Whole-Cell Currents from Noncultured Human Lens Epithelium

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Perforated patch techniques were used to measure whole-cell ionic currents in freshly dissociated human lens epithelial cells that had not been subjected to culture media or serum. With a 150 mmol/l K⁺ internal solution, the cells had resting voltages of $-27.4 \pm 4.7$ mV (mean ± standard deviation [SD]) and capacitances of $10.4 \pm 2.8$ pF (mean ± SD). The input resistance of the cells was $1.6 \pm 0.7$ GΩ (mean ± SD) at large negative voltages. A delayed outwardly rectifying K⁺ current was found in most cells studied. Current magnitudes of 1–2 nA at $+80$ mV were common. The current had selectivities, activation time constants, deactivation time constants, open probability versus voltage relationships, and inactivations similar to those of the delayed rectifying K⁺ current found in many cell types and studied previously in cultured human lens epithelium. These results verify the existence, at high density, of these currents in noncultured human epithelial cells. Invest Ophthalmol Vis Sci 33:2262–2268, 1992

During the past few years, lenses of many species have been found to contain several varieties of K⁺-selective channels in their epithelial cell membranes. At least three currents occur ubiquitously.¹⁻⁶ One is a highly inwardly rectifying current similar to that reported in heart cells and elsewhere but different from most reported inward rectifiers because the outward currents do not appear to be blocked by internal Mg++.³ This current has been found in the lens epithelium of rat, mouse, rabbit, chick, and cultured human lens epithelial cells. The current flows through a channel that allows larger inward than outward currents for the same driving force. It is blocked sensitively by submillimolar concentrations of external Cs⁺ or Ba++. The second K⁺ current in lens membranes is caused by a large conductance K⁺-selective channel essentially identical to BK and activated by both internal Ca++ and membrane depolarization.⁴ Such Ca++-activated K⁺ currents occur in the lens epithelium of humans, rabbits, and chicks. The channels that give rise to this current are largely quiescent at commonly found intracellular Ca++ concentrations and require positive transmembrane voltages for substantive activation at resting intracellular Ca++ concentrations. It is likely that these channels are major contributors to lens membrane properties only after an intracellular Ca++ increase. These channels are blocked by many compounds including tetraethylammonium (TEA), quinidine, Ba++, decamethonium, and charybdotoxin. The third common K⁺ current is similar to the delayed rectifier found in many excitable membranes.⁷ The current shows steep voltage dependence with most channels being closed at voltages more negative than $-30$ mV. It is outwardly rectifying; outward currents are larger than inward currents for the same driving force. This rectification is derived from the finding that the channels close at voltages where current flow would be inward. The channels responsible for the currents open after a delay when a transmembrane voltage step sufficient to cause channel opening occurs. These channels are blocked by TEA and 4-aminopyridine but are relatively insensitive to external Ba++ and Cs+. This current was found in the lens epithelium of rabbits, chicks, and humans. Although several other kinds of K⁺-selective currents have been seen in lens epithelium, these three are the most common in reports published currently.

Virtually all electrophysiology done on human lens epithelium has been done on cultured epithelium.¹²⁻⁹ The properties of a delayed rectifying K⁺ current in epithelial cells cultured from human autopsy lenses were quantified.¹ In these studies, Ca++-activated K⁺ currents and tetrodotoxin (TTX)-blockable Na⁺ currents also were found. When dealing with cultured cells, we question whether the tissue culturing might cause the expression of channels not usually found in intact natural tissue and/or might not allow expres-
sion of channels that usually are seen in such tissue. During the past few years, in comparing freshly dissociated and cultured epithelial cells from various ocular tissues, we have seen many instances of both kinds of problems. We, therefore, thought it important to compare freshly dissociated human lens epithelial cells that had not been exposed to culture media or serum with those that we previously studied in tissue culture. This comparison is the focus of this report.

Materials and Methods

Tissue Preparation

All cells studied were obtained from autopsy globes 6-24 hr after death. The lenses used varied in age from 45-84 yr. Each lens was removed from the globe by careful microdissection and then was prepared for epithelial cell dissociation according to previously described protocols. Briefly, the lens capsule and its adhering epithelium were removed with jeweler’s forceps and pinned (epithelial cells up) to a small Sylgard no. 184 disc (Dow Corning, Midland, MI). All excess capsule and fiber cells were trimmed away, leaving only the monolayer of the epithelium and its capsule. The epithelium and Sylgard disc were placed in a solution of 0.125% collagenase (Type IV; Worthington, Freehold, NJ) and 0.05% protease (Type XXIV; Sigma, St. Louis, MO) in 10 ml of a low Ca++ Na+ aspartate Ringer’s solution at pH 7.350. The epithelium and capsule were incubated for 1.5-2 hr at room temperature. Then the capsule with epithelial cells attached was removed gently from the enzyme solution and placed in 5 ml of a standard NaCl-containing Ringer’s solution. The cells were removed from the capsule by gentle trituration with a fire-polished Pasteur pipette. The solution with suspended cells was changed for fresh Ringer’s solution, and the cells were resuspended by gentle trituration.

Recording Techniques

Approximately 100 µl of the dissociated cells was added to the well in an acrylic plastic chamber positioned under a compound microscope. The bottom of the chamber was lined with a small piece of microscope slide glass that had been cleaned with ethanol. The cells settled to the bottom, and many adhered securely to the glass substrate. Patch electrodes were pulled from either 7760 or 8161 glass, coated with Sylgard no. 184 to within 100 µm of their tips, and fire polished under direct observation to a consistent end point. The electrodes used had resistances from 1.5-3 MΩ. The tips were filled by briefly dipping them into the desired filling solution and then backfilling with the same solution, which also contained 240 µg/ml of amphotericin B (A-4888; Sigma). The electrodes were mounted in a holder in an Axopatch 200 patch clamp (Axon Instruments, Foster City, CA) and sealed to the cells with suction under direct observation. Within 5-10 min, the amphotericin B partitioned into the membrane patch in the pipette to produce a low resistance access into the cell interior; this allowed voltage clamping. Cell capacitance was measured using the circuitry inherent in the Axopatch 200. Resting voltages were determined either from the reversal potential of the whole-cell current in the voltage clamp or from the zero current potential in the current clamp. The records were capacity compensated using circuitry provided in the Axopatch 200 for that purpose. We did not use leak subtraction.

Voltage step protocols were delivered to the input of the patch clamp and the currents, filtered at 1 KHz through an eight-pole Bessel filter, and digitized at 2-4 KHz using an Axolab computer interface and pClamp software (Axon). The data were analyzed using the custom-designed software and macros in Microsoft Excel (Redmond, WA). Curve fitting was done using Clampfit, a program included with pClamp. The final plots were done using Excel, PaintBrush 4 (ZSoft, Marietta, GA), Draw Perfect (WordPerfect, Orem, UT), and SigmaPlot (Jandel, Corte Madera, CA).

Solutions

The solutions used for the study are listed in Table 1.

Results

Resting Voltage and Capacitance

Our results were derived from 26 cells from 13 different human lenses varying in age from 45-84 yr. The capacitance of the cells was 10.4 ± 2.8 pF (mean ± standard deviation [SD], n = 11). The resting voltage from eight cells characterized in normal Ringer’s solution was -27.4 ± 4.7 mV (mean ± SD) when the pipette was filled with the standard 150 mmol/l K+ filling solution (Table 1). The cells had input resistances that ranged from 450 MΩ to 3.6 GΩ and averaged 1.6 ± 0.7 GΩ (mean ± SD, n = 19).

Form of the Currents

The freshly dissociated human lens epithelium was dominated by a single current over a voltage range of -70 to +80 mV. In every instance where we were able
to make a reasonable whole-cell current measurement, a delayed rectifying K⁺ current like that shown in Figure 1A was seen. These records were generated by an activation protocol where the cell was maintained at a fixed holding voltage, stepped to a series of other voltages, and returned to the holding voltage after each step. In general, the maximum currents were particularly large (range, 1–2 nA at +80 mV). The currents first activated in the −40 to −20-mV range. They activated with a delay after onset of the pulse, and the time course of activation became faster as the transmembrane voltage was made more positive. At the outset, these general properties seemed similar to those reported previously for a current measured from cultured human lens epithelial cells. When the cells were bathed in an isosmotic KCl Ringer’s solution, recordings similar to Figure 1B were obtained. Even under these circumstances, the outward currents were larger than the inward currents, showing the outwardly rectifying property. The major difference between the currents in a Na⁺ versus a K⁺ Ringer’s solution was that the tail currents (arrows) generated at the turn off of the voltage step were larger when the bath K⁺ concentration was elevated. In the activation protocol of Figure 1A, the increasingly positive voltages activated an increasing number of channels to enter the open state. Because the opened channels take time to deactivate (close), the instantaneous current quantified immediately after turning off the activating voltage pulse was a measurement of the number of the channels in the open state at the end of the activating pulse. The size of the current, however, also depended on the concentration of ions available to carry the current. Therefore, the tail currents were much larger in 150 mmol/L K⁺ Ringer’s solutions. This allowed easy resolution of the time course of the deactivation that occurred as the channels closed to assume the open probability appropriate for the voltage used to generate the tail currents. In summary, this predominant current acti-

![Fig. 1. (A) A typical family of whole-cell currents recorded from a single dissociated human lens epithelial cell bathed in normal Ringer. The voltage step protocol used to generate the currents is shown. The holding potential was −70 mV. Voltage steps were in 10-mV increments to a maximum of +80 mV. The recording parameters were: bandwidth = 1 kHz, access resistance = 6 mΩ (perforated patch), series resistance compensation = none. (B) A family of whole-cell currents recorded from a single dissociated human lens epithelial cell bathed in 150 mmol/L K⁺ Ringer. Bandwidth = 1 kHz, access resistance = 4 mΩ (perforated patch), with no series resistance compensation.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933392/)
vated with a delay after a voltage step. It was also outwardly rectifying, and it therefore was a member of the family of currents known as delayed rectifiers. In no instance, did we see Na+ currents blockable by TTX, currents which were common in cultured human lens epithelial cells.

Selectivity

The selectivity of the current can be investigated by a tail current protocol. The channels first were activated with a voltage pulse sufficiently positive that essentially all channels were opened. Then tail currents were generated by steps to a series of different voltages (Fig. 2A). Use of the instantaneous tail current (arrow) at each voltage allowed construction of a current–voltage relationship whose reversal potential could be used to assess the selectivity of the current. Figure 2B overplots several such current–voltage relationships generated with different K+ concentrations in the bathing solution surrounding the cells. The reversal potentials shifted to more positive voltages, and the inward conductance increased as the outward K+ concentration became larger. Figure 2C overplots these reversal potentials for several different bath K+ concentrations with the relationship expected for a perfectly selective K+ channel. All these results show that the channel was highly selective for K+ over Na+. In Figure 2D, we show current records

![Fig. 2. (A) A family of whole-cell tail currents generated from a single human lens cell bathed in 150 mmol/l K+ Ringer. The voltage step protocol is shown. The cell voltage was held at −70 mV, briefly stepped to +70 mV to open all the channels, and then stepped to a series of different voltages in 10-mV increments to generate a series of tail currents (arrow). Bandwidth = 1 kHz, access resistance = 5 MΩ (perforated patch), with no series resistance compensation. (B) IVs from instantaneous tail currents (current amplitude is that of the first current point following the onset of the voltage step to generate the tail currents). Bathing solutions were: squares = 150 mmol/l K+, closed triangle = 75 mmol/l K+ −75 mmol/l Na+, open triangles = 40 mmol/l K+ −110 Na+, closed circles = 20 K+ −130 Na+, and open circles = 4.7 K+ −146 Na+. Both the slopes of the IVs and the reversal potentials are a function of the external K+. (C) Overplots of reversal potentials vs. external [K+] for three different cells obtained from data similar to those in Figure 2B. The open squares are the values expected for a perfectly selective K+ channel. (D) A family of whole-cell currents from a human lens epithelial cell bath in 150 mmol/l Rb+ Ringer to show that Rb+ effectively carries the tail current. Bandwidth = 1 kHz, access resistance = 6 MΩ, no series resistance compensation.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933392/ on 08/09/2018)
generated from an activation protocol when the bath contains 150 mmol/l RbCl Ringer's solution. The tail currents were nearly as large as those generated in a K⁺ solution (Fig. 1B), suggesting that Rb⁺ was nearly as good at carrying current through the channel as K⁺. This was a distinguishing feature of this channel because, although many other K⁺ channels are as permeable to Rb⁺ as to K⁺, their conductance for Rb⁺ usually is much less than their K⁺ conductance.

Open Probability Versus Voltage

By measuring the magnitude of the instantaneous tail currents generated at a single voltage after the activation protocol, it was possible to measure the probability that the channels were in the open state (Pₒ). The magnitude of that instantaneous current reflected the fraction of total channels open at the previous voltage. In Figure 3, we plotted the magnitude of the instantaneous tail current from a representative cell as a function of voltage before the voltage step that generated the tail currents. The tail currents began to increase in the -40 to -20 mV range where the channels began to open and became maximal by +60 mV or so. By fitting a Boltzmann distribution through these data:

$$\ln\left(\frac{P_o}{1 - P_o}\right) = \frac{-qF}{RT} (V - V_o)$$

where q = effective gating charge, Vₒ = voltage where Pₒ = 0.5, F = Faraday's constant, R = universal gas constant, and T = absolute temperature, we found that the 50% open probability was at -18.8 ± 7.1 mV and the effective gating charge was 2.7 ± 0.9e (mean ± SD, n = 26). This analysis assumed that the channel had two states (open and closed) and that Pₒ = 1 at the plateau current at positive voltages.

Gating Kinetics

The time course of the activation of the current (Figs. 1A–B) was complicated (as shown previously in cultured lens cells) and could not be fitted by a simple exponential relationship. Therefore, we determined the time period required for the current to increase from 10% to 90% of its steady-state value following steps to the voltages shown. Data are from two cells held at -70 mV. Data are obtained from records like those shown in Figure 1A.

Using currents generated by a tail current protocol (Fig. 2A), it was possible to fit single exponentials to the tail current decay with time at each voltage and generate time constants for deactivation. The results of this analysis are plotted as a function of voltage in Figure 5. The deactivation became slower and slower as the transmembrane voltage was depolarized.

The recordings in Figure 1B show that the outward current reached a peak and then began to decay slowly with time. This represents inactivation of the channel on a long time scale, a time scale requiring several seconds in most preparations studied to date. The time course of this inactivation process was variable from one dissociated cell to another, and we did not try to quantify the inactivation time constant. However, another way of investigating the inactivation process is to look at the holding voltage dependence of these currents. In Figure 6, we present current–voltage relationships from a representative cell activated with the same activation protocol but
Fig. 5. The time constant, in milliseconds, for the activated channels to deactivate following steps to the voltages shown. Data are obtained by fitting a single exponential to the tail current at each voltage. The tail currents are generated as shown in Figure 2A.

with the cell held at different holding potentials as shown. When the cell was held at increasingly depolarized voltages, this delayed outwardly rectifying K⁺ current decreased in magnitude; therefore, there was an inactivation state for the channel that generated this current. All these kinetic properties (activation, deactivation, and inactivation) were similar to those reported earlier in cultured human lens epithelial cells.¹

Blockade by Tetraethylammonium (TEA)

The substance TEA is known to be an effective blocker of delayed rectifying K⁺ channels.¹⁷ Therefore, we tested its effects on the currents in freshly dissociated human lens epithelium. Figure 7 overplots the steady-state current–voltage relationships obtained from a single cell bathed in 150 K⁺ Ringer's solution, with and without 20 mmol/l TEA. It was found that TEA reduced the current at all voltages where the channel was open. The current–voltage relationship obtained in the presence of TEA was almost the same form as that determined with a cell held at 0 mV (Fig. 6). The residual current in both cases was not ohmic; this suggests that a second current type probably existed in these cells. We have not studied this second current yet. These results using TEA were comparable with those obtained with the current found in cultured human lens epithelium and provide additional evidence that the delayed rectifying K⁺ current was the same in the cultured and freshly dissociated human lens epithelium.

Other Currents

Two of the 26 cells contained inward rectifier potassium currents in addition to the delayed rectifier. In addition, currents from Ca²⁺-activated potassium channels were seen in inside-out patches from freshly isolated human lens epithelial monolayers and in one of two freshly dissociated cells tested. We did not test for Ca²⁺-activated currents in all cells and, therefore, cannot estimate their frequency of occurrence or magnitude in the cell population we studied.

Discussion

Our results show that freshly dissociated human lens epithelial cells contained predominantly a delayed outwardly rectifying K⁺ current that was similar (if not identical) to that previously recorded in cultured human lens epithelial cells. Both showed approximately the same selectivity for K⁺ over Na⁺, had similar time courses of activation and deactivation with voltage, and showed evidence of inactivation on a long time scale. In addition, both currents began activating in the -20 to -40 mV range and achieved maximum open probability by about +20 mV. We found TEA was an effective blocker of the delayed rectifier in both cultured and freshly dissociated human lens epithelial cells.

One major difference between the cultured and freshly dissociated cell populations was that we did not see TTX-blockable Na⁺ currents in the freshly dissociated cells.¹¹² These Na⁺ currents were sufficiently common in the cultured cells that we would have seen them if they occurred in the freshly dissociated cells with the same frequency. We do not believe that TTX-blockable Na⁺ currents in lens tissue are entirely a tissue-culture artifact because we have recorded such currents from freshly dissociated frog lens epithelial cells (unpublished observations), and
other investigators have implicated them in the lens response to low Ca++ bathing solutions. A second difference is that the freshly dissociated cells were much smaller than cultured cells. The average cell capacitance was 10.4 pF/cell in the fresh cells compared with approximately 50 pF/cell in the cultured cells. This does not mean that the cultured cells were swollen, only that their growth was regulated in culture differently from that in intact human lenses.

Our results showed that delayed rectifying K+ currents, once thought to occur only in excitable tissues, occurred ubiquitously in freshly dissociated human lens epithelial cells and were not a result of culture artifacts in previous studies. Previously, TEA blockable K+ conductances in lens membranes have been reported, and it has been suggested that lens membranes have properties similar to excitable cells. The cells we studied also contained inward rectifiers and Ca++-activated potassium channels, and therefore, we confirmed that tissue cultured cells of human lens epithelium can provide relevant information about the K+ conductance of intact tissue. Our results also support the hypothesis that additional studies on the properties of this delayed rectifying K+ current are warranted in the cell culture model where the cells are more available than from normal autopsied globes.

Key words: lens, epithelium, patch clamp, currents, human

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References