Characterization of an Inwardly Rectifying Potassium Channel in the Rabbit Superior Lacrimal Gland

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PURPOSE. To characterize the properties of an inwardly rectifying K⁺ (Kᵢᵢ) current in fresh, enzymatically isolated acinar cells from the rabbit superior lacrimal gland.

METHODS. New Zealand White rabbits of both sexes were killed by injecting 45 mg/kg pentobarbital sodium, and the glands were excised. Single acinar cells were isolated enzymatically from these glands. Standard patch-clamp techniques were used to record ion currents.

RESULTS. Hyperpolarizing voltages evoked Kᵢᵢ currents that had a conductance of 2.7 ± 0.16 nS (n = 6) in the range —50 mV to —160 mV. The Kᵢᵢ current was activated with steep voltage dependence on hyperpolarization, and the conductance was approximately dependent on the square root of the external K⁺ concentration. Increasing the pipette Cs⁺ concentration from 10⁻⁹ M to 10⁻⁶ M increased the conductance to 5.3 ± 0.45 nS (n = 7). Internal substitution of K⁺ with various cations gave the following permeability sequence: K⁺ (1.0) > Rb⁺ (0.83) > Li⁺ (0.15). The Kᵢᵢ current was inhibited by 4-aminopyridine (5 mM). The single-channel conductance was 43 ± 2.7 pS (n = 11), and the relationship between single-channel conductance (γ) and external K⁺ concentration ([Kᵢₒ]) is given by: γ = 7.04[Kᵢₒ]⁻⁰.₃⁷ (pS, r² = 0.99, P < 0.05). The relationship between [Kᵢₒ] and zero current potential (Eᵦᵦᵦ) is given by: Eᵦᵦᵦ = 35.5 log([Kᵢₒ]) — 77.8 (mV; r² = 0.99, P < 0.05).

CONCLUSIONS. The Kᵢᵢ current identified in these lacrimal acini has a similar dependence on [Kᵢₒ] as other inward rectifiers of excitable tissues and exocrine glands. However, this study highlights that there are interspecies variations and similarities between Kᵢᵢ channels that could be related to their individual physiological roles. The authors’ investigations suggest that one role of the Kᵢᵢ channel in the rabbit superior lacrimal gland acinar cells is to set and stabilize the resting membrane potential. However, this Kᵢᵢ channel may also be involved in secretion, as has been shown to occur in the sheep parotid gland. (Invest Ophthalmol Vis Sci. 1998;39:308–314)

Inwardly rectifying currents have been described in a wide variety of excitable cells, including skeletal muscle and cardiac muscle, and nonexcitable cells, including renal distal tubule, rat hepatocytes, lens epithelial cells, rat osteoclasts, rabbit parotial cells, sheep parotid, and retinal Müller cells. In these tissues, they have been reported to have several physiological roles. First, the inward rectifier is involved in setting and stabilizing the resting membrane potential. Second, in some cells, including glial cells, its function is to buffer transient increases in extracellular K⁺ concentration. Third, it may be involved in removing K⁺ to prevent its accumulation in the cell caused by increased activity of the Na⁺-H⁺ antiporters. In this manner, accumulated K⁺ ions may then leave through the inward rectifier. Finally, in the sheep parotid, an inwardly rectifying K⁺ channel is thought to be involved in spontaneous secretion, which is secretion in the absence of nerve stimulation and a unique characteristic of this salivary gland. It is of interest that this inwardly rectifying K⁺ (Kᵢᵢ) channel has not been previously reported in other lacrimal glands. Therefore, in the present study, we investigated the properties of this channel in the rabbit superior lacrimal gland.

MATERIALS AND METHODS

Preparation of Lacrimal Gland Cells

New Zealand White rabbits of both sexes (1.5–2.5 kg) were killed by injecting 45 mg/kg pentobarbital sodium (Nembutal; Abbott Laboratory, Irving, TX) administered intravenously in accordance with the Australian National Health and Medical Research Council guidelines for animal ethics and the tenets of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The superior lacrimal glands were excised and placed in a petri dish containing 5 ml Dulbecco’s modified Eagle’s medium supplemented with 5-glutamine (0.584 g/l) and D-glucose (4.5 g/l). The glands were cut into small pieces and gently centrifuged for 1 minute at 25g using a hand-operated centrifuge (Model 1011; Hettich, Tuttingen, Germany). The supernatant was discarded, and the glandular fragments were then incubated in 1 mg/ml trypsin (Type IX; Sigma Chemical, St. Louis, MO) for 8 minutes at 37°C in an oscillating water bath (130 oscillations/minute).

After centrifugation of the trypsin solution for 1 minute at 25g, the supernatant was discarded and the cellular pellet was
rinsed with 1 mg/ml soybean trypsin inhibitor (Type 1-S; Sigma). The cellular pellet was then transferred to a 25-ml Erlenmeyer flask and incubated in the standard NaCl-bathing solution (see Solutions and Chemicals) containing 200 U/ml collagenase (Type II; Worthington Biochemical, Freehold, NJ) and 600 U/ml hyaluronidase (Type V; Sigma) for 48 minutes at 37°C in the oscillating water bath. The enzyme solution was filtered through a 100-μm, nylon-mesh filter and centrifuged for 2 minutes at 25g. The cellular pellet was washed twice with the standard NaCl bath solution containing 2 mg/ml bovine serum albumin (Sigma). After each wash, the cells were centrifuged for 2 minutes at 25g, and the supernatant was discarded. After the second wash, the cells were resuspended in 6 ml Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (P.A. Biologicals, Trace Biosciences, Sydney, Australia). All of the results described in this study were recorded from these freshly isolated acinar cells.

**Path-Clamp Recording and Analysis**

Whole-cell and single-channel currents were recorded by standard patch-clamp techniques at room temperature (20–25°C). The channel currents were amplified and filtered at 1 kHz (−3 dB) using an Axopatch 200A and CV201A headstage (Axon Instruments, Foster City, CA) and sampled online by a microcomputer (IBM 486-compatible) using commercial software and associated analog-to-digital hardware (pClamp 5.5 1/Labmaster TL-1-125; Axon Instruments and Scientific Solution, Foster City, CA). Patch pipettes (4–10 MΩ) were fabricated from thin-walled borosilicate glass (Vitrex Microhematocrit tubes; Modulomat I/S, Denmark). The reference electrode was an electrode of Ag–AgCl immersed in a KCl solution (140 mM) that was connected to the bath by an agar bridge. In experiments in which the salt bridge was not used, the pipette potential was corrected for the liquid junction potentials between the pipette solution and bath solution. In both the whole-cell and the cell-attached configurations, positive current was defined as positive ions moving out of the headstage and was shown by an upward deflection in all traces. Conversely, positive current in the whole-cell configuration corresponds to the outward membrane current and was shown by an upward deflection in all traces. Conversely, positive current in the cell-attached configuration corresponds to the patch inward current and is represented by upward deflections in single-channel traces. Because it has been shown in rat lacrimal glands that furosemide (1 mM) inhibits inward Cl− current activity, it was added to the extracellular bath solution during whole-cell K+ current recording.

**Results**

The resting cell membrane potential under physiological conditions (KCl-rich pipette solution and NaCl-rich bath solution) was −40 ± 2 mV (n = 17). This was similar to the resting membrane potential of −37 ± 8 mV (n = 50) obtained by Kikkawa using intracellular electrodes. Figure 1A shows the steady state, whole-cell, current-voltage (I-V) relationship from a sample of 12 single rabbit superior lacrimal gland acinar cells before and after the addition of 10 mM tetraethylammonium (TEA) to the extracellular bath solution. This caused a shift of the reversal potential from −39 ± 2 mV to −1 ± 2 mV. The total membrane conductances for inward currents was reduced by 18% and for outward currents by 72%, which suggested that TEA-sensitive currents comprised the majority of the outward currents and was a minor component of the inward currents.

Figure 1B shows the effect of TEA on single-channel current responses to voltage steps over time. Addition of TEA (10 mM) to the pipette solution totally inhibited single channel activity. Similarly, the K+ current showed inhibition by the addition of either Cs+ (5 mM) or Ba2+ (100 μM) to the pipette solution, with single-channel current responses identical with those shown in Figure 1B. In comparison, the K+ current was insensitive to 4-aminopyridine (1 mM and 5 mM) with no inhibition of single channel activity observed.

The steady state, whole-cell, current-voltage relationship of single rabbit superior lacrimal gland acinar cells with Cs+ (5 mM) added to the extracellular bath solution is shown in Figure 2. The K+ current was inhibited by Cs+ (5 mM), and the reversal potential shifted from −37 ± 2 mV to −26.8 ± 0.5 mV (n = 5).

The slope conductance of the whole-cell K+ current increased from 2.7 ± 0.16 nS to 5.2 ± 0.45 nS (n = 6, Fig. 3) after an increase in the free Ca2+ concentration from 1.0 × 10−9 M to 1.0 × 10−6 M.

Figure 4A shows representative cell-attached recordings at various voltages in a single rabbit superior lacrimal gland acinar cell, under physiological conditions. An I-V curve representing the single-channel amplitude as a function of voltage for the cell described in Figure 4A is shown in Figure 4B. K+ currents recorded from the rabbit superior lacrimal gland were shown to have a single-channel conductance of 43 ± 2.7 pS (n = 11). Figure 5B shows the current-voltage relationship of the K+ ion channel in cell-attached patches in rabbit superior lacrimal gland cells in which the K+ concentration was varied among 5, 20, 70, and 140 mM by the substitution of Na+ for K+. Representative cell-attached recordings, at a pipette potential of 50 mV for each of the four pipette K+ concentrations, are shown in Figure 5A. The relationship between single-channel conductance and pipette K+ concentration (Fig. 6) is described by the Michaelis-Menten equation using a Km of 19.2 mM and a maximum single-channel conductance (Vmax) of 46.1 pS. A log-log plot of the single-channel conductance (γ), as a func-
FIGURE 1. (A) Steady state, whole-cell, current-V relations of single rabbit superior lacrimal gland acinar cells before (○, mean of 12 experiments) and after (△, mean of 8 experiments) the addition of 10 mM tetraethylammonium (TEA) to the extracellular bath solution. In each experiment the data were recorded under physiological conditions; that is, the pipette contained a KCl-rich solution (140 mM) and the extracellular bath contained a NaCl-rich solution. The vertical bars represent the SEM when they were larger than the symbols. (B) The effect of TEA (10 mM) on the inwardly rectifying K⁺ channel in cell-attached recordings. Current responses were to voltage steps between −60 mV and 80 mV from a holding potential of 0 mV. Similar results were obtained with Cs⁺ (5 mM) and Ba²⁺ (100 µM). The data were recorded under physiological conditions with the different blocking agents added to the pipette. Control recordings of the inwardly rectifying K⁺ channel activity in cell-attached patches not exposed to TEA are shown in Figure 4A.

The extrapolated $E_{rev}$ values versus log $[K^+]_o$ are described by the equation, $E_{rev} = 35.5 \log [K^+]_o - 77.8$ mV ($r^2 = 0.99, P > 0.05$). The $E_{rev}$ was determined by extrapolating the linear portion of the single-channel I-V curves shown in Figure 5B.

Ion substitution experiments were performed in which the pipette K⁺ was replaced with the cations Rb⁺ and Li⁺. The channel was highly selective for potassium ions, with the permeability sequence of $K^+ (1.0) > Rb^+ (0.83) > Li^+ (0.15)$. The numbers in parentheses indicate the numerator in the fraction $P_X/P_K$, where X is the test ion.

**DISCUSSION**

Using cell-attached, patch-clamp techniques, this study demonstrated the existence of inwardly rectifying K⁺ currents in...
rabbit superior lacrimal gland acinar cells. The \( K_m \) current we identified in these rabbit lacrimal acini satisfies the criteria for identifying an inward rectifier: The channels open with steep voltage dependence on hyperpolarization and the conductance is approximately dependent on the square root of the external \( K^+ \) concentration. A similar current has not been reported in the rat lacrimal gland, although an inwardly rectifying, nonspecific cation channel with a single-channel con-

![Graph showing current-voltage relationships](image-url)

**FIGURE 3.** Steady state, whole-cell, current-voltage relationships of single rabbit superior lacrimal gland cells in the voltage range between \(-160 \, \text{mV} \) and \(-20 \, \text{mV} \) with differing free \( \text{Ca}^{2+} \) concentrations in the pipette solution: \( 10^{-9} \, \text{M} \) (●) and \( 10^{-6} \, \text{M} \) (○). The *vertical bars* represent the SEM when they were larger than the symbols.

![Single-channel activity and I-V relationship](image-url)

**FIGURE 4.** (A) Inwardly rectifying \( K^+ \) single channels recorded from a cell-attached patch from a rabbit superior lacrimal gland acinar cell. Single-channel activity was recorded using the standard pipette and bath solutions described in Materials and Methods. Representative channel activity is shown at the pipette potentials of \(-60, -50, -40, -30, 0, 30, \) and \( 70 \, \text{mV} \). For each trace, the *solid horizontal arrow* indicates the zero current level at which there are no channels open. (B) Single-channel \( I-V \) relationship for the cell shown in A. The single-channel amplitude is plotted against the negative of the pipette potential \((-V_p)\).
ductance of 25–30 pS,20,30 which is equally selective for Na⁺, K⁺, and Cs⁺, has been recorded. We have not observed this type of nonspecific cation current in rabbit lacrimal acinar cells. This interspecies variation suggests that the mechanism of secretion differs between the rat and the rabbit and suggests further that a model of secretion based on other species is not totally applicable to the human model.

The Kir current that we report contributes the minor component of the total inward current (18%) in rabbit lacrimal acinar cells. It appears that the main physiological role for this current in rabbit lacrimal acinar cells is to stabilize the resting membrane potential. The evidence for this centers on changes observed in the resting membrane potential when cells were exposed to TEA and Cs⁺. The resting membrane potential in the rabbit lacrimal gland under physiological conditions was -40 ± 2 mV (n = 17), which was similar to the resting membrane potential of -37 ± 8 mV (n = 50) obtained by Kikkawa28 using intracellular electrodes. The addition of TEA to the bath solution shifted the reversal potential from -40 mV to -1 mV, and, similarly, extracellular application of Cs⁺ caused the membrane potential to shift from -37.4 ± 1 mV to -26.8 ± 0.5 mV (n = 3).

Single-channel recording methods revealed a 43-pS channel that displayed inward rectification, which is within the range of conductance (22–47 pS) reported for similar channels in other tissues. The Kir current we report here is Ca²⁺ activated, whereas the Kir in sheep parotid19 and rat hepatocytes14 is Ca²⁺ insensitive. However, there is a conflicting report31 that demonstrates the presence of Ca²⁺-activated inwardly rectifying K⁺ channels in rat hepatocytes. The Kir current is inhibited by TEA, whereas in the sheep parotid19 and mouse lens epithelial cells15 the inwardly rectifying K⁺ current was insensitive to TEA. However, in the mouse lens epithelial cells, TEA (10 mM) reduced the inward current at -160 mV to 64 ± 5% (n = 4) of its control value. In this study TEA (10 mM) added to the extracellular bath caused the reversal potential to shift from -40 mV to -1 mV. The Kir current is insensitive to 4-aminopyridine (5 mM), which is similar to the reports of the inwardly rectifying K⁺ current in mouse lens epithelial cells.15 The divalent cation Ba²⁺ is a characteristic blocker of inwardly rectifying K⁺ current in a variety of tissues, including sheep parotid,19 mouse lens epithelial cells,15 murine macrophage cell line J774.1,32 starfish egg cells,33 frog skeletal muscle,4,34 calf Purkinje cells,35 and rat osteoclasts.17 In this study, Ba²⁺ totally inhibited Kir single-channel activity in rabbit superior lacrimal gland cells. The Kir current is also sensitive to Cs⁺, and this sensitivity is similar to the current found in other species.7,15,17,19

**Figure 5.** (A) Representative cell-attached recordings of inwardly rectifying K⁺ (Kir) at a pipette potential of -50 mV for different pipette (extracellular) K⁺ concentrations. For each trace, the solid horizontal arrow indicates the zero current level at which there are no channels open. (B) Single-channel I-V relationship of the Kir channel in cell-attached patches for pipette (extracellular) K⁺ concentrations of 140 mM (●, n = 11), 70 mM (○, n = 8), 20 mM (●, n = 5), and 5 mM (□, n = 5). The vertical bars represent the SEM when they were larger than the symbols. The single-channel amplitude is plotted against the transmembrane potential (Vm) calculated by subtracting the pipette potential (Vp) from the average membrane potential (-40 mV). The single-channel amplitude is plotted against the transmembrane potential (Vm) calculated by subtracting the pipette potential (Vp) from the average membrane potential (-40 mV) reported in Results.

**Figure 6.** Plot of conductance as a function of pipette (extracellular) K⁺ concentration in cell-attached patches. The curve is a nonlinear least-squares fit of the Michaelis–Menten equation giving values of KM = 19.2 mM and VM_max = 46.1 pS (r² = 0.95).
The single-channel conductance of the $K_{in}$ channels we report here has dependence on $[K_\text{o}]$, similar to that of other inward rectifiers observed in exocrine glands\textsuperscript{19} and other tissues.\textsuperscript{14,15,32} However, there is some interspecies variation in the degree to which conductance varies by a particular power dependency on $[K_\text{o}]$. For example, $[K_\text{o}]^{0.5}$ in guinea pig ventricular myocytes,\textsuperscript{7} $[K_\text{o}]^{0.55}$ in sheep parotid cells,\textsuperscript{19} $[K_\text{o}]^{0.56}$ in the murine macrophage cell line J774.1,\textsuperscript{32} and $[K_\text{o}]^{0.37}$ in the rabbit superior lacrimal gland from this study. These small differences in the degree with which conductance varies by a particular power dependency on $[K_\text{o}]$ may not be closely related to the functional role of the channel. For example, in murine macrophages\textsuperscript{32} and the rabbit lacrimal gland, the $K_{in}$ channel is responsible for stabilizing the cell membrane potential, whereas in the sheep parotid,\textsuperscript{19} the inwardly rectifying $K^+$ channel is not involved in stabilizing the cell membrane potential and its role requires further investigation.

Many physiological processes are dependent on temperature. For example, the dynamic process of regulatory volume increase in rat lacrimal gland acini\textsuperscript{46} does not occur in HEPES-buffered solutions at 20°C but is present in these solutions at 37°C. This observation suggests that the active NaK2Cl cotransporter is inactive at the lower temperature. Although we characterized $K_{in}$ at room temperature, the probable role of $K_{in}$ is in setting and stabilizing the membrane potential. Such a role depends on ion diffusion processes rather than active transport mechanisms and is less likely to be greatly affected by changes in temperature. Indeed, reports of the single-channel current of ClC-0 chloride channels show only a weak dependence on temperature.\textsuperscript{37} Similarly, the conductance of inwardly rectifying $K^+$ whole-cell currents in guinea pig ventricular myocytes\textsuperscript{38} is weak ($Q_{10}$ of 1.28), and the conductance of adenosine triphosphate-dependent $K^+$ channels in rat ventricular myocytes\textsuperscript{39} is relatively weak ($Q_{10}$ of 1.4-1.6).

Previous authors have reported functional roles of the inwardly rectifying $K^+$ channels apart from setting and stabilizing the membrane potential. Inwardly rectifying $K^+$ channels may buffer transient increases in extracellular $K^+$ as has been suggested in glial cells.\textsuperscript{20,22} It has also been shown in salivary glands that, when stimulated, cells lose $K^+$ to their surroundings.\textsuperscript{50,41} However, the parotid is the only salivary gland that has an inward rectifier,\textsuperscript{19,42} and it has been suggested by Ishikawa et al.\textsuperscript{19} that, in the sheep parotid, the NaK2Cl cotransporter plays no part in secretion as opposed to other salivary glands in which $K^+$ uptake occurs through the cotransporter,\textsuperscript{43-45} and, hence, $K^+$ uptake occurs through the inward $K^+$ rectifier. It has been suggested\textsuperscript{19} that the inward rectifier in the sheep parotid is involved with spontaneous secretion by the salivary glands. Current models of secretion are based on the efflux of Cl$^-\textsuperscript{-}$ across the luminal membrane being balanced by the efflux of $K^+$ through channels in the basolateral membrane. It has been shown that some inward rectifiers do allow some outward current flow,\textsuperscript{7,21} and this may be sufficient to drive Cl$^-\textsuperscript{-}$ efflux across the luminal membrane leading to spontaneous secretion. In other cells, it has been suggested that the inward $K^+$ rectifier is involved in removing $K^+$ from the cell to prevent its accumulation in the cell in response to Na$^+$-dependent transport processes such as the Na$^+$-H$^+$ exchange.\textsuperscript{25} As H$^+$ is extruded by the cell, Na$^+$ ions accumulate within the cell. This activates the Na$^+$-K$^+$ pump, which extrudes Na$^+$ ions and leaves an excess of $K^+$ ions in the cell. These $K^+$ ions may then leave the cell through the inward rectifier, which can serve as a vehicle for the outward movement of $K^+$ ions from the cell as mentioned above.

In conclusion we have identified a Ca$^{2+}$-activated inwardly rectifying $K^+$ channel in the rabbit superior lacrimal gland with a single conductance of 43 pS, which is dependent on the extracellular $K^+$ concentration. The current is blocked by TEA, Ba$^{2+}$, and Cs$^+$ but is insensitive to 4-aminopyridine. From our data, the primary role of this inward rectifier seems to set and stabilize the resting membrane potential.

References


