bridge. Currents were recorded using a patch clamp amplifier (Dagan 8900, Dagan Corporation, Minneapolis, MN), filtered at 1 kHz and sampled at 1 kHz to 3 kHz using a laboratory interface (CED 1401, Cambridge, UK). Voltage stimuli were driven, and current analysis was performed with the EPC software package (CED, Cambridge, UK). Voltages are given as −Vp (mV) and upward deflections represent outward current.

Analysis Techniques

The rapid current fluctuations seen with this channel precluded use of standard amplitude histograms and open probability analysis. Instead, the unitary channel conductance was determined by fitting an on-screen ruler to current levels observed during constant voltage steps, and constructing a current/voltage plot from these data. Channel activity was determined by integrating the mean current flowing through the patch. This value is the product of the channel open probability, number of channels and current amplitude added to the leak current. Because changes in mean current could have been caused either by increases in channel activity or increases in leak current, records were checked to be certain that channels closed to constant baseline. Records in which the baseline shifted were rejected.

Solutions

During seal formation, cells were bathed in standard extracellular solution containing (in mM): 125 NaCl, 4.4 KCl, 2 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES, 10 $NaHCO_3$, 5 glucose and 20 sucrose. For all experiments except for block and selectivity, electrodes contained 140 NaGluconate, 10 HEPES, 20 sucrose and 2 CaDi-isethionate. The bath contained 140 NaGluconate, 10 HEPES, 20 sucrose, 5×10^{-3} $CaCl_2$ and 1.1 EGTA for a free Ca^{2+} concentration of 10^{-7} M. For selectivity and blocking experiments, electrodes contained 130 NaCl, 10 HEPES, 20 sucrose, and either 5×10^{-3} $CaCl_2$ and 1.1 EGTA or 2 $CaCl_2$. The control bath solution contained 130 NaCl, 10 HEPES, 20 sucrose, 5×10^{-3} $CaCl_2$ and 1.1 EGTA. Dilute bath solution contained 33 NaCl, 10 HEPES, 200 sucrose, 5×10^{-3} $CaCl_2$ and 1.1 EGTA. Concentrated bath solution contained 520 NaCl, 10 HEPES, 20 sucrose, 5×10^{-3} $CaCl_2$ and 1.1 EGTA. The pH was adjusted to 7.3 with NaOH. All experiments were performed at room temperature.

Drugs

Gadolinium was applied directly to the bath from a 100 mM $GdCl_3$ stock solution to give a working concentration of 100 μM or 4 mM. SITS, DIDS and NPPB were perfused onto the cells at the concentrations indicated in the text. SITS was stored as a 100 mM stock solution in distilled water at 5°C. DIDS was stored as a 50 mM stock solution in distilled water at −20°C. NPPB was mixed shortly before use as a 1% stock solution in DMSO and kept in the dark at room temperature. NPPB was a kind gift from SmithKline Beecham, Welwyn, Herts, UK. All other chemicals were obtained from Sigma, UK.

Permeability Calculations

Channel selectivity was determined by exposing excised patches to an asymmetric mixture of NaCl. Ionic permeability was calculated by manipulating the constant field equation [8]. All selectivity and offset calculations were based upon ion activities determined from activity coefficients [19].

When the bath solution was changed, patch potentials were corrected for offset potential by subtracting the liquid junction potential from the zero-current potential [15]. The liquid junction potential was measured in patch-free conditions to be 4 mV in dilute NaCl solution and −4 mV in concentrated NaCl solution. Potentials shown here have been corrected for such offsets.

Values are given as mean ± SE.

Abbreviations

DIDS	4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid
DMSO	dimethyl sulfoxide
EGTA	ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid
HEPES	N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid
NPPB	5-nitro-2-[3-phenypropylamino] benzoic acid
SITS	4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid

Results

Single Channel Currents

The repolarizing current observed in the whole cell oscillations by Stelling and Jacob [23] was activated by hyperpolarization, so we specifically looked for single channel currents activated by hyperpolarizing pulses. To avoid contamination from K^+ [9] and possible Cl^- currents in these cells, experiments were initially performed on excised, inside-out patches bathed in symmetrical NaGluconate solutions. When excised patches were hyperpolarized by 80 or 100 mV, a noisy, large conductance channel was observed in over 90% of recordings. The channel usually appeared 1-to-4 mins after the hyperpolarization began. Once activated, channel activity could be detected at all hyperpolarized patch potentials tested (Fig. 1). It was less active at depolarized potentials. The maximum conductance was found to range from 187 to 363 pS, with a mean of 285.5 ± 17.7 pS (n = 13).

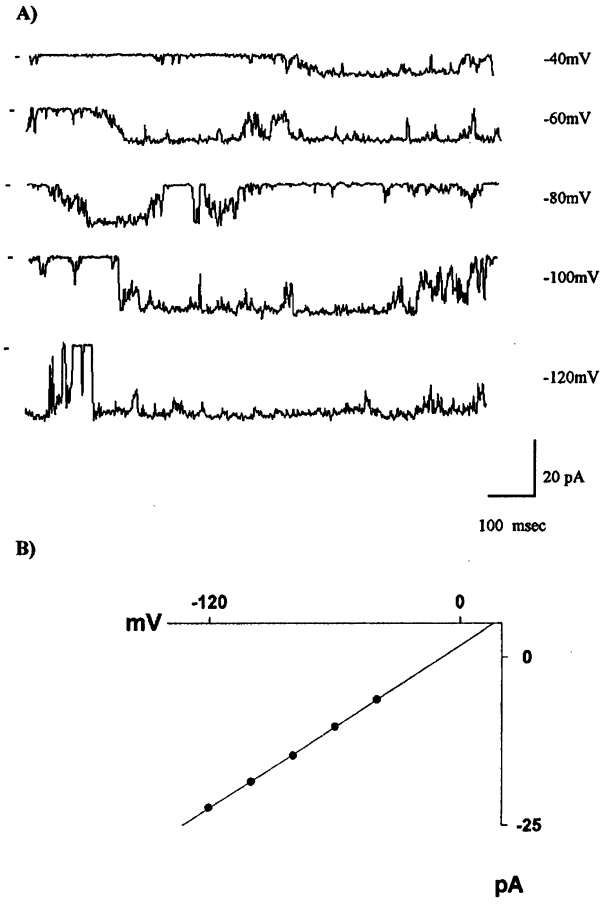

Fig. 1. Single channel current from an excised patch bathed in symmetrical NaGluconate. The negative of the pipette potential is shown to the right of each trace, and the closed channel level is indicated by a dash to the left of each trace. Downward deflections indicate inward current through open channels. Although records were characterized by transient openings that did not reach a constant open current, the fully open level can be seen clearly at each potential. This open current value was used to construct a current/voltage plot shown; the conductance of this channel was 215 pS.

The recordings were characterized by flickery transitions in between the fully open and the fully closed levels. These transitions could not be fully resolved as square current steps even when the sampling frequency was increased from 1 kHz to 3 kHz, but remained as flickery, multiple-level openings. Although the nature of these levels precluded thorough analysis, it is unlikely that these flickery levels represent a separate channel type because; (i) sublevels were not observed before channel activation by extended hyperpolarization; (ii) sublevels were not superimposed upon the fully open state. The channel occasionally spent several minutes in this flickery mode. The most obvious explanation is that the channel enters an unstable mode when the rate of ion movement through the channel is constantly changing and it is unable to open fully.

Selectivity

The large conductance flickery current of this channel resembles that reported for channels with a low anion to cation selectivity [2, 10, 16, 26, 27, 28]. A series of experiments was thus designed to determine the selectivity of the channel in our preparation. Excised patches were exposed to asymmetric concentrations of NaCl. Control extracellular concentrations of NaCl were used in the patch electrode. The bath solution alternated between 130 mM NaCl and either 33 mM or 520 mM NaCl solutions. Voltage ramps were then applied from ± 100 or ± 50 mV to provide a quick and accurate value of channel reversal potential [2].

When the bath contained 33 mM NaCl, the channel always reversed at or near 0 mV (Fig. 2). The chloride-to-sodium ion permeability ratio (P_{Cl}/P_{Na}) was determined to be 1.32 ± 0.01 (n = 15). However, when the bath contained 520 mM NaCl, the channel showed slight anion selectivity, with P_{Cl}/P_{Na} = 3.66 ± 0.45 (n = 5). This slight anion selectivity under these conditions has been reported previously [4, 22] and raises the possibility that the channel functions as a multi-ion pore. However, the patch was under osmotic strain during these experiments, and it is possible that this shift in reversal potential was caused by the interdependent movement of anions and water [7]. The presence of 2 mM calcium in the pipette did not alter the selectivities.

The characteristics of the channel were not effected by replacing NaGluconate with NaCl; the rapid fluctuations in current remained. When the bath contained 33 mM NaCl, the maximum conductance was 281.2 ± 29.3 pS (n = 15). When the bath contained 520 mM NaCl, the conductance was 308 ± 23.4 pS (n = 5). Neither of these values are significantly different from those obtained with symmetrical NaGluconate (285.5 ± 17.7 pS, n = 13).

Channel Block

Gadolinium (150–200 μM) has been found to block the whole-cell hyperpolarization-activated nonselective current in 6/9 PCE cells tried (Stelling & Jacob, *unpublished observations*) and Gd^{3+} blocked the nonselective channel at a concentration of 4 mM (n = 5) (Fig. 3), but was ineffective at 100 μM (n = 2). The block was reversible; when the gadolinium was washed out channel activity returned to previous levels with the same degree of flicker. While the concentration of gadolinium required is larger than that used to block cation channels [5], the use of Gd^{3+} in the mM range is not unprecedented [3]. High concentrations (0.4 mM) were used to cause complete inhibition of pressure-sensitive channels in *E. coli* since low concentrations were stimulatory [5].

Attempts to block the channel with SITS (500 μM), NPPB (500 μM) and DIDS (100 μM) were unsuccessful.

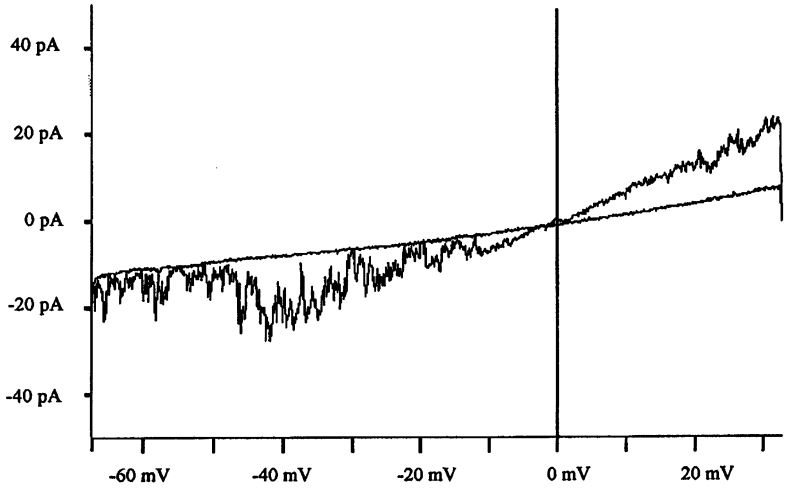

Fig. 2. Channel selectivity. This excised patch had dilute NaCl bathing the cytoplasmic face, 130 mM NaCl in the electrode and was polarized from −70 to +30 mV. The 100 mV ramp had a duration of 2 secs. The current traces indicating the open channel and leak levels intersect at −4 mV. This indicates a $P_{Cl}/P_{Na} = 1.33$. The maximum conductance of this channel was 280 pS.

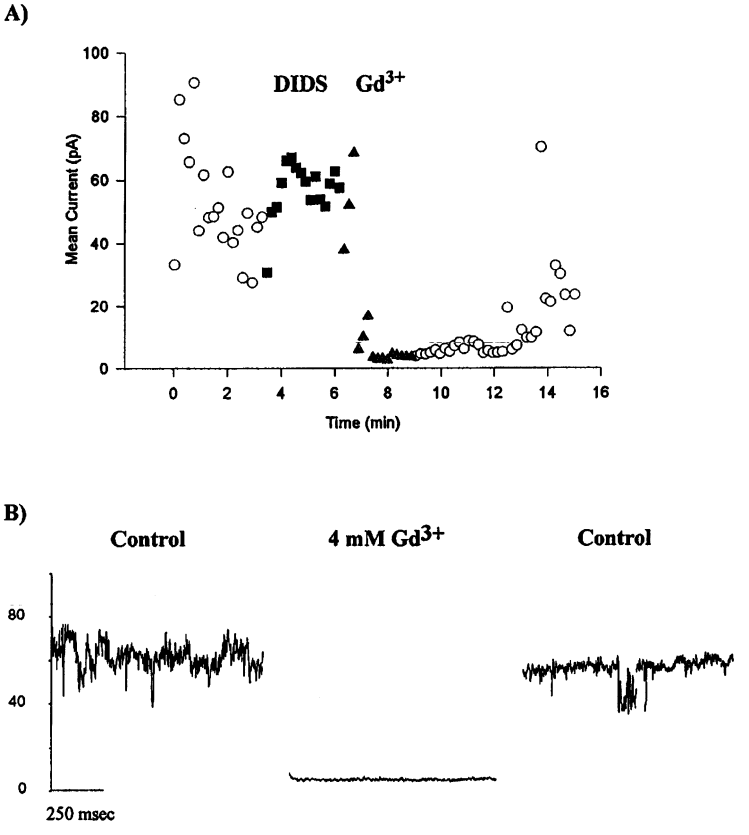

Fig. 3. Gadolinium block. (*A*) Mean current recorded from an excised patch depolarized by 70 mV with 130 mM NaCl in the electrode. Channel activity was high when the bath contained control 130 mM NaCl (◯) and was not affected by 100 μM DIDS (■). However, addition of 4 mM Gd^{3+} to the bath led to a rapid reduction in current (▲). Activity slowly returned when the Gd^{3+} was replaced by control solution (◯). At least three channels were present in the patch. (*B*) Single channel currents from the experiment in (*A*). Each trace lasts for 1 sec. Activity decreased rapidly after application of Gd^{3+}, and gradually returned once the Gd^{3+} was washed off. A similar block by 4 mM Gd^{3+} was observed in 5 patches.

Time and Voltage-dependence of Activation/Inactivation Kinetics

The kinetic analysis of the nonselective channel activity revealed several characteristics which suggest it might contribute to the repolarizing current observed in the whole cell recordings. The first such characteristic was found when trying to activate the channel. As previously mentioned, channel activity was usually not seen immediately after patch excision; it was necessary to hyperpolarize excised patches by −80 or −100 mV for 1–4 mins to activate this channel. This is also true of the hyperpolarizing-activated current in the whole-cell recordings.

To relate single channel activation to the oscillations seen in whole cell recordings, a staircase voltage protocol was devised which consisted of steps from 0 to −140 mV and back (Fig. 4*A*). This can be thought of as an up/down voltage ramp but with an extended dwell at each potential. Each step was 2-secs long, and the potential was changed by 20 mV at each step. We then

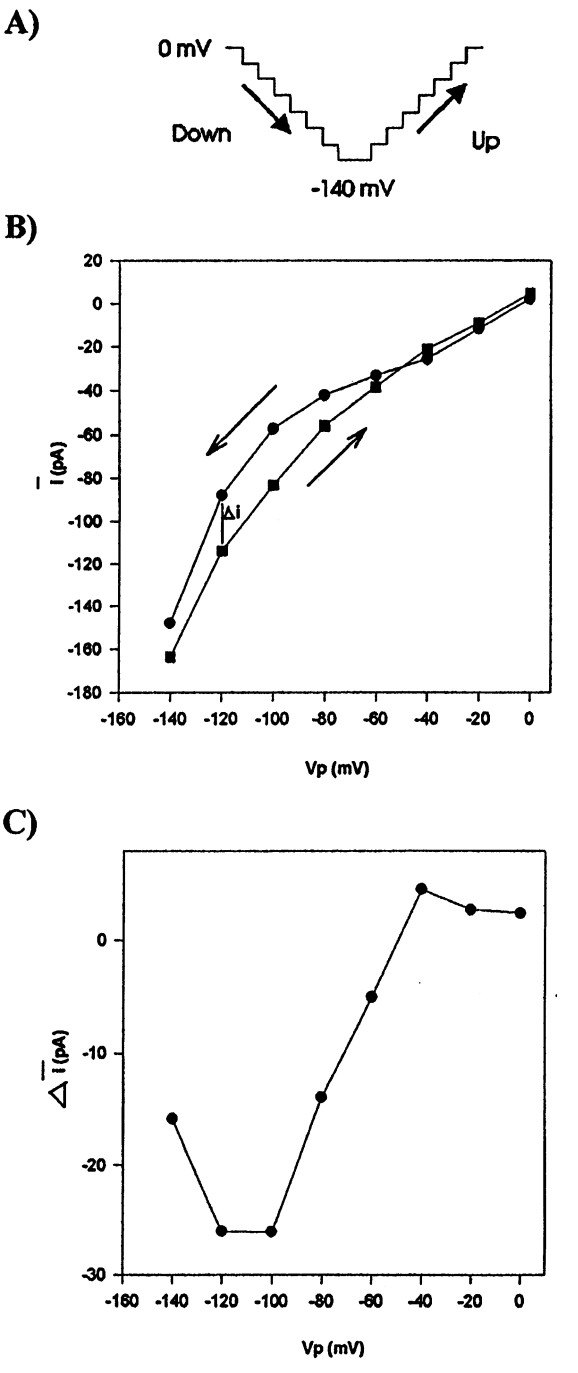

Fig. 4. Channel response to staircase protocol. (*A*) The staircase protocol used to mimic membrane oscillations (for explanation *see* text). The potential was changed by 20 mV after each of the 2 second steps. Excised patches were polarized from 0 to −140 or −120 mV and back again. (*B*) Mean current evoked from each step of a staircase protocol: ● = downward steps; ■ = upward steps. Each point is the average of three cycles. At potentials more hyperpolarized than −50 mV, the current was clearly greater, and thus the channel more active, during the returning branch of the protocol. The current evoked during the returning phase is less than that of the ascending phase between −50 and 0 mV. The mean current is not leak subtracted. (*C*) The difference in mean current, Δi is shown for each potential. Assuming that the leak current remains constant, this value reflects a difference in channel activity induced by the previous patch polarization. This difference current was greatest between −100 and −120 mV. The data shown in (*B*) and (*C*) are from an excised patch bathed in symmetrical NaGluconate; similar results were observed in 10 patches under the same conditions.

averaged the patch current during the step. When the mean current at each step was measured and plotted against voltage (*see* Materials and Methods), the current was larger during the ascending, returning branch of the staircase protocol over the range of −120 to −60 mV in 10 out of 12 patches examined (Fig. 4*B*). This indicates that channel inactivation lags behind activation at this stage of the staircase protocol. In 6 of the 10 experiments showing this long term activation, the ascending current was found to be less than the descending current between −50 and 0 mV, indicating a voltage dependence of the activation.

To clarify the voltage-dependence of this channel activity, the term difference current (Δi) was defined as the difference in current between the descending and ascending steps at the same potential during the staircase protocol. The difference current, Δi, was largest between −100 and −120 mV for the 10 patches showing this increased activity. However, Δi reversed direction near −50 mV in six of these patches (Fig. 4*C*). These observations suggest that the balance of activation and inactivation in the nonselective channel would lead to a voltage-dependent increase in channel activity during the repolarizing phase of the oscillation in the whole cell.

Cell-attached Kinetics

In cell-attached patches, spontaneous channel activity was occasionally observed at the resting membrane potential. However, activity was usually evoked by hyperpolarizing the patch by 80 or 100 mV for 1–3 mins, as with inside-out records. The currents showed frequently flickery transitions (Fig. 5*A*) and reversed approximately 70 mV more positive than the cell membrane potential (Fig. 5*B*). As the electrode contained K^+-free solution, this is predicted to be close to 0 mV, the reversal potential of a nonselective channel.

When the staircase protocol was applied to cell-attached patches, the pattern of activation was similar to that observed in inside-out patches. The activity was greater during the ascending, repolarizing phase, but the difference current was greatest between −80 and −100 mV (n = 3) (Fig. 5*C*). This is more depolarized than the maximum observed with inside-out patches and is consistent with the presence of the resting cell membrane potential.

Discussion

We have described a high-conductance, nonselective ion channel in pigmented ciliary epithelial cells that can be

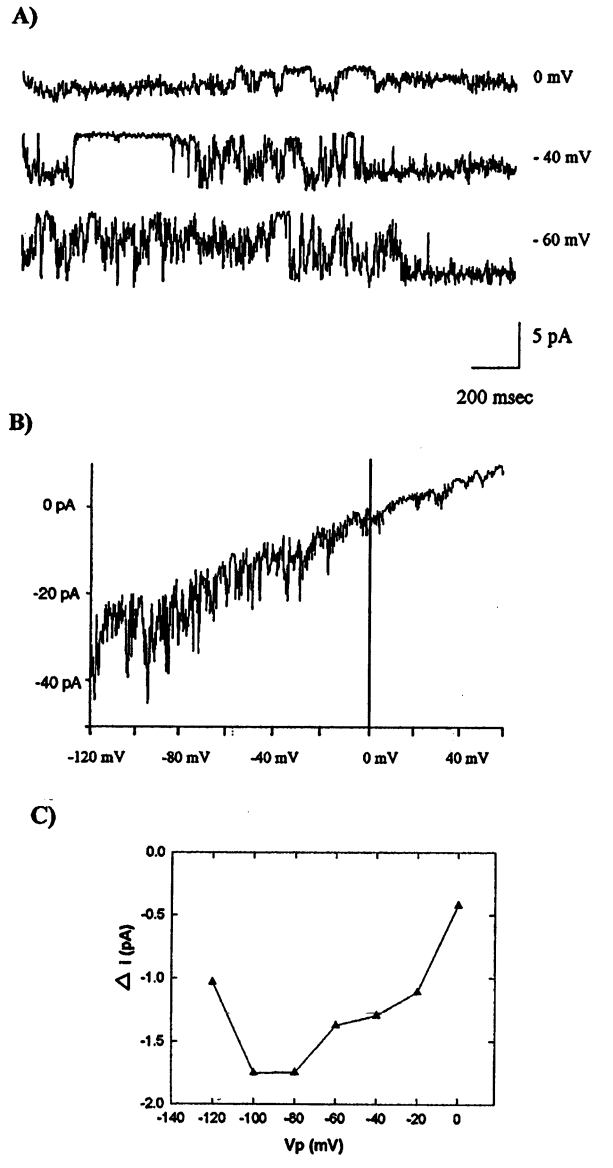

Fig. 5. Cell-attached activation. (*A*) Cell-attached recordings showing inward currents. Fully open and closed levels can be detected, but the channel spends a substantial proportion of the time in transition between the two states. The electrode contained NaGluconate solution and the cells were bathed in standard extracellular solution. The potentials indicated to the right of each trace are with respect to the cell membrane potential. (*B*) In this cell-attached recording, a 2-sec voltage ramp was applied from −120 to +60 mV. The cell membrane potential (pipette potential of 0 mV) is indicated by the vertical bar. Although the channel spends most of its time in transition between the open and closed states, it clearly reversed approximately 70 mV more positive than the cell membrane potential. The electrode contained NaGluconate and the bath contained physiological saline. (*C*) The difference current, Δi, obtained when the staircase protocol was applied to a different cell attached patch. The electrode contained NaGluconate, the bath contained physiological saline and potentials are given with respect to the cell membrane potential. The difference current reaches a maximum at a less hyperpolarized point than in the excised patches reflecting the presence of the membrane potential.

readily identified by its rapid fluctuations at submaximal current levels. The activation properties of this channel are voltage dependent and characterized by an exceptionally long time dependence. These characteristics suggest that this channel may contribute to the repolarizing phase of the oscillations seen in whole cell recordings [23]. The high conductance of this channel and its frequency of occurrence in membrane patches suggest that it makes a large contribution to the whole-cell current. Stelling and Jacob [23] reported a type of hyperpolarizing oscillation during which a large nonselective current was activated. Activation of this current increased the whole-cell conductance by a factor of 4 to 1.7pS. Thus 1.3pS could be ascribed to this nonselective current which would suggest 60 channels per cell, assuming an open probability (P_o) of 0.1, or 600 channels per cell, assuming a value for P_o of 0.01.

The channel usually required continuous hyperpolarization before activity appeared in cell-attached or excised patches. The staircase protocol illustrates the phenomenon of time-dependent activation and how the slow time course of this hyperpolarization-dependent activation might be balanced by inactivation caused by reducing the patch potential. The rate of inactivation appears to be voltage dependent, increasing as the patch potential approaches 0 mV. This property, of a residual effect of hyperpolarization, has been observed in other channels such as the large conductance non-selective anion channel in B lymphocytes [11] and it has been suggested that it is related to a mechanism for membrane ''memory.''

Selectivity

The results from the asymmetric concentration experiments suggest that the channel cannot discriminate between anions and cations when present in low concentrations at the intracellular face of excised patches. Such weak selectivity is not unusual; the major intrinsic protein (MIP) channel of lens fiber membranes has a P_{Cl}/P_K of 1.8 in a 1.0:0.1 M asymmetrical KCl gradient [4] and a large conductance channel in T84 cells has a P_{Cl}/P_{Na} of 0.67 in a 100:50 mM NaCl gradient [25]. A nonselective channel has been described in *Aplysia* neurones that has similar permeabilities to anions and cations in the presence of external Na ions [2]. The changing rate of ion flow through a channel permeable to both anions and cations would produce rapid fluctuations in current observed in this paper. Such low selectivity can be understood if these channels do not show permanent charges, allowing the channel to the polarized [2] or if a fixed charge in the channel can be screened by counter ions [7].

Some large-conductance anion channels have considerable cation permeabilities [12, 16, 21, 27]. While the nonselective channel in this study shares several characteristics with the large-conductance anion chan-

nels, significant differences exist between the channel types. Once activated, the nonselective channel in ciliary epithelial cells is open at all potentials while most of the large conductance anion channels close when polarized more than ±30 mV away from 0 mV [11, 12, 16]. In addition, the nonselective channel was not blocked by SITS, DIDS or NPPB, while anion channels have been reported to be blocked by SITS [1, 16, 27], DIDS and NPPB [25]. The nonselective channel here most closely resembles the channel in T84 cells [26] which was active at all potentials, had a very low anion to cation selectivity, and was not blocked by SITS or DIDS at concentrations up to 5 mM.

Physiological Role

The purpose of this study was to look for the channel(s) underlying the depolarizing phase of the oscillations seen in these cells in previous whole-cell recordings [23, 24]. We believe that the nonselective channel identified in this paper contributes to the repolarizing whole-cell current for several reasons. (i) Currents detected with both the whole-cell and single channel methods were characterized by considerable flicker. (ii) The whole-cell current reversed close to 0 mV, consistent with the reversal potential of a nonselective channel. (iii) In both whole-cell and single channel records, the channel activated after hyperpolarization. (iv) The difference current seen at the single channel level (Fig. 4 and 5) reached a maximum at −100 mV, close to the voltage at which the repolarizing phase of the oscillations turned on. (v) After hyperpolarization, the current observed at the single channel level inactivated near −50 mV, close to the potential at which the repolarizing phase of the oscillations ended and the whole-cell current turned off. Stelling and Jacob [24] have suggested that the oscillations in membrane potential may be involved the process of ion loading — the first phase in aqueous humour secretion. The high conductance nonselective channels described in this report may thus contribute to the secretion of aqueous humour by allowing ion entry across the basal (serosal) side of the tissue.

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References

1. Bosma, M.M. 1989. Anion channels with multiple conductance levels in a mouse lymphocyte cell line. *J. Physiol.* **410:**67–70
2. Chesnoy-Marchais, D., Evans, M.G. 1986. Nonselective ionic channels in *Aplysia* neurons. *J. Membrane Biol.* **93:**75–83
3. Cui, C., Smith, D.O., Alder, J. 1994. Characterisation of mechanosensitive channels in *Escherichia coli* cytoplasmic membrane by whole-cell patch clamp recording. *J. Membrane Biol.* **144:**31–42
4. Ehring, G.R., Zampighi, G., Horwitz, J., Bok, D., Hall, J.E. 1990. Properties of channels reconstituted from the major intrinsic protein of lens fibre membranes. *J. Gen. Physiol.* **96:**631–664
5. Filipovic, D., Sackin, H. 1991. A calcium-permeable stretch-activated channel in renal proximal tubule. *Am. J. Physiol.* **260:**F119–F129
6. Fox, J.A., Pfeffer, B.A., Fain, G.L. 1988. Single-channel recordings from cultured human retinal pigment epithelial cells. *J. Gen. Physiol.* **91:**193–222
7. Franciolini, F., Nonner, W. 1987. Anion and cation permeability of a chloride channel in rat hippocampal neurons. *J. Gen. Physiol.* **90:**453–478
8. Goldman, D.E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27:**37–60
9. Jacob, T.J.C. 1991. Two outward currents in bovine ciliary epithelial cells: $I_{K(Ca)}$ and $I_{K(V)}$. *Am. J. Physiol.* **261:**C1055–C1062
10. Krouse, M.E., Schneider, G.T., Gage, P.W. 1986. A large anion-selective channel has seven conductance levels. *Nature* **319:**58–60
11. McCann, F.V., McCarthy, D.C., Keller, T.M., Noelle, R.J. 1989. Characterisation of a large conductance non-selective channel in B lymphocytes. *Cellular Signalling* **1:**31–44
12. McGill, J.M., Basavappa, S., Fitz, J.G. 1992. Characterisation of high-conductance anion channels in rat bile duct epithelial cells. *Am. J. Physiol.* **262:**G703–G710
13. Mitchell, C.H., Jacob, T.J.C. 1993. Single channel memory: the open probability of a large conductance cation channel in isolated bovine pigmented ciliary epithelial cells. *J. Physiol.* **467:**335P
14. Mitchell, C.H., Jacob, T.J.C. 1994. Two maxi-conductance channel types in bovine pigmented ciliary epithelial cells. *Invest. Ophthalmol. Vis. Sci.* **35:**(Suppl.) 1455
15. Neher, E. 1992. Correction for liquid junction potentials in patch clamp experiments. *Methods Enzymol.* **207:**182–191
16. Nelson, D.J., Tang, J.M., Palmer, L.G. 1984. Single-channel recordings of apical membrane chloride conductance in A6 epithelial cells. *J. Membrane Biol.* **80:**81–89
17. Rae, J.L., Levis, R.A. 1985. Patch voltage clamp lens epithelial cells: theory and practice. *Molec. Physiol.* **6:**115–162
18. Raviola, G. 1971. The fine structure of the ciliary zonule and ciliary epithelium. *Invest. Ophthalmol. Vis. Sci.* **10:**851–869
19. Robinson, R.A., Stokes, R.H. 1968. Electrolyte Solutions. Rev. 2nd Ed. p 481. Butterworths, London
20. Sather, W.A., Bodoia, R.D., Detwiler, P.W. 1985. Does the plasma membrane of the rod outer segment contain more than one type of ion channel? *Neurosci. Res.* **2:**S89–S99
21. Schlichter, L.C., Grygorczyk, R., Pahapill, P.A., Gryorczyk, C. A large, multiple-conductance chloride channel in normal human T lymphocytes. *Pfluegers Arch.* **416:**413–421
22. Schwarze, W., Kolb, H-A. 1984. Voltage-dependent kinetics of an anionic channel of large unit conductance in macrophages and myotube membranes. *Pfluegers Arch.* **402:**281–291
23. Stelling, J.W., Jacob, T.J.C. 1992. The inward rectifier K^+ current underlies oscillatory membrane potential behaviour in bovine pigmented ciliary epithelial cells. *J. Physiol.* **458:**439–456
24. Stelling, J.W., Jacob, T.J.C. 1993. Membrane potential oscillation from a novel combination of ion channels. *Am. J. Physiol.* **265:**C720–C727
25. Sun, X.P., Supplisson, S., Torres, R., Sachs, G., Mayer, E. 1992. Characterization of large-conductance chloride channels in rabbit colonic smooth muscle. *J. Physiol.* **448:**335–382
26. Vaca, L., Kunze, D.L. 1992. Anion and cation permeability of a large conductance anion channel in the T84 human colonic cell line. *J. Membrane Biol.* **130:**241–249
27. Velasco, G., Prieto, M., Alvarez-Rieva, J., Gascon, S., Barros, F. 1989. Characteristics and regulation of a high conductance anion channel in GBK kidney epithelial cells. *Pfluegers Arch.* **414:**304–310
28. Wold, K.H., Leibowitz, M.D., Neumcke, B., Hille, B. 1987. A high-conductance anion channel in adult amphibian skeletal muscle. *Pfluegers Arch.* **410:**632–640