The Large-Conductance Potassium Ion Channel of Rabbit Corneal Epithelium Is Blocked by Quinidine

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Basal layers of the rabbit corneal epithelium contain a large-conductance potassium ion (K⁺) selective channel (160–170 pS in 150 mM KCl). This channel previously was shown to be blocked by cesium and barium ions applied to its outer surface. By direct patch clamp experiments, it is shown that the channel also is blocked by quinidine in the 0.1–1 mM range when applied either to the outside or inside of membrane patches containing these channels. This additional pharmacologic tool should aid in identifying the individual currents that compose the macroscopic currents from corneal epithelial cells and eventually should help to provide a detailed assessment of their function. Invest Ophthalmol Vis Sci 33:286–290, 1992

The basolateral membrane of either rabbit or frog corneas has been shown to contain primarily potassium ion (K⁺) conductance.1−3 This conductance has been reported to be increased by chloride ion secretagogues,2 but this, in part, has been disputed.3 Recently, evidence was found for two kinds of K⁺ conductances in these membranes, one blocked by barium ions (Ba++) but not quinidine and the other blocked by both Ba++ and quinidine.4 The quinidine-blocked conductance apparently is involved in volume regulation.

We recently described a large-conductance K⁺ channel in rabbit corneal epithelium that might be the basis for at least one of these previously described conductances.5 In a survey of a large number of K⁺ channel blockers, we found that cesium ion (Cs⁺) and Ba++ block the single channels, but we found no quinidine effect. Recently, while measuring whole-cell currents from single dissociated rabbit corneal epithelial cells, we found that most of the K⁺ current was blocked by external quinidine. Therefore, we restudied the effect of quinidine on the single channel currents. We now report that, under the circumstances described in this report, quinidine is an effective blocker of the single channels when applied either from the inside or outside.

Materials and Methods

Our studies were done on isolated single corneal epithelial cells from New Zealand white rabbits. These cells were dissociated as previously described.5 Briefly, the entire corneal epithelium was removed as described previously.6'7 It then was placed in a centrifuge tube containing 5 ml of 0.008% trypsin in calcium-free rabbit Ringer's solution (146 mM NaCl, 4 mM KCl, and 5 mM HEPES) and triturated with a fire-polished Pasteur pipette for approximately 8 min at room temperature. These cells were spun down at 300 × g and then resuspended in 5 ml of rabbit Ringer's solution (same solution as described with 2.5 mM CaCl₂ and 15 mM glucose). This solution was placed in a well under our experimental microscopes where the cells could be patch clamped with direct observation. All experiments were done using either cell-attached or inside-out patches. In some experiments, outside-out patches would have been desirable, but we found that outside-out patches usually contained several K⁺ channels. Because of their inherently flickery kinetics and the additional flicker imparted by quinidine, the desired currents were impossible to quantify in outside-out patches. All single-channel measurements were done using an Axopatch 200 integrating patch clamp (Axon Instruments, Foster City, CA). The data were stored on a modified video tape recorder.8 The single-channel currents were digitized from the tape through a 2-kHz 8-pole Bessel filter using a sampling frequency of 5 kHz. Single bursts of the channel were identified using a custom-made cursor-driven computer program. The cursors identified on the display screen the time at which the channel opened and the time at which it finally closed. The current at all times between these
two were averaged to give the mean open channel current. From this we subtracted the mean baseline current to obtain what we report here as the "channel mean current."

All experiments were done using 150 mM KCl, 2.5 mM CaCl₂, and 5 mM HEPES in the pipette with quinidine concentrations ranging from 0–1 mM. In the cell-attached patch recordings, the cells were bathed in a 150 mM K⁺ methane sulfonate Ringer’s solution with 2.5 mM CaCl₂ and 5 mM HEPES, pH 7.4. In some experiments, the membrane patch was excised from the cell, and currents were measured in the inside-out configuration with 150 mM K⁺ solutions in the bath containing 0–1 mM quinidine. The bath was changed frequently to avoid any concentration change caused by evaporation.

The blockade was analyzed as described by others for a nonpermeating blocker interacting with a single binding site in the channel. The following relationship is expected to describe the ratio of currents in the presence and absence of a blocker:

\[
\frac{I_b}{I_o} = \left[1 + \frac{[b]}{K_d(0) \exp\left(-\frac{5ZVF}{RT}\right)}\right]^{-1}
\]

where \(I_b\) = mean current in the presence of blocker; \(I_o\) = mean current in the absence of blocker; \([b]\) = blocker concentration; \(K_d(0)\) = blocker concentration when current is half blocked at 0 mV; 5 = the electrical distance of the binding site along the transmembrane field; \(V\) = voltage; \(F\), \(R\), and \(T\) are Faraday’s constant, gas constant, and temperature (Kelvin), respectively; and \(Z\) is valence.

**Results**

Figure 1 shows single-channel currents from two representative cell attached patches. In Figure 1A, the pipette contained 150 mM KCl Ringer’s solution. In Figure 1B, the pipette contained 1.0 mM quinidine in addition. In the presence of quinidine, both the inward and outward currents showed additional flicker and voltage-dependent reductions in single-channel amplitude.

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933390/)
Both the concentration and voltage dependence of this blockade are depicted in Figure 2A. The channel mean current was plotted as a function of the transmembrane voltage for several different concentrations of quinidine in the pipette. These data came from ten different membrane patches (see Figure legend). There was a small, but easily discernible, reduction in the current at all voltages when the pipette quinidine was as low as 0.1 mM. The extent of the block increased as the quinidine concentration increased. The block was not very voltage dependent, although it was clear that the outward currents were blocked more than the inward currents even when the quinidine was applied from the outside. The block was not complete at a quinidine concentration of 1 mM. These data were fitted to a one-site blocking model in Figure 2B. They reasonably well fit a single blocking site located where it could sense approximately 20% of the membrane field with a $K_d$ at 0 mV near 1.2 mM. This analysis predicted that approximately 5 mM external quinidine would be necessary to produce “complete” blockade at 0 mV.

When quinidine was applied to the inside surface of inside-out patches, it again blocked in a weakly voltage-dependent way (Fig. 3A). At each concentration and voltage, the blockade was more substantial for internally applied quinidine than for externally applied. Fitting these data to a one-site blocking model (Fig. 3B) required a blocking site that again saw approximately 20% of the membrane field and had a $K_d$ near 0.6 mM at 0 mV, about one half that for externally applied quinidine.

**Discussion**

These results show clear and substantial blockade of this $K^+$ channel by either internal or external quini-
Fig. 3. (A) IV plots obtained from a representative inside-out patch where both the pipette and bath contained a 150 mM K⁺ Ringer (see text). Following measurement in a quinidine-free bath, quinidine in the concentrations shown was added to the bath starting at low concentrations and proceeding to the highest. (B) Fits of the data from (A) to a one-site blocker model. For 0.1 mM quinidine, δ = 0.2 and Kᵦ = 0.71. For 0.5 mM quinidine, δ = 0.17 and Kᵦ = 0.62. For 1.0 mM quinidine, δ = 0.22 and Kᵦ = 0.55.

dine and contradict our previous results in which we did not see blockade from externally applied quinidine. We do not know the reason for this discrepancy. One possibility is that corneal epithelium contains two large-conductance K⁺ channels, one blocked by quinidine and one not. A second possibility is that the previous blocker overview experiments done in Chicago using New Zealand white rabbits from a different supplier may have studied a different large-conductance channel than the one studied in Minnesota and reported in our recent publication.⁵ A third possibility is that the quinidine in the solutions used for those experiments was not active. It is not possible to determine in retrospect which, if any, of these possibilities correctly explains the discrepancy.

Our current studies show quinidine to be an effective blocker of this channel. Quinidine, therefore, can be used as a pharmacologic tool along with Cs⁺ and Ba²⁺ blockade to help identify the specific currents that cause macroscopic conductance in basolateral corneal epithelium. The concentration and voltage dependence of quinidine blockade reported here will not explain completely the results of others,⁵ in which 0.2 mM quinidine largely blocked their volume-induced K⁺ conductance increase in both bullfrogs and rabbits. There is no physiologically realizable voltage at which 0.2 mM quinidine would block our channel completely. However, because our channel was blocked by both Ba²⁺ and quinidine, it must be considered a possible pathway in the swelling-induced K⁺ conductance these authors found. Further progress on these issues is expected from whole-cell current measurements with single dissociated corneal epithelial cells in which voltage clamp, cell swelling, and measurement of macroscopic currents can be done simultaneously.
**Key words:** cornea, epithelium, rabbit, patch-clamp, potassium channel, blocker, quinidine

**References**