Swelling-activated potassium channel in porcine pigmented ciliary epithelial cells.

Swelling-Activated Potassium Channel in Porcine Pigmented Ciliary Epithelial Cells

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ABSTRACT

Purpose: Ion channels in the ciliary epithelium play critical roles in the formation of aqueous humor in the eye. The present study identified a novel, swelling-activated K^+ current in freshly dissociated porcine pigmented ciliary epithelial cells.

Methods: Ciliary epithelial cells were freshly dissociated from porcine eyes. Whole-cell currents were recorded using the patch-clamp technique in pigmented and non-pigmented ciliary epithelial cell (PCE-NPCE) pairs or single PCE cells.

Results: The zero-current potential was -49 ± 13 mV in PCE-NPCE cell pairs (n = 97), and -52 ± 12 mV in single PCE cells (n = 30). Whole-cell currents in these cells were dominated by an outwardly rectifying K^+ current activated by potentials more positive than -90 mV, which never inactivated during prolonged depolarization. The K^+ current was significantly augmented by hypotonic cell perfusion. External Ba^{2+} was a blocker of this K^+ conductance (IC_{50} of 0.38 mM), but the conductance was insensitive to external TEA^+. Linopirdine, a specific inhibitor of KCNQ channels, effectively blocked the K^+ current in these PCE cells. **Conclusions:** Porcine PCE cells express a swelling-activated K^+ channel, which may be a member of the KCNQ/Kv7 channel family. This K^+ channel is active near resting potentials and could contribute to the regulation of cell volume and water transport via the ciliary epithelia.

INTRODUCTION

The ciliary body epithelium is a transporting epithelium, composed of two epithelial layers, a non-pigmented ciliary epithelial (NPCE) cell layer facing the vitreal space, and a pigmented ciliary epithelial (PCE) cell layer facing the ciliary stroma. By transporting ions and water between the ciliary body and vitreal space, the ciliary epithelium plays critical roles in the formation of aqueous humor in the eye.

Studies on ion transport via the ciliary epithelium have mainly focused on components underlying chloride secretion, which limit aqueous humor production.[1, 2] In particular, Cl^- channels in NPCE cells mediate the final step of Cl^- release and thereby regulate the rate of aqueous humor secretion. The molecular identity of NPCE Cl^- channels has not been precisely elucidated, but several lines of evidence indicate that the ClC-3 member of the ClC family of channels and transporters likely plays a major role in Cl^- inflow.[3,4] In addition to anion channels, epithelial potassium channels also contribute to critical cellular functions including the secretion of ions and water across the epithelia. The rate of anion secretion depends on the activity of K^+ channels since cell hyperpolarization caused by the opening of K^+ channels increases the driving force for anion efflux.[5] Several previous studies have described some K^+ channels expressed in the ciliary epithelium, including voltage-gated delayed rectifiers,[6-8] Ca^{2+}-activated outward K^+ channels[7,9,10] and an inward rectifier activated by hyperpolarization.[6,11] It is unknown whether these K^+ currents in the ciliary epithelium are sensitive to cell swelling.

In the retinal pigment epithelium (RPE), another ocular secretory epithelium, K^+ conductance is mainly composed of two types of K^+ channels, an inward rectifier Kir7.1[12,13] and a M-type K^+ channel.[14,15] The latter was termed from its resemblance to

the M-current, a K^+ current distributed in neurons and other excitable cells. This M-type K^+ current in RPE is a voltage-gated K^+ channel with an activation threshold near the equilibrium potential for K^+, and thus, contributes to the resting potential of the cell. One of the characteristic properties of the M-type K^+ current in the RPE is its insensitivity to tetraethylammonium (TEA), a classical K^+ channel blocker. [14,15]

In this study, we identified a swelling-activated, non-inactivating outward K^+ current in freshly dissociated ciliary pigmented epithelial cells. The K^+ current was essentially insensitive to TEA and resembled the M-type K^+ current of RPE in terms of voltage-dependence, kinetics and blocker sensitivities.

METHODS

Solutions

External and internal solutions were buffered with HEPES as in previous patch-clamp studies of corneal epithelium,[16] and retinal pigment epithelium.[15] Briefly, the standard Ringer's solution consisted of (in mM): 135 NaCl, 5.0 KCl, 10 HEPES, 10 glucose, 1.8 $CaCl_2$ and 1.0 $MgCl_2$, and was titrated to pH 7.4 with NaOH. The osmolarity of standard Ringer's solution was 302 ± 6 mosmol/l. The standard pipette solution consisted of (in mM): 30 KCl, 83 potassium gluconate, 5.0 HEPES, 5.5 EGTA-KOH, 0.5 $CaCl_2$ and 2.0 $MgCl_2$, and was titrated to pH 7.2 with KOH. The osmolarity of standard pipette solution was 257 ± 5 mosmol/l. An amphotericin B-stock solution (1.2 mg amphotericin B / 50 μl DMSO) was added to 2.0 ml of the pipette solution to give a final concentration of 240 μg/ml. The cell isolation medium was similar to that used in a previous study on RPE and contained (in mM): 135 N-methyl-D-glucamine (NMDG)-Cl, 5.0 KCl, 10 HEPES, 3.0 EDTA-KOH, 10 glucose, 3.0 cysteine, 1.0

glutathione, 1.0 L(+)-ascorbic acid, 1.0 taurine and 0.2 mg/ml papain (Type III), and was titrated to pH 7.4 with NaOH. In experiments where the concentrations of K^+, or tetraethylammonium$^+$ (TEA^+) were varied, NaCl was replaced by an equimolar amount of the appropriate Cl^- salt. A hypotonic solution was made by diluting standard Ringer's solution to 20% and adjusting $[K^+]$ to 5 mM to give an osmolarity of 239 ± 2 mosmol/l. Linopirdine was dissolved in DMSO and then diluted in Ringer's solution. Throughout the experiments, the final concentration of DMSO in the perfusates was no more than 0.2%, which did not affect whole cell currents in this study.

NaCl, KCl, $CaCl_2$ and L(+)-ascorbic acid were obtained from Wako Chemical Co. (Osaka, Japan). All the other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Cell Isolation

All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Porcine eyes were enucleated just after death at a local slaughterhouse and transferred to the laboratory within 30 minutes. A piece of ciliary body with epithelium was cut out and incubated in cell isolation medium for 30 to 40 minutes. The tissue was transferred to standard Ringer's solution containing 0.1 % bovine serum albumin for 3 minutes, and then incubated in standard Ringer's solution for 10 minutes followed by gentle vortexing. Isolated cells were observed in a sample of the suspension by phase-contrast microscopy. The cell suspension was stored at 4 ^{o}C for up to 24 hours before use.

Cell Perfusion

Isolated ciliary epithelial cells were transferred to a Lucite perfusion chamber

(RC-5/25, Warner Instrument, Hamden, CT) and settled for 10 to 20 minutes before perfusion. The perfusate flowed into the chamber under gravity at a flow rate of 0.7 ml / min by a perfusion system (BPS-4, ALA Scientific Instruments, Westbury, NY) and was continuously removed by suction. Fluid height was adjusted to give a chamber volume of approximately 0.5 ml and a complete solution exchange within 2 minutes. All experiments were conducted at room temperature (20 to 25 ^{o}C).

Electrophysiological Methods

Patch pipettes were pulled from borosilicate glass tubing (BF150-110-10, Sutter Instruments, San Rafael, CA) with a multistage programmable puller (P-97, Sutter Instruments, San Rafael, CA). The pipette input resistance was between 1 and 3 MΩ. Under phase-contrast microscopy (ECLIPSE TE300, Nikon, Tokyo, Japan), a target cell was selected, and a pipette tip was pressed onto the cell membrane using a micromanipulator (MP-285, Sutter Instruments, San Rafael, CA) to establish a gigaohm seal. Currents under voltage-clamp were recorded by a Heka amplifier system (EPC-8, Heka, Lambrecht/Pfalz, Germany). The built-in low pass filter was set to 3 kHz unless noted otherwise. Recordings were referenced to an Ag-AgCl electrode (EP-2, WPI, Sarasota, FL). The membrane capacitance was compensated by built-in circuits. The apparent membrane potential was corrected for the pipette tip potential (10 mV). Statistical data are presented as mean $\pm$ S.D. Data were fitted by a non-linear, least-squares fitting using IGOR Pro (Wavemetrics, Lake Oswego, OR).

RESULTS

Whole-Cell Currents

Pairs of pigmented and non-pigmented ciliary epithelial cells (PCE and NPCE cells) with broad contact area (Figure 1) were selected for the recording. The PCE-NPCE cell pairs are typically similar to that observed in a previous report.[17] In the initial and major phase of this study, we found M-type K^+ current[14,15] expressed in the PCE-NCE cell pairs, as shown in Figure 2. Thereafter, we tried to record from isolated single ciliary epithelial cells, and found the identical M-type K^+ current was observed in single PE cells (n = 30) but never in single NPE cells (n = 11). Therefore, data obtained from PCE-NPCE cell pairs (n = 97) and single PCE cells (n = 30) were pooled in this study. The zero-current potential (V_0) was measured in the zero-current clamp mode after establishment of a perforated patch configuration: the mean V_0 was -49 ± 13 mV in PCE-NPCE cell pairs (n = 97) and -52 ± 12 mV in single PCE cells (n = 30). The membrane capacitance and input resistance were 47 ± 34 pF and 489 ± 324 Mohm in PCE-NPCE cell pairs (n = 93), which were somewhat larger than those values of 37 ± 29 pF and 389 ± 277 Mohm measured in single PCE cells (n = 29). Student's *t*-tests showed no significant difference in these values between the two groups.

Effect of Holding Potential.

Representative families of whole-cell currents in a PCE-NPCE cell pair are depicted in Figure 2. The currents in Figure 2A were generated by a series of voltage pulses from a holding potential of -10 mV. Hyperpolarizing pulses produced time-dependent relaxations in current that reversed around -80 mV. The current relaxations reflect the deactivation of a conductance. The following tail currents elicited by a depolarizing voltage step to the holding potential (-10 mV) from various hyperpolarized prepulses reflect activation of a conductance (see later). Figure 2B shows currents in the same cell evoked by a series of voltage steps to the same test potentials from a holding potential of -70 mV. Voltage pulses to potentials in the range between -110 and -70 mV produced time-independent currents with no rectification. On the other hand,

depolarizing voltage pulses to potentials more positive than -60 mV produced a slowly activating current that did not inactivate during the 2 sec depolarization.

Figure 2C compares the steady-state current-voltage relationships generated from holding potentials of -70 mV (open circle) and -10 mV (closed circle). The two I-V curves were nearly identical, indicating that the conductance underlying this outward current was fully open when the membrane was held at -10 mV and that it did not inactivate during the 10 sec interval between pulses. Electrophysiological properties of this K^+ current in PCE cells were quite similar to those of the M-type K^+ current in RPE[13, 14].

Selectivity.

With 5 mM K^+ in the bath, the current relaxations produced by hyperpolarizing pulses reversed between -90 and -80 mV (Figures. 2B and 3A). The reversal potential (V_r) was analyzed by interpolation from plots of the deactivation current amplitude versus membrane voltage (not shown). For 4 cells, V_r averaged -84 $\pm$ 2 mV, which is very close to the theoretical E_K (-83 mV). To evaluate sensitivities for K^+, V_r was also examined at higher $[K^+]_o$. Figure 3B shows an example of the K^+ current activated in 140 mM K^+ Ringer, which reversed between –7 and +3 mV. In 3 cells superfused with 140 mM K^+ Ringer (E_K = 0 mV), V_r averaged -1 $\pm$ 3 mV. Overall, V_r was not significantly different from E_K indicating that the time-dependent outward current is largely carried by K^+.

Conductance-Voltage Relationship.

The data in Figure 2B indicated that the M-type K^+ current activated at -60 mV but at more negative potentials, the activation of a conductance could be underestimated because of its small size and slow kinetics. Therefore, we analyzed the tail current elicited by a depolarizing voltage step to a set potential from various hyperpolarized prepulses in order to determine the voltage activation range of K^+ conductance. Figure

4A depicts a family of tail currents produced by voltage steps to -10 mV following 2 sec prepulses to the potentials indicated. When the prepulse potentials were in the range of -110 to -90 mV, the tail current began from the same initial level and then rose exponentially to a new steady-state. In contrast, prepulses to potentials between -80 and -50 mV produced smaller tail currents that began at a higher initial level but rose to the same steady-state. Finally, when the voltage was stepped to -10 mV from -40 and +10 mV, the initial current level was roughly the same as the steady-state current. The results of these experiments indicate that the M-type K^+ current was completely deactivated at -90 mV and more negative potentials, partially activated between -80 and -30 mV, and completely activated at -20 mV and more positive potentials.

The conductance at each prepulse potential, $G(V)$, can be expressed as:

$$G(V) = G_{max} - \Delta G(-10)$$

where G_{max} is maximum conductance and $\Delta G(-10)$ is the change in conductance produced by stepping the voltage to -10 mV, calculated by dividing the tail current amplitude by the driving force on K^+ at -10 mV (= 73 mV). Similar results were obtained in 11 other cells, giving a mean value for G_{max} of 2.3 ± 0.9 nS (n=12). Figure 4B summarizes the results of these experiments and plots normalized conductance as a function of prepulse potential. The continuous curve is the least squares fit of the data to the Boltzmann function: $\dfrac{G(V)}{G_{max}} = \dfrac{1}{1 + \exp\left[(V_{1/2} - V_m)/\ k_n\right]}$,

with values for $V_{1/2}$ and k_n of -63.3 mV and 10.8 mV, respectively.

Blocker Sensitivity

To further identify channel type, we examined the sensitivity of the M-type K^+ current in the PCE cells to several K^+ channel blockers. M-type K^+ current in the retinal pigment epithelium[14, 15] is known to be sensitive to Ba^{2+} and Cs^+ but not to TEA^+. Figure 5A depicts families of whole-cell currents recorded in the absence (top) and the presence (bottom) of 50 mM external TEA^+. TEA^+ had little effect on the amplitude or

kinetics of the time-dependent K^+ current. The conductance-voltage relationship obtained from tail currents are depicted in Figure 5B, which shows that even in 50 mM TEA^+ the maximum conductance was 15 % smaller than it was in the absence of TEA^+. The maximum K^+ conductance changed to 101 ± 3 % for 4 cells in the presence of 20 mM TEA^+, and 91 ± 6 % for other 4 cells in the presence of 50 mM TEA^+. Ba^{2+}, an effective blocker of the retinal pigment epithelium M-type K^+ current, also inhibited the K^+ current in PCE. Figure 6A shows that 2 mM Ba^{2+} considerably inhibited the K^+ current by 82% compared to the control. For 3 cells, the best-fit of the data to a first order equation indicates an apparent IC_{50} of 0.38 mM (Figure 6B). Electrophysiological properties and blocker sensitivities indicate that the swelling-activated K^+ current in PCE belongs to a family of KCNQ channels.[18] Thus, we investigated the effect of linopirdine, a specific inhibitor of KCNQ channels. Figure 6C represents families of whole-cell currents recorded in the absence (top) and the presence (bottom) of linopirdine. The K^+ current component (tail currents) was almost completely blocked by 10 μM linopirdine. In 4 cells, linopirdine (10 to 25 μM) depolarized the resting potentials to -41 ± 8 mV from the control value of -59 ± 4 mV. Figure 6D summarizes the results of experiments in which the effects of various concentrations of linopirdine were tested on the K^+ current, which indicated an apparent IC_{50} of 0.54 μM.

Activation by Hypotonicity

Modulation of the M-type K^+ current by cell swelling was evaluated. Cell perfusion with a 20% diluted Ringer's solution (239 ± 2 mosmol/l) induced a significant increase in the K^+ currents in the PCE-NPCE cell pairs as shown in Figure 7. This current augmentation by hypotonicity was reversible when the perfusate returned to the standard osmolarity (Figure 7B). In fourteen PCE-NPCE cell pairs, exposure to 20% hypotonic solution increased the maximum conductance to 250 ± 86% of the control value. Similar augmentations of *M-type K^+ current* were also observed in single PCE cells (n = 3, ratio; 184 ± 82%). The reversal potential of the hypotonicity-induced

current component averaged -78 $\pm$ 8 mV (n=7), a value more positive than the theoretical E_K (-83 mV), which indicates that chloride conductance was also activated somewhat and thereby the reversal potential shifted to the direction of theoretical E_{Cl} (-33mV). The current component augmented by exposure to 20 % hypotonic solution as was blocked by linopirdine (20 μM, n =3), but essentially insensitive to external TEA^+ (40 mM, n = 3) as shown in Figure 7C.

DISCUSSION

In the present study we identified a non-inactivating, outward K^+ current in porcine pigmented ciliary epithelial cells that is likely different from the previously reported K^+ channels in the ciliary epithelium. To date, two types of outward K^+ currents have been identified in the ciliary epithelium. Voltage-activated, delayed rectifier K^+ currents were reported in studies of cultured rabbit PCE cells,[6] cultured NPCE cells,[8] and fresh bovine PCE cells.[7] All of these currents were inhibited by TEA^+. Another type of current is the Ca-activated K^+ current reported in rabbit PCE cells, which was also sensitive to TEA^+.[9] Carbachol-induced transient outward K^+ currents described in fresh bovine PCE cells was also Ca^{2+}-sensitive,[10] but its sensitivities for channel blockers are unknown.

K^+ current in the present study closely resembled the M-type K^+ current expressed in retinal pigment epithelium[14,15] in terms of activation kinetics, voltage dependence and blocker sensitivities. The common properties of these currents are that they are non-inactivating and activate with a threshold of -60 mV or more negative voltages, and, thus, they contribute to the resting potential. In addition, they are inhibited by Ba^{2+} but relatively insensitive to TEA^+. The M-type K^+ current of retinal pigment epithelium was termed from its resemblance to the M-current, a K^+ current identified in certain neurons and other excitable cells. The underlying channel of the M-

current is encoded by KCNQ genes.[18, 19] The KCNQ/Kv7 channel family is composed of a group of five voltage-gated K^+ channel subunits (Kv7.1-Kv7.5) with six-transmembrane domain-spanning proteins encoded by the KCNQ genes (KCNQ1-5). The channels are formed by homo-tetramers of the same KCNQ α-subunits or co-assembly of different KCNQ proteins. Linopirdine, a specific blocker of KCNQ channels, inhibited the K^+ channel in this study suggesting that the K^+ current in PCE cells belongs to KCNQ channel members. Sensitivities of KCNQ/KV7 channels to the classical K^+ channel blocker TEA vary by member: KCNQ1 and KCNQ2 are relatively sensitive to TEA, with an IC_{50} ranging from 0.3 to 5.0 mM, whereas KCNQ3, KCNQ4 and KCNQ5 are substantially insensitive to TEA, with an IC_{50} of 30 mM or more.[18, 20] The K^+ channel in this study was little affected even by treatment with 50 mM TEA; hence, it is likely to belong to the latter members.

One of the physiological roles of this K^+ channel in PCE cells is likely to consist of a major K^+ conductance of the apical and/or basolateral membranes, which is supported by the fact that the channel is open near the resting potential and that linopirdine, a specific blocker of this K^+ channel depolarized the zero-current potential of the PCE cells (-59 $\pm$ 4 to -41 $\pm$ 8 mV). Another role of this K^+ channel in PCE cells may be to mediate cell volume and water transport. As with the K^+ channel in this study, some KCNQ channels have been reported to be activated by cell swelling. For instance, the KCNQ1 channel, which is expressed in secretory epithelia, like the distal colon or airway, is regulated by small changes in cell volume and participates in the secretion of salt and water.[21] In addition, recent evidence suggested that KCNQ3 and KCNQ5 also could mediate basal anion secretion from an airway epithelial cell line[22] and regulate Na^+ transport across lung epithelial cells.[23]

In conclusion, we identified a swelling-activated K^+ channel in freshly dissociated porcine pigmented ciliary epithelial cells. Electrophysiological and

pharmacological properties of this channel indicate that it is a member of the KCNQ/Kv7 channel family. This K^+ channel is active near resting potentials and may contribute to the regulation of cell volume and water transport via the ciliary epithelia.

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Figure legends

FIGURE 1

Photomicrograph of isolated porcine ciliary epithelial cells.

A pair of pigmented and non-pigmented ciliary epithelial cells (PCE and NPCE cells) freshly isolated from porcine eyes. In this experiment, a <u>PCE</u> cell membrane was clamped by a patch-pipette. Bar, 50 μm.

FIGURE 2

Whole-cell current in porcine ciliary epithelium and its dependence on holding potential.

(**A**) Whole-cell currents in a porcine PCE-NPCE pair were elicited by 1s-voltage steps ranging from +10 to -110 mV in -10 mV increments from a holding potential (HP) of -10 mV. (**B**) In the same cell, currents were elicited by same range voltage steps from a HP of -70 mV. (**C**) I-V relationships are depicted with a HP of -10 (●) and -70 mV (○). The steady-state current amplitudes were averaged between 900 and 1000 ms in the whole-cell currents in **A**.

FIGURE 3

Reversal potential of the time-dependent K^+ current and its relationship to extracellular K^+ concentration.

(A) Superfused with standard Ringer solution ($[K^+]_o = 5$ mM), currents elicited by membrane voltage steps ranging from –100 to –70 mV from a holding potential of –10 mV. The polarity of the deactivating currents reversed between –90 and –80 mV.   (B)

Superfused with a high K^+ solution ($[K^+]_o = 140$ mM), K^+ currents elicited by membrane voltage steps ranging from -37 to $+33$ mV from a holding potential of -107 mV. In 140 mM $[K^+]_o$ solution, the activating currents reversed between -7 and $+3$ mV.

FIGURE 4

Voltage dependence of the K^+ conductance.

(A) Tail current activation at -10 mV following prepulses to the potentials indicated. Tail current amplitude is inversely proportional to the fraction of K^+ channels activated at prepulse potential. (B) Conductance-voltage relationship. K^+ conductance at each voltage is normalized to maximum conductance. Data represents mean $\pm$ SEM for 12 cells. The continuous curve is the least squares fit of the data to a Boltzmann function:

$$\frac{G(V)}{G_{max}} = \frac{1}{1 + \exp\left[\left(V_{1/2} - V_m\right) / k_n\right]}, \quad \text{where } V_{1/2} \text{ and } k_n \text{ are -63.3 mV and 10.8 mV,}$$

respectively.

FIGURE 5

Effect of TEA on the K^+ current. (A) Families of whole-cell currents recorded in the absence (top) and the presence (bottom) of 50 mM TEA. Currents were elicited by membrane voltage steps ranging from $+10$ to -130 mV from a holding potential of -10 mV. (B) Effect of TEA on steady-state conductance-voltage relationship. The K^+ conductance in the absence (closed circle) and the presence (open circle) of 50 mM TEA was calculated from tail currents in A.

FIGURE 6

Inhibitory effects of Ba^{2+} and linopirdine on the K$^+$ channels. (A) Whole cell currents elicited by voltage steps ranging from +10 to -130 mV were obtained in the absence and the presence of 2 mM Ba^{2+}. (B) Dose response curve. Relative current amplitude at each concentration was measured and summarized in 3 cells. Smooth curves represent fitting the data to the first order equation: $I_{Inhibitor} / I_{control} = 1 - \{[Inhibitor] / ([Inhibitor] + IC_{50})\}$, with an IC$_{50}$ of 0.38 mM. All error bars represent SEM. (C) Whole cell currents elicited by voltage steps ranging from +10 to -110 mV were obtained in the absence and the presence of 10 μM linopirdine. (D) Dose response curve. Each data point depicts measurements in 3 cells. Smooth curves represent fitting the data to the same equation above, where the IC$_{50}$ was 0.54 μM. All error bars represent SEM.
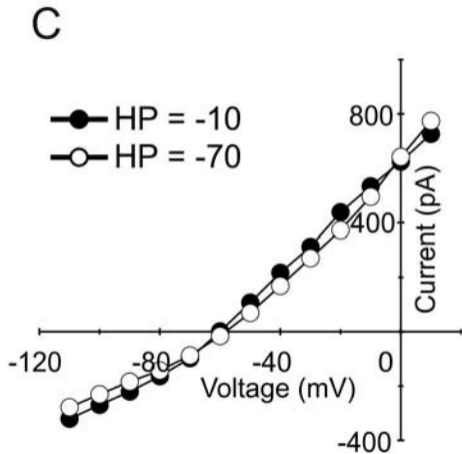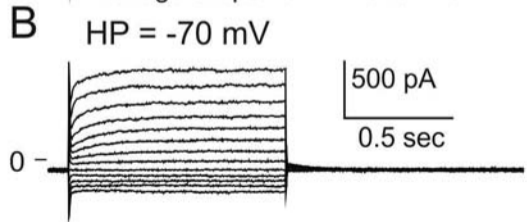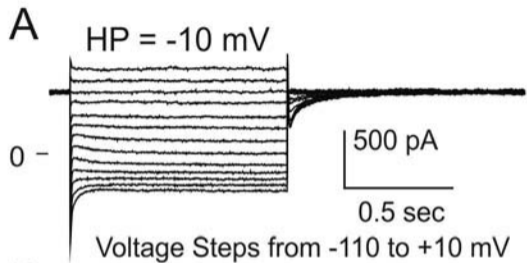
FIGURE 7

Effect of hypotonicity on the K$^+$ current.

(A) Families of whole-cell currents recorded when a cell was superfused with standard Ringer's solution (302 ± 6 mosmol/l, left panel) and 20% diluted hypotonic solution (239 ± 2 mosmol/l, right panel). Currents were elicited by membrane voltage steps ranging from +10 to -120 mV from a holding potential of –10 mV. (B) Time course of the M-type K$^+$ current. Maximum amplitude of the tail currents was monitored in the same cell in A. (C) Effects of high TEA and linopirdine in hypotonic condition. In another cell, families of whole cell current were recorded in standard Ringer's solution, 20% diluted hypotonic solution, hypotonic (20% dilution) 40 mM TEA solution, and 20 μM linopirdine in hypotonic solution. Currents were elicited by membrane voltage steps ranging from +10 to -110 mV from a holding potential of –10 mV.

précis summary

A swelling-activated potassium channel was identified in freshly dissociated porcine pigmented ciliary epithelial cells. This potassium channel may be a member of the KCNQ/Kv7 and could contribute to the regulation of water transport via the ciliary epithelia.

A HP = -10 mV

0 —

500 pA

0.5 sec

Voltage Steps from -110 to +10 mV

B HP = -70 mV

0 —

500 pA

0.5 sec

C

● HP = -10
○ HP = -70

800

Current (pA)

400

-120   -80   -40   0

Voltage (mV)

-400

A

-10 mV

-80
-90

-70

-100 mV

100 pA

0.5 sec

B

+33 mV
+23
+13
+3
-7
-17

-107

-37 mV

100 pA

0.5 sec

A

-20 to -10

-40, -30

-70

-80

-90 to -110 mV

0

100 pA

200 msec

B

Normalized Conductance

Voltage (mV)

A

Control

0 –

500 pA

500 ms

50 mM TEA

0 –

B

Conductance (nS)

4

3

2

1

−120   −80   −40   0

Voltage (mV)

A

Control   Hypotonic Ringer (20% dilution)

0-

0-

500 pA

0.5 sec

B

Current (pA)

800

600

400

Hypotonic Ringer

0 2 4 6 8 10

Time (min)

C

Control   Hypotonic Ringer (20% dilution)

0-

0-

Hypotonic 40 mM TEA solution

200 pA

500 msec

0-

Linopirdine in Hypotonic Ringer

0-