Identification of a Kv3.4 Channel in Corneal Epithelial Cells

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PURPOSE. Voltage-gated K+ channels maintain salt and water balance and normal function of corneal epithelial cells. To determine their identity, Kv channel types were sought in cultured rabbit corneal epithelial cells and in the intact rat corneal epithelium.

METHODS. Immunohistochemistry and Western blot analysis were performed to detect K+ channels in the membrane and cell lysates of rat and SV-40–transformed rabbit corneal epithelial (RCE) cells, using specific antibodies. The whole-cell patch clamp was used to characterize the biophysical and pharmacologic properties of the K+ current in RCE cells.

RESULTS. Expressions of K+ channel types in corneal epithelial cells were detected by using a panel of specific anti-K+ channel antibodies. Western blot analysis, using specific anti-K+ channel antibodies including anti-Kv1.1, -2.1, -2.2, -2.3, -2.4, -4.2, and -4.3, demonstrated that in corneal epithelial cells, Kv3.4 channel was highly expressed in whole-cell lysates and in cell membrane preparations. The anti-Kv3.4 channel antibody produced intense immunoreactivity in both RCE cells and rat corneal epithelium. Fluorescence immunostaining and avidin-biotin-peroxidase complex immunostaining confirmed localization of Kv3.4 channels in the cell membrane of both RCE and rat corneal epithelial cells. Voltage depolarization-activated K+ currents in RCE cells were inhibited by applications of either 4-aminopyridine (4-AP, at micromolar levels), α-dendrotoxin at nanomolar levels, or blood-depressing substance-I at nanomolar levels.

CONCLUSIONS. Biochemical and pharmacological profiles of the voltage-gated, 4-AP-sensitive K+ channel in rat and RCE cells resemble characteristics of a Kv3.4 channel, a member of the Shaw subfamily. This channel may play important roles in maintaining normal function of corneal epithelium. (Invest Ophthalmol Vis Sci. 2004;45:1796–1803) DOI:10.1167/iovs.03-1056

The corneal epithelium functions as a physical barrier to prevent noxious agents from infecting this tissue and the underlying stroma. In addition, it serves to protect inner ocular tissues from environmental insults. These protective functions rely on the ability of the corneal epithelium to undergo continuous renewal to replace terminally differentiated layers, which are being continuously sloughed off into the tears. The cornea is covered by a transparent tear film surface that forms a refractive layer, assuring visual acuity. Net ion transport activity across the corneal epithelium makes an important contribution to the maintenance of the osmotic gradients needed for eliciting sufficient fluid flows that adequately hydrate the tear layer.1,2 This secretory process can be sustained, provided there is adequate expression and modulation of ion channel and transporter activity underlying net ion transport from the stroma toward the tears. Furthermore, the mitogenic response of corneal epithelial cells to growth factors requires maintenance of intracellular homeostasis and cell volume regulated by ion channels and transporter activity. This dependence is evident, because both the mitogenic response to growth factors and the ability of the corneal epithelium to elicit volume regulatory responses control are attenuated by blockade of ion channels and transporters.3–5 This association is also apparent in other tissues, because pharmacologic blockade of ion channels and transporters disrupts cell volume control and leads to inhibition of cell proliferation.6–9 It has been shown that cell volume modulation is an important mediator of cell cycle control in several tissues.10–13 This dependence may stem from the realization that metabolic, mitotic, and migratory activities are all elevated during proliferation compared with cells arrested in the G0/G1 stage of the cell cycle. Elevated synthetic activity increases the amounts of critical factors that are needed for orderly progression through the G1 phase of the cell cycle. Migration is also dependent on cell swelling because it is needed to permit extension of the cytoskeleton and cell elongation.14 These considerations point to the importance of obtaining a better understanding of how changes in ion channel and transporter expression and activity modulate cell cycle progression.15

K+ channels are important mediators of cell function. They play crucial roles in cell volume regulation, membrane potential maintenance, fluid secretion, and cell growth control.16 In rabbit and human corneal epithelial cells, there are voltage-gated K+ channels in the apical membrane of the superficial layers, and a large-conductance K+ channel was also found in rabbit and human basal cell layers.17–19 These channels are stimulated by fenamate, cGMP, carbachol, cell swelling, membrane stretching, CO2, and acidification18,20–25 and are inhibited by barium, quinidine, diphenyl-2-aminoethanol, and fluoxetine (Prozac; Eli Lilly, Indianapolis, IN).18,26 The whole-cell currents were further characterized in cultured human corneal epithelial cells.27 They contain a depolarization-activated, outward-rectifying K+ current; a hyperpolarization-activated, outward-rectifying K+ current; and an inward-rectifying K+ current.27 Recently, mRNA of the inward-rectifying Kv (Kir2.1) channel has been reported in this tissue.28 The bovine corneal epithelium exhibits its two types of outward K+ current: an inactivating voltage-gated K+ current that is inhibited by arachidonic acid and a noisy, sustained K+ current.29 Our previous results indicated that there are voltage-gated and 4-AP–sensitive K+ channels in rabbit corneal epithelial (RCE) cells, which are regulated by EGF16 and play a role in mediating cell death induced by UV irradiation.30–32 However, the specific subfamily members

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contribute to the K⁺ currents have yet to be evaluated in corneal epithelial cells.

Kv3.4 channels belong to the mammalian shaker-related K⁺ channels and are a member of the Shab subfamily. These channels exhibit A-type fast inactivation, as do the Kv1.4, -1.1, -4.1, and -4.3 channels. Kv3.4 expression has been found in the hippocampus, cerebellum, brain stem, spinal cord, skeletal muscle, arterial smooth muscle cells, and pancreatic acinar cells. It performs an important role in modulating electrical excitability of neurons and muscle fibers. Yet, there has been, no report that Kv3.4 channels are expressed in corneal epithelium. The purpose of the present study was to identify what type of K⁺ channels is expressed in corneal epithelial cells. We used immunohistochemistry, Western blot analysis, and -4.3 (Alomone Labs, Ltd., Jerusalem, Israel). Sections were washed antibody 1:100 to 1:200 dilution in PBS-T. The primary antibodies that feature (RT), and then incubated overnight at 4°C for 1 hour with 10% normal horse serum (NHS) in PBS-T at room temperature. phosphate-buffered saline (PBS) and 0.1% Triton X-100 (PBS-T), blocked for 30 minutes with phosphate-buffered saline (PBS) or for 10 minutes with optimal cutting temperature (OCT) compound. Sections (10 μm) were cut with a cryostat and mounted on gelatin-coated slides. RCE cells (70%–80% confluence) were grown on microscope slides, washed twice with PBS, and fixed for 10 minutes with 4% paraformaldehyde in 0.1 M PB or for 10 minutes with –20°C methanol. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, using protocols approved and monitored by the Animal Care Committee of Wright State University School of Medicine.

Immunoblot Analysis

The samples were centrifuged at 12,000g for 5 minutes at 4°C and 25 μL supernatant was loaded per lane. After fractionation on a 6% SDS-polyacrylamide gel, proteins were electrotransferred to a PVDF membrane. The membrane was blocked in PBS-0.05% Tween 20 (PBS-T) containing 5% nonfat milk for 1 hours at RT and then, incubated with the respective antibody overnight at 4°C. After three washes with PBS-T buffer, the membrane was incubated with alkaline phosphatase (AP)-linked secondary antibody for 1 hour at RT. The proteins were detected with a Western blot detection kit (Phototope-Star; Cell Signaling Technology, Beverly, MA).

Electrophysiology

The nystatin perforated patch-clamp technique was used for whole-cell K⁺ current recording. Pipettes with a resistance of 3 to 4 MΩ when filled with 150 mM KCl solution were manufactured with a two-stage pipette puller (PP-83; Narishige, Tokyo, Japan) and fire-polished before use. The pipette tip was filled with a solution containing (in mM) 140 KCl, 2 MgCl₂, 0.5 CaCl₂, 2 adenosine triphosphate (ATP), 0.05 guanosine triphosphate (GTP), 1 EGTA, and 10 HEPES (titrated with KOH to pH 7.2). The remainder of the pipette was back-filled with the same pipette solution supplemented with 200 μg/mL nystatin. The bath solution composition was (in mM) 140 NaCl, 2 KCl, 1 CaCl₂, and 10 HEPES (pH 7.4). The whole-cell current recording was performed by using a patch-clamp amplifier (Axopatch 200A; Axon Instruments, Inc., Union City, CA), and data were collected and analyzed by computer (pCLAMP software; Axon Instruments, Inc.). All experiments were performed at RT (21–23°C). K⁺ channel blockers were used in the study including 4-aminopyridine (4-AP), α-dendrotoxin (DTX; Sigma-Aldrich), and blood-depressing substance-I (BDS4; Alomone Laboratories).

Statistical Analysis

Data obtained from patch-clamp studies are shown as original values or as the mean ± SE, where indicated. Significant differences between all values were determined by paired Student’s t-test at the confidence interval P < 0.05 or as indicated in the legends.

Results

Distribution of the Kv3.4 Channel in Rat Corneal Epithelium

In the previous study, we reported a voltage-gated and 4-AP-sensitive K⁺ channels are present in RCE cells. To reveal the

Material and Methods

Culture of Rabbit Corneal Epithelial Cells

A rabbit corneal epithelial (RCE) cell line was used in the study. RCE cells were grown in DMEM/F-12 (1:1) culture medium containing 10% fetal bovine serum, 5 μg/mL insulin, 10,000 U/mL penicillin, and 10,000 mg/mL streptomycin and maintained in an incubator supplemented with 95% air and 5% CO₂ at 37°C. The medium was replaced every 2 days, and the cells were passaged with 0.05% trypsin-EDTA digestion.

Tissue and Cell Preparation

Corneas were rapidly excised from adult Wistar rats (140–180 g) that were killed in an adjoining neurophysiological laboratory, fixed for 1 hour in 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4), dehydrated with 30% (wt/vol) sucrose in 0.1 M PB, and frozen with optimal cutting temperature (OCT) compound. Sections (10 μm) were cut with a cryostat and mounted on gelatin-coated slides. RCE cells (70%–80% confluence) were grown on microscope slides, washed twice with PBS, and fixed for 10 minutes with 4% paraformaldehyde in 0.1 M PB or for 10 minutes with –20°C methanol. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, using protocols approved and monitored by the Animal Care Committee of Wright State University School of Medicine.

Immunohistochemistry

The sections were membrane permeabilized for 30 minutes with phosphate-buffered saline (PBS) and 0.1% Triton X-100 (PBS-T), blocked for 1 hour with 10% normal horse serum (NHS) in PBS-T at room temperature (RT), and then incubated overnight at 4°C with the primary antibody 1:100 to 1:200 dilution in PBS-T. The primary antibodies that were used in the study included anti-Kv1.1, -2.1, -3.1b, -3.2, -3.4, -4.2, and -4.3 (Alomone Labs, Ltd., Jerusalem, Israel). Sections were washed three times in PBS and then incubated with fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG antibody (1:100; Jackson Immunoresearch Laboratories, West Grove, PA) for 2 hours at RT. Sections were washed and mounted in antifade medium ( Vectashield; Vector Laboratories, Burlingame, CA). Analysis was performed with an epifluorescence or a confocal microscope (×60 immersion objective lens, n/p, cerebellum, brain stem, spinal cord, skeletal muscle, arterial smooth muscle cells, and pancreatic acinar cells). For avidin-biotin complex (ABC) staining, after primary antibody incubation, sections were incubated with biotinylated goat anti-rabbit IgG antibody (1:200; Vector Laboratories) for 2 hours at RT, and incubated with avidin-biotin-peroxidase complex (ABC kit; Vector Laboratories) in PBS for 2 hours at RT. After an additional wash with PBS, sections were incubated with 0.02% diaminobenzidine (DAB) and 0.01% H₂O₂ diluted in 0.05 M Tris buffer (pH 7.4) for 5 to 15 minutes. Finally, sections were dehydrated and mounted in dextropropoxyphene (DPX; BDH Laboratory Supplies, Poole, UK).

Membrane Solubilization and Whole-Cell Lysate Preparation

Membrane fractionation of RCE cells was performed as described. Briefly, confluent cell cultures were washed twice with cold PBS and scraped with a policeman onto ice in PBS containing 1 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation at 1000 rpm for 5 minutes at 4°C, cell pellets were homogenized in TE buffer (50 mM Tris [pH 7.4] and 1 mM EDTA) plus 1 mM PMSF, 10 μg/mL aprotinin, and 10 μg/mL leupeptin and centrifuged at 21,000g for 30 minutes at 4°C. The pellets (crude membrane fraction) were suspended in SDS sample buffer (Laemmli buffer), sonicated, and boiled at 100°C for 5 minutes. The samples were either used for direct immunoblot analysis or stored at –80°C for future use. For preparation of the whole-cell lysate, confluent RCE cells in 60-mm culture dishes were washed twice with cold PBS, scraped onto ice in 0.5 mL SDS buffer, sonicated on ice for 2 seconds, and then boiled at 100°C for 5 minutes.
Confocal Microscopic Analysis of Kv3.4 Immunoreactivity in RCE Cells and Rat Cornea Epithelium

Confocal microscopy was used to characterize the cellular location of Kv3.4 in RCE cells (Fig. 3A) and the rat cornea epithelium (Fig. 3B). Confocal images of fluorescence-labeled Kv3.4 channel appeared as punctate lines representing immunocomplex clusters in the cell membrane. No intracellular labeling was found in either RCE cells or the rat corneal epithelium. Confocal images confirm that Kv3.4 channels are mainly located in the cell membrane of corneal epithelial cells.

Blockage of Kv Current in RCE Cells by α-DTX

The nystatin-perforated whole-cell patch clamp technique was performed to record the whole-cell currents in RCE cells. The whole-cell currents were elicited by a series of voltage steps from a holding potential of -60 mV to +60 mV in +20 mV increments. The whole-cell K+ currents were voltage dependent with rapid activation and gradual inactivation during the period of stimulation. α-DTX from the venom of the Eastern green mamba snake selectively inhibits K+ currents.45 To determine whether the Kv channel in RCE cell is sensitive to α-DTX, we applied α-DTX to the bath solution. Figure 4 shows that α-DTX inhibited Kv currents in a dose-dependent manner. The half-maximum inhibitory effect (IC50 = 25 nM) was achieved within 5 minutes. The Kv current sensitivity to inhibition by α-DTX indicates that the K+ channels in RCE cells may belong to a particular member of the Kv channel subfamily.

Blockage of Kv Current in RCE Cells by BDS-I

In patch-clamp studies, we used α-DTX to inhibit the Kv channel in RCE cells. To determine further whether the Kv3.4 channel is indeed a functional channel in RCE cells, we measured the effect of BDS-I, a specific blocker of Kv3.4 channels on whole-cell K+ current in the cells. BDS-I is a 43-amino-acid peptide from sea anemone (Anemonia sulcata) and has high specificity to Kv3.4 channels. It is reported that BDS-I revers-
ibly inhibits Kv3.4 channels (IC$_{50}$ = 100–200 nM) and fails to block other voltage-gated K$^+$ channels in the Kv1, -2, -3, and -4 subfamilies. By adding BDS-I to the bath solution, we found that 400 nM BDS-I dramatically inhibited the Kv current (Fig. 5A). The whole-cell K$^+$ current was suppressed more than 70% by BDS-I compared with the control, at a membrane potential of +40 mV (Fig. 5B). The time course shows that BDS-I (400 nM) suppressed by 50% the peak amplitude of the K$^+$ current within 5 minutes (Fig. 5C). The inhibitory effect of BDS-I on the K$^+$ current in RCE cells suggests that Kv3.4 channels are present in these cells.

**Effect of 4-AP on Kv Currents**

One of the hallmarks of K$^+$ channels displaying A-type current behavior is their sensitivity to 4-AP. Our previous studies show that voltage-gated K$^+$ channels in RCE cells are very sensitive to 4-AP. The data from cell-attached patch clamp techniques show that 4-AP blocked the Kv channel activity in a dose-dependent manner. As described, 4-AP (100 µM) effectively inhibited the Kv currents (Fig. 6A). To confirm that both 4-AP and α-DTX inhibit the same Kv current, α-DTX (200 nM) was added to the bath solution to inhibit the Kv current followed by washing it out with fresh bath solution. After recovery of the Kv current, 4-AP (100 µM) was perfused into the patch chamber. 4-AP also suppressed this current to an extent similar to that observed during exposure to α-DTX (Fig. 6B). This agreement between their effects suggests that both α-DTX and 4-AP affect the same type of Kv3.4 channels.

**Expression of Kv3.4 Channel Protein in Corneal Epithelial Cells**

To determine whether Kv channel proteins are expressed in corneal epithelial cells, we performed Western blot analysis on whole-cell lysates and membrane fractions derived from RCE and rat cornea. Kv2.1 and -3.4 with a molecular mass of ~116

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**Figure 2.** Expression of Kv3.4 channel in RCE cells. (A) Immunostaining of RCE cells with anti-Kv3.4 channel antibody or (B) with PBS in the absence of anti-Kv3.4 antibody. (C) Detection of Kv3.4 channel expression in detached RCE cells with anti-Kv3.4 antibody. (D) Detection of Kv3.4 channel expression in RCE cells with ABC immunostaining with anti-Kv3.4 channel antibody. RCE cells grown on glass slides were fixed with 4% paraformaldehyde in 0.1 M PBS. Micrographs were taken with a fluorescence microscope with a 40× objective lens.

**Figure 3.** Cellular distribution of Kv3.4 channels in RCE cells and rat corneal epithelium. Kv3.4 channel immunoreactivity was localized by confocal images in the membrane of cultured RCE cells (A) and rat corneal epithelium (B).
kDa were detected in whole-cell lysates of RCE cells (Fig. 7A), whereas no specific bands were detected with any of the other types of Kv channel antibodies. Further experiments confirmed that this subtype is expressed only in whole-cell lysates, in membrane fractions of both RCE cells (Fig. 7B), and in the rat cornea (Fig. 7C). In addition, preincubation of the Kv3.4 antibody specificity was validated by showing that preincubation with the antigenic peptide blocked the immunodetection of this protein (data not shown). The results suggest that both the Kv3.4 and probably Kv2.1 channel subtypes are expressed in RCE cells and in rat corneal epithelium.

**DISCUSSION**

We determined the K⁺ channel subtype expressed in corneal epithelial cells because previous studies have shown that changes in K⁺ channel activity modulate essential corneal epithelial functions needed for tissue homeostasis.¹,² In these studies, it was shown that such changes are essential for me-

**FIGURE 4.** Effect of α-DTX on K⁺ current in RCE cells. (A) Inhibition of whole-cell K⁺ currents by α-DTX. Different concentrations of α-DTX were applied to the patch chamber, and K⁺ currents were activated by a depolarization pulse protocol (top). (B) Current-voltage (I-V) relationship obtained in the presence and absence of α-DTX. (C) Time course of K⁺ current blocked by various concentrations of α-DTX. K⁺ currents were normalized as a fraction of I_DTX/I C, where I_DTX and I_C represent the peak K⁺ current measured before and after addition of α-DTX, respectively. A voltage pulse protocol was generated from the resting potential of −60 to +60 mV for 2 seconds in 20-mV increments, to depolarize the membrane potential. (B, C) Data are expressed as the mean ± SE of results in four independent experiments (P < 0.05).

**FIGURE 5.** Effect of BDS-I on K⁺ current in RCE cells. (A) Inhibition of whole-cell currents by 400 nM BDS-I. (B) I-V relationship measured before and after addition of 400 nM BDS-I. (C) Time-course of K⁺ current blocked by 400 nM BDS-I. Whole-cell currents were recorded under the conditions described Figure 4. K⁺ currents were activated by depolarization of the membrane potential from a holding potential of −60 mV to +60 mV for 2 seconds in 20-mV increments. Data are presented as in Figure 4.
diately changes in net ion transport, cell cycle progression, and apoptosis resulting from exposure to cAMP-mobilizing agonists, growth factors, and ultraviolet light, respectively. Each of these responses is dependent on K⁺ channel activation. Therefore, corneal epithelial renewal, which is a result of a balance between cell proliferation, apoptosis, and differentiation, is dependent on K⁺ channel activation by stimuli that affect each of these responses. Given the importance of K⁺ channel modulation in the control of epithelial renewal, our identification of Kv3.4 expression provides a drug target for more specific modulation of each of these responses. The development of novel, specific, Kv3.4 modulators could affect more selective stimulation of the epithelial renewal process in a clinical setting.

Our approach to characterizing the specific Kv channel subfamily expressed in corneal epithelial cells included first evaluating their distribution with immunohistochemistry. After screening with a panel of selective Kv antibodies, we detected plasma-membrane–delimited, specific Kv3.4 expression. Our finding that preadsorption of the anti Kv3.4 antibody with the antigenic peptide blocked the immunocytochemical response validated the specificity of this staining reaction. Furthermore, omission of the primary antibody also resulted in no staining. Cell membrane localization was confirmed with confocal microscopy because punctate labeling was found at the cell membrane. It is noteworthy that such labeling was more intense in the basal proliferating layer, which is consistent with the known importance of K⁺ channels in mediating mitogenic responses by this cell layer to serum-containing growth factors. Another line of evidence supporting Kv3.4 expression in corneal epithelial cells is that Western blot analysis revealed its presence in a plasma-membrane–enriched fraction. On the contrary, Kv5.1, -4.2, and -4.3 channel proteins were not detected with this method. Our finding that there was no detectable Kv3.4 expression in the cytoplasm supports its functional importance in mediating the membrane voltage changes required to elicit these responses. Such changes may be necessary to elicit activation of a host of different signaling pathways that are needed for the control of cell cycle progression, apoptosis, and modulation of net ion transport.

Patch-clamp experiments demonstrated that the whole-cell K⁺ current in RCE cells is voltage dependent, with fast activation and gradual inactivation. The pharmacologic profile of the whole-cell K⁺ current in RCE cells is similar to that described for the Kv3.4 channel. Both 4-AP and α-DTX inhibited whole-cell K⁺ currents in RCE cells in a dose-dependent manner, indicating that the Kv channel in RCE cells is Kv3.4. Additional evidence from BDS-I experiments further support this notion because BDS-I, a highly specific blocker of Kv3.4 channel, does not block other types of voltage-gated K⁺ channels, such as Kv1.4, -2.1, -4.1, -4.2, and -4.3. Additional evidence from BDS-I experiments further support this notion because BDS-I, a highly specific blocker of Kv3.4 channel, does not block other types of voltage-gated K⁺ channels, such as Kv1.4, -2.1, -4.1, -4.2, and -4.3. BDS-I significantly reduced K⁺ currents in RCE cells confirming that the Kv channel in corneal epithelial cells indeed belongs to the Kv3.4 subfamily.

Electrophysiologic characterization of the whole-cell K⁺ currents was performed with the whole-cell patch clamp. The activation pattern of the whole-cell K⁺ current in RCE cells is similar to that described for Kv3.4 channels in the brain and in the skeletal muscle, in which the activation phase is fast. However, the inactivation phase of the whole-cell K⁺ current in RCE cell is much slower than the Kv3.4 channels found in

**Figure 6.** Effect of 4-AP on K⁺ currents in RCE cells. (A) Inhibition of whole-cell K⁺ currents by application of 100 μM 4-AP. (B) Time-course of K⁺ current reversibly blocked by α-DTX (10 μM) and then blocked by 4-AP. Whole-cell currents were activated under the conditions described Figure 4. K⁺ currents were activated by depolarization of the membrane potential from a holding potential of −60 mV to +60 mV for 2 seconds in 20-mV increments.

**Figure 7.** Detection of Kv channel protein expressions in RCE cells and rat cornea. (A) Expression of Kv channel proteins in RCE cells detected by Western blot analysis with a panel of various antibodies. (B) Expression of Kv3.4 channel protein in RCE cells by Western blot analysis with anti-Kv3.4 antibody. (C) Expression of Kv3.4 channel protein in the rat cornea by Western blot analysis with anti-Kv3.4 antibody. Whole-cell lysates, nuclear extracts, and membrane fractions of cultured RCE cells and the rat cornea were fractionated by PAGE and analyzed by Western blot with polyclonal rabbit anti-Kv channel antibodies.
excitable tissues. A recent report shows that there is a voltage-gated K⁺ channel in bovine corneal epithelial cells. This K⁺ current has a pattern similar to that of the Kv 5.1 current in RCE cells. The possible explanation for the difference of Kvs channels in these tissues serve different functions. In summary, our present study indicates that a Kv 5.4 channel is functionally expressed in the cell membrane of corneal epithelial cells and in rat corneal epithelium. This channel subfamily member may provide essential contributions to mechanisms underlying important corneal functions.

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