J Membrane Biol (2013) 246:167–175
DOI 10.1007/s00232-012-9517-4

# Ionic Currents of Human Trabecular Meshwork Cells from Control and Glaucoma Subjects

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Received: 31 July 2012 / Accepted: 15 October 2012 / Published online: 8 November 2012


**Abstract** Increasing evidence suggests that trabecular meshwork (TM) cells participate in the regulation of intraocular pressure by controlling the rate of filtration of the aqueous humor. Ionic conductances that regulate cell volume and shape have been suggested to play an important role in TM cell volume regulation. Here, we compared ionic currents from TM cells derived from a normal subject (CTM) and from an individual affected by glaucoma (GTM). We found that while the ionic current types were similar, the current amplitudes and percentage of cells endowed with specific current at baseline were different in the two cell lines. Thus, we found that the majority of CTM cells were endowed with a swelling-activated $Cl^-$ current at baseline, whereas in the majority of GTM cells this current was not active at baseline and became activated only after perfusion with a hypotonic solution. An inward rectifier $K^+$ current was also more prevalent in CTM than in GTM cells. Our work suggests that disregulation of one or more of these ionic currents may be at the basis of TM cell participation in the development of glaucoma.

**Keywords** Trabecular meshwork · ION channel · Glaucoma

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## Introduction

One of the hallmarks of glaucoma is elevated intraocular pressure (IOP). Elevated IOP is thought to result in optic nerve damage and eventually blindness. The IOP is balanced by the rate of production and drainage of the aqueous humor (Morrison and Acott 2003). The aqueous humor is filtered through the trabecular meshwork (TM), a tissue located in the anterior chamber angle and drained via Schlemm's canal (Morrison and Acott 2003; Tripathi 1972). The rate of filtration through the TM is influenced by the tone of the ciliary muscle, which sends tendons into the TM tissue (Kaufman 2008; Tamm 2009). However, increasing evidence suggests that the TM tissue itself can modulate the filtration rate by changing the shape and volume of the cells that compose it (Goel et al. 2012). The volume and shape of cells are regulated by various systems including the cytoskeleton (Kaufman 2008) and membrane ionic conductances (Ellis 2011; Stumpff and Wiederholt 2000). For example, studies in different types of tissues, including TM cells, have established that chloride currents that are activated by cell swelling participate in cell volume regulation (Bond et al. 1998; Soto et al. 2004; Volk et al. 2008). Other types of channels sensitive to membrane stretch or sheer force can influence cell shape, thus affecting the outflow rate (Goel et al. 2012; Stumpff and Wiederholt 2000). Finally, the ion channel makeup of TM cells has the potential to impact the function of the TM tissue and, consequently, the outflow rate of the aqueous humor. Therefore, in order to fully understand the contribution of the TM tissue to the filtration rate of the aqueous humor, a good understanding of the ionic conductances present in these cells is imperative. Moreover, a comparison between the ionic conductances present in TM cells from normal and glaucoma subjects can give insight into the mechanisms that are disregulated in glaucoma.

Previous studies by other laboratories identified and characterized the ionic conductances of TM cells (Comes et al. 2006; Dismuke and Ellis 2009; Gasull et al. 2003; Llobet et al. 2001; Nilius et al. 1998; Soto et al. 2004; Stamer et al. 1995). A swelling-activated $Cl^-$ current, an inward rectifier $K^+$ current and an outward rectifier $Ca^{2+}$-sensitive $K^+$ current, among others, were found to be present in TM cells (Dismuke and Ellis 2009; Gasull et al. 2003; Llobet et al. 2001; Mitchell et al. 2002; Soto et al. 2004). These studies were focused only on TM cells isolated from normal subjects or from animal models. The goal of the present work was to compare the ionic currents present in human TM cells from normal and glaucoma subjects. We show here that, similarly to what was previously reported, TM cells are endowed with at least three major distinct types of currents: a swelling-activated $Cl^-$ current, an outwardly rectifying $K^+$ current and an inwardly rectifying $K^+$ current. However, the ratio of cells in which each current is prevalent varies considerably in normal versus glaucoma-derived TM cells. Our work shows that intrinsic electrophysiological properties are distinct between normal and glaucoma TM cells and suggests that these differences could be considered possible contributors to the development of the disease state.

## Methods

### Ethics Statement

This study adhered to the guidelines of the institutional review board of the University of Miami. Human samples were handled in accordance with the principles expressed in the Declaration of Helsinki. Cadaver human eyes were obtained from National Disease Research Interchange, Philadelphia, PA, with the approval (exempt under category 4 of NIH guidelines) of the institutional review board of the University of Miami. The human TM cell culture protocol was approved by the institutional review board of the University of Miami.

### Cell Line Development and Culture

Two TM cell lines were developed, from a normal (CTM35) and a glaucoma (GTM35) donor. Both donors were Caucasian males aged 35 years at the time of death and enucleation. The eyes were collected within 3 h of death, stored immediately in precooled Optisol GS and subsequently maintained at 4 °C. These eyes were dissected within 24 h under a dissecting microscope into four quadrants of the anterior chamber. The trabecular meshwork with minimum contamination of sclera and cornea was prepared from each quadrant. The excised tissue was immersed in 70 % ethanol, and all subsequent procedures were carried out in a sterile hood with sterile instruments. Tissues from each quadrant were placed in 10-cm plates containing enough sterile phosphate-buffered saline to cover approximately $\sim$1 mm height. With a fine scalpel, the cells from the middle of these dissected tissues were scraped, and scraped cells were removed into areas away from the tissue mass and further gently meshed with forceps. These cells were allowed to grow in DMEM with 10 % FBS for 3 days. Both cell types were subjected to stable transfection with a Myc T58A mutant construct (Sears et al. 2000; Yeh et al. 2004). Cells were selected in the presence of neomycin for stable transfection. These cell lines show continuous growth beyond 30 passages, whereas the primary TM cells fail to grow in culture beyond 20–22 passages. However, cells used for the current investigation were stored from the same initial stock and are approximately from the sixth to tenth passages. Both CTM35 and GTM35 cell lines were maintained in DMEM with 10 % FBS and 1 % antibiotic/antimycotic. For electrophysiology, cells were plated at roughly 10 % confluence in dishes containing 12-mm round glass coverslips and allowed to adhere overnight before electrophysiological recordings.

### Electrophysiology

Ionic currents were recorded at room temperature using the whole-cell configuration of the patch-clamp technique. An Axopatch 200 B, Digidata 1322A and the pCLAMP suite of programs (Molecular Devices, Sunnyvale, CA) were used for data acquisition and analysis. Patch pipettes were pulled from borosilicate capillaries (World Precision Instruments, Sarasota, FL) and fire-polished to have a resistance of 2–4 MΩ. Pipettes were filled with a solution containing (in mM) K-aspartate 150, $MgCl_2$ 3, $CaCl_2$ 1, EGTA 5 and HEPES 10, pH 7.3 (265 mOsm). The isotonic extracellular solution contained (in mM) NaCl 145, KCl 5, $MgCl_2$ 3, $CaCl_2$ 1 and HEPES 10, pH 7.4 (295 mOsm); the hypotonic extracellular solution contained (in mM) NaCl 80, KCl 5, $MgCl_2$ 3, $CaCl_2$ 1 and HEPES 10, pH 7.4 (184 mOsm); the solution at high $K^+$ concentration contained (in mM) KCl 150, $MgCl_2$ 3, $CaCl_2$ 1 and HEPES 10, pH 7.4 (289 mOsm). The extracellular solutions in which the concentration of $Cl^-$ was reduced contained (in mM) NaCl 40, Na-aspartate 40, KCl 5, $MgCl_2$ 3, $CaCl_2$ 1 and HEPES 10, pH 7.4 (180 mOsm), and Na-aspartate 80, KCl 5, $MgCl_2$ 3, $CaCl_2$ 1 and HEPES 10, pH 7.4 (176 mOsm). The osmolarity of the solutions was measured using an osmometer (Advanced Micro-osmometer Model 3MO plus; Advanced Instruments, Norwood, MA). Values of cell capacitance were derived from amplifier reading after correction of the pipette capacitance. The average cell capacitance was 67.7 ± 9.7 pF ($n$ = 19) and 51.2 ± 5.5

pF ($n = 47$) for CTM and GTM cells, respectively. Means were not statistically different. 5-Nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) and 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) were acquired from Tocris (Abingdon, UK) and Invitrogen (Grand Island, NY), respectively. Stock solutions were prepared in DMSO and $H_2O$ at concentrations of 0.1 and 0.5 M, respectively.

## Results

### TM Cells from a Normal Subject Express a Swelling-Activated $Cl^-$ Current

To determine the types of ionic currents present in normal TM cells (CTM), we applied the patch-clamp technique in the whole-cell mode. We used extracellular and pipette solutions that mimic the ionic composition of the extracellular and intracellular spaces, respectively. In 11 of the 12 CTM cells we patched, we detected a rapidly activating outwardly rectifying current (Fig. 1a). This current was reminiscent of the swelling-activated $Cl^-$ current previously described in TM cells (Comes et al. 2006; Soto et al. 2004). Thus, to establish if the current we detected in CTM cells was sensitive to cell swelling, we perfused the cells with a hypotonic solution containing 80 instead of 145 mM NaCl. We found that the outward rectifying current increased in amplitude in hypotonic solution (Fig. 1b, d). The reversal potential of the current also shifted toward less negative potentials (Fig. 1c). These results suggested that the swelling-activated current might be a chloride current.

To test this hypothesis, we used the $Cl^-$ channel blocker NPPB (Mitchell et al. 2002). We found that application of 100 μM NPPB substantially blocked the swelling-activated current (Fig. 2a–d). We conclude that the majority of CTM cells express a swelling-activated $Cl^-$ current.

### An Outward $K^+$ Current in the Majority of GTM Cells

Surprisingly, when we studied ionic currents in GTM cells, we found that the majority of these cells expressed an

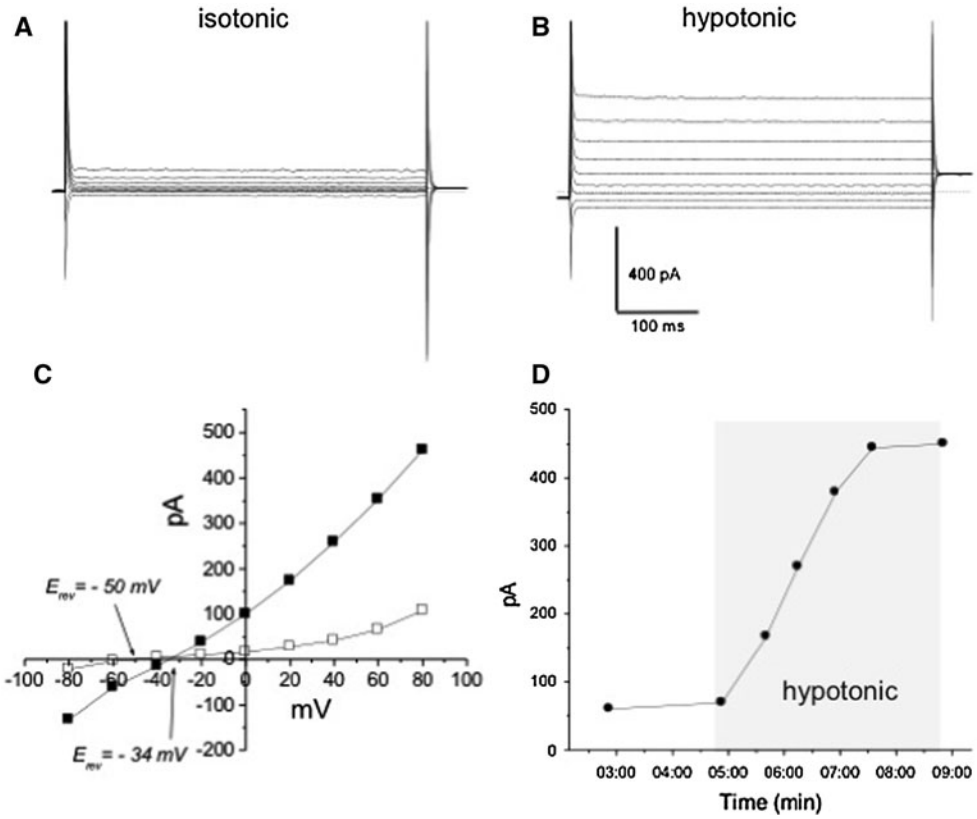


**Fig. 1** CTM cells express a swelling-activated current. **a** Example of ionic currents recorded in a CTM cell perfused with an isotonic physiological solution. Currents were elicited by voltage steps from − 80 to + 80 mV (20-mV increments) from a holding potential of − 50 mV. **b** Same as in **a** while perfusing the cell with a hypotonic solution. In both panels *dashed line* represents the zero current level. **c** Current–voltage relationships of currents shown in **a** and **b**. *Arrows* point to the reversal potentials of the currents. In isotonic solution ($[Cl^-]_o = 158$ mM, *open squares*) the reversal potential was − 50 mV; perfusion with hypotonic solution ($[Cl^-]_o = 93$ mM, *closed squares*) shifted the reversal potential to −34 mV. **d** Time course of current activation at +80 mV. *Shaded area* represents perfusion with hypotonic solution

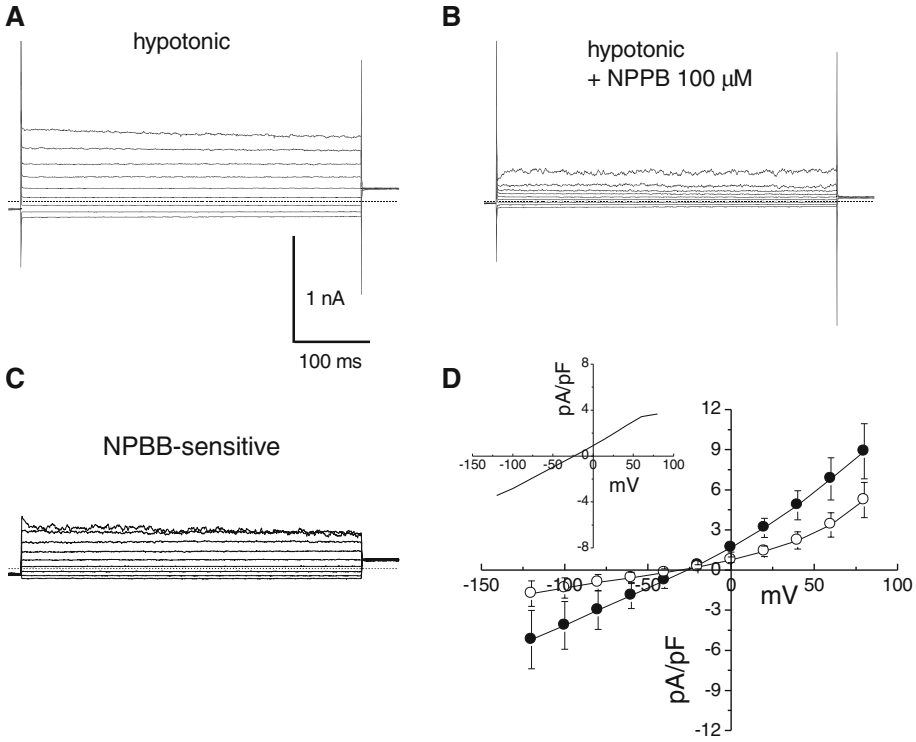


**Fig. 2** Swelling-activated current of CTM cells is inhibited by NPPB. **a** Example of currents recorded in a CTM cell perfused with hypotonic solution. Currents were elicited by voltage steps from −120 to +80 mV in 20-mV increments. Holding potential was −50 mV. **b** The same cell shown in **a** was perfused with hypotonic solution plus 100 μM NPPB, a chloride channel blocker. **c** NPPB-sensitive current obtained by subtracting current shown in **b** from current shown in **a**. In **a**–**c** *dashed line* represents the zero current level. **d** Average current–voltage relationships normalized by the cell capacitance in hypotonic solution (*filled circles*, $n = 5$) and hypotonic solution plus 100 μM NPPB (*open circles*, $n = 5$). *Inset* Current–voltage relationship of the NPPB-sensitive current

outwardly rectifying current with features distinct from the current we detected in CTM cells (Fig. 3a, b). First, this current did not activate instantaneously but followed a time course that became more rapid at more depolarized voltages. Second, it reversed at more negative potentials (Fig. 3b, c). These features suggested that this current is likely carried by outwardly rectifying $K^+$ channels. To confirm this, we perfused cells with a high-$K^+$ solution. We found that under these conditions the reversal potential of the current shifted toward more positive potentials (from −75 to −7.5 mV) and that a substantial inward component at negative potentials appeared (Fig. 3d–f). These results confirmed that, indeed, this current is carried by $K^+$ ions.

In approximately one-third of GTM cells, though, we did detect the swelling-activated $Cl^-$ current in isotonic solution (Fig. 4a, b). When we compared the amplitudes of the swelling-activated $Cl^-$ current in CTM and GTM cells in isotonic solution, we found that they were similar (Fig. 4c). Moreover, in GTM cells in which the current was not active in isotonic solution, this could be activated by perfusion with the hypotonic solution to amplitudes similar to the ones seen in CTM cells (Fig. 4d). Moreover, the swelling-activated $Cl^-$ current of GTM cells was blocked by NPPB to an extent similar to the swelling-activated current of CTM cells (Fig. 4e). In addition, we found that 500 μM DIDS blocked the swelling-activated current of GTM cells by a similar degree, further supporting that this is a $Cl^-$ current. Using GTM cells, we also tested the effect of changing the concentration of extracellular chloride on the reversal potential of the current, while maintaining all the other permeant ions at a constant concentration. We found that lowering the extracellular $Cl^-$ concentration shifted the reversal potential toward less negative values (Fig. 4f; Table 2), as expected for a current carried by $Cl^-$ ions. To conclude, we found that the prevalent current present in GTM cells at baseline is an outwardly rectifying $K^+$ current and that in only about one-third of these cells was the swelling-activated $Cl^-$ current active in isotonic solution. However, the swelling-activated $Cl^-$ current could be activated in GTM cells by perfusion with hypotonic solution. In contrast, the swelling-activated $Cl^-$ current is prevalent in isotonic solution in CTM cells (Table 1). Our data suggest that the swelling-activated $Cl^-$ current is present in both GTM and CTM cells but that it is active at baseline in a higher percentage of CTM than GTM cells.

Since we detected the outwardly rectifying $K^+$ current in only one CTM cell, we wondered whether the swelling-

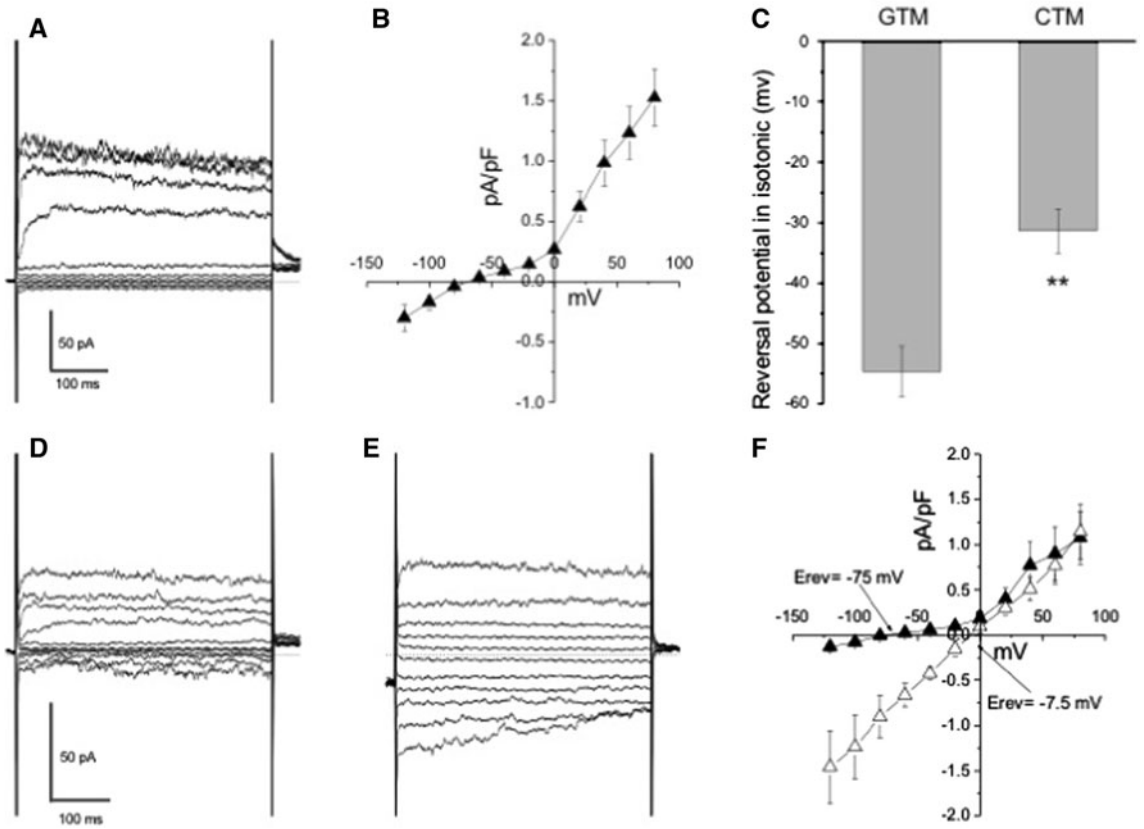


**Fig. 3** Outward $K^+$ currents in GTM cells. **a** Example of outward $K^+$ currents in a GTM cell perfused with isotonic solution. Voltage steps were from −120 to +80 mV in 20-mV increments. Holding potential was −50 mV. *Dashed line* is the zero current level. **b** Average current–voltage relationship for currents similar to the ones shown in **a** normalized by the cell capacitance in GTM cells ($n = 14$). **c** Average reversal potential of currents recorded in GTM ($n = 21$) and CTM ($n = 12$) cells in isotonic solution. $^{**}p \leq 0.01$ by *t*-test. **d** Another example of outwardly rectifying currents in GTM cells, recorded in a physiological isotonic solution. The same voltage protocol used in **a** was used here. **e** The same cell shown in **d** was perfused with the high-$K^+$ solution. **f** Average current–voltage relationships for the outwardly rectifying $K^+$ current recorded in physiological solution (*filled triangles*) and in high-$K^+$ solution (*open triangle*). Note the shift in reversal potential (from −75 to −7.5 mV) consistent with this current being carried by $K^+$ ions ($n = 4$)

activated $Cl^-$ current present at baseline in most CTM cells masked the outward $K^+$ current that can be readily recorded in GTM cells. To test this possibility, we analyzed the NPPB-resistant currents in both CTM and NTM cells. We found that these currents were slowly activating, outwardly rectifying and reversed at $\sim -50$ mV (Fig. 5). These results suggest that these are likely $K^+$ currents. We conclude that in CTM cells the outward $K^+$ current is likely present and just masked by the swelling-activated $Cl^-$ current.

### An Inwardly Rectifying $K^+$ Current in Normal and Glaucoma TM Cells

We found that 8 out of 12 (66 %) CTM and 8 out of 21 (38 %) GTM cells expressed an inward rectifier current, whose amplitude increased and whose reversal potential shifted from negative to 0 mV when extracellular [$K^+$] was increased from 5 to 150 mM (Fig. 6a–c). These features indicate that this is an inwardly rectifying $K^+$ current (Llobet et al. 2001). Note that in the few cells in which the inwardly rectifying $K^+$ current was the major one present, it displayed strong inward rectification (Fig. 6a, b). However, the strong inward rectification was in most cells less apparent due to the presence of other overlapping currents, including the swelling-activated $Cl^-$ current (Fig. 6c, d). When we compared the amplitude of the inwardly rectifying $K^+$ current in CTM and GTM cells, we found that it was larger in CTM cells (Fig. 6d). To conclude, while an inwardly rectifying $K^+$ current was found in both CTM and GTM cells, it was larger and present in a higher percentage of CTM cells.

## Discussion

In this work we compared ionic currents from two cell lines derived from a normal subject (CTM) and from an

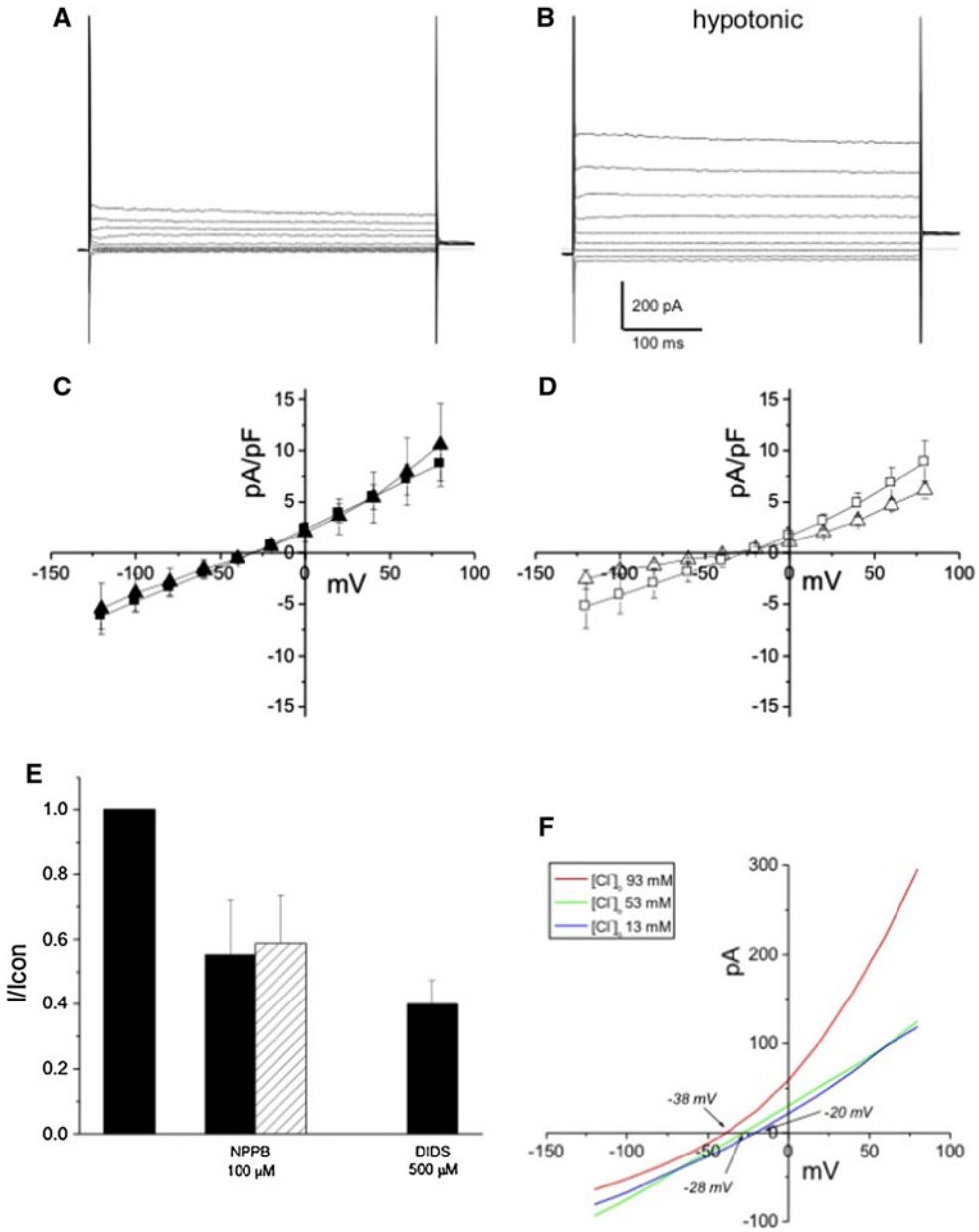


**Fig. 4** Swelling-activated chloride current in GTM cells. **a** Example of ionic current recorded in a GTM cell perfused with isotonic solution. **b** The same cell was perfused with hypotonic solution. The voltage protocol used in Fig. 1a, b was used here. *Dashed line* is the zero current level. **c** Average current–voltage relationships normalized by the cell capacitance of the swelling-activated chloride current in CTM (*filled squares*, $n = 11$) and GTM (*filled triangles*, $n = 6$) cells, recorded in isotonic solution. **d** Same as in **c** for cells in which the current was activated by hypotonic solution. CTM (*open squares*, $n = 5$) and GTM (*open triangles*, $n = 9$) cells. **e** Effect of NPPB and DIDS on the swelling-activated current in GTM (*black bars*, $n = 4$ and 3) and CTM (*dashed bar*, $n = 5$) cells. **f** Current–voltage relationships for the swelling-activated $Cl^-$ current recorded while perfusing a GTM cell with 93, 53 and 13 mM $Cl^-$ in extracellular solution. *Arrows* indicate the reversal potential of the current

individual affected by glaucoma (GTM), respectively. We found that three major ionic currents are expressed in both cell lines but that current amplitudes and the fraction of cells that express them at baseline are different. More specifically, we found that while in the majority of CTM cells a swelling-activated $Cl^-$ current is prevalent at baseline in isotonic solution, in the majority of GTM cells this current is activated only upon perfusion with hypotonic solution. Moreover, an inwardly rectifying $K^+$ current is of larger amplitude in CTM versus GTM cells.

A swelling-activated $Cl^-$ current has been previously described in TM cell primary cultures from humans and animal models (Mitchell et al. 2002; Soto et al. 2004). Based on pharmacological and selectivity properties, it was

**Table 1** Percentage of CTM and GTM cells endowed with the hypotonically activated $Cl^-$ current at baseline, while being perfused with isotonic solution

| | Cells with hypotonically activated $Cl^-$ current |
|---|---|
| CTM | 11/12 (91 %) |
| GTM | 7/20 (35 %) |

**Table 2** Average shift in reversal potential of hypotonically activated $Cl^-$ current when the extracellular concentration of $Cl^-$ was changed from 158 to 93 mM (ΔV1), from 93 to 53 mM (ΔV2) and from 53 to 13 mM (ΔV3)

| ΔV1 | ΔV2 | ΔV3 |
|---|---|---|
| 22.6 ± 6.7 ($n = 6$) | 4.7 ± 1.2 ($n = 6$) | 11.9 ± 1.7 ($n = 6$) |

Values were obtained from GTM cells. Data are expressed as mean ± SE

proposed that this current is likely ICl swell, a chloride current found in many cell types and involved in regulatory volume decrease (RVD). RVD is a phenomenon by which cells that undergo hypotonic shock resulting in swelling of the cell are able to return to their baseline volume. Return to baseline volume requires efflux of $Cl^-$ and $K^+$, which is followed by water outflow (Cala 1980). ICl swell is thought to be the major $Cl^-$ current that mediates $Cl^-$ efflux, which might influence RVD (Bond et al. 1998). Following the discovery of ICl swell in TM cells, it was suggested that this current might play a role in RVD in these cells following osmotic shock with hypotonic solution (Soto et al. 2004). This hypothesis was tested using tamoxifen, a blocker of the swelling-activated $Cl^-$ current. It was indeed found that in TM cells treated with tamoxifen RVD was reduced and slowed significantly (Soto et al. 2004). Because RVD of TM cells was suggested to be involved in aqueous humor outflow, Soto and colleagues also tested the outflow facility of the aqueous humor following osmotic shock in the absence and presence of tamoxifen. They found that blocking of ICl swell significantly reduced outflow. Reduction of outflow rate of the aqueous humor is associated with increase of IOP, which is a hallmark of glaucoma. These results suggest that one of the features of TM cells of glaucoma patients might be reduced amplitude of the ICl swell current. In our experiments, we found that ICl swell is present in a lower percentage of glaucoma TM cells compared to normal cells, when the cells are perfused with isotonic solution (at baseline). However, when we challenged the cells with the hypotonic solution, ICl swell was activated in GTM cells and its amplitude reached the amplitude found in CTM cells. These results suggest that GTM cells might have disregulated ICl swell channels rather than a lower number of them. One possibility is that ICl swell channels in GTM cells are less sensitive to membrane stretch. Alternatively, an intracellular molecule that regulates ICl swell amplitude might be at lower or

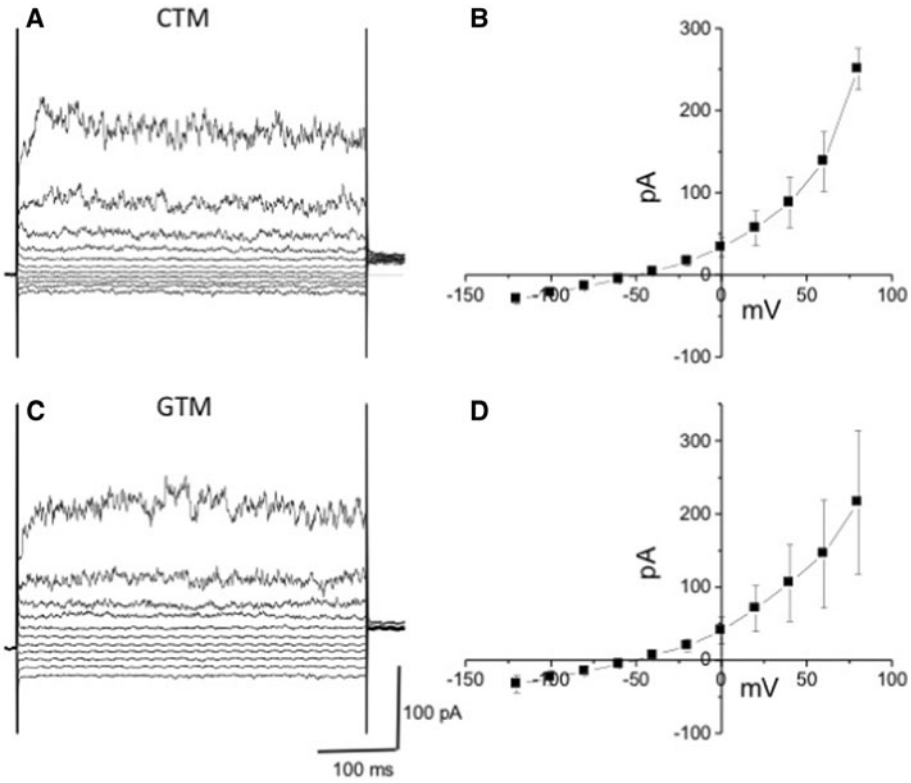


**Fig. 5** Outward $K^+$ current in CTM cells. **a** Example of outwardly rectifying NPPB-resistant currents in a CTM cell. The cell was perfused with hypotonic solution + 100 μM NPPB. Voltage steps were from −120 to +80 mV in 20-mV increments. Holding potential was −50 mV. *Dashed line* is the zero current level. **b** Average current–voltage relationship obtained from currents similar to the one shown in **a** ($n = 3$). **c**, **d** Same as **a** and **b** for GTM cells, $n = 5$

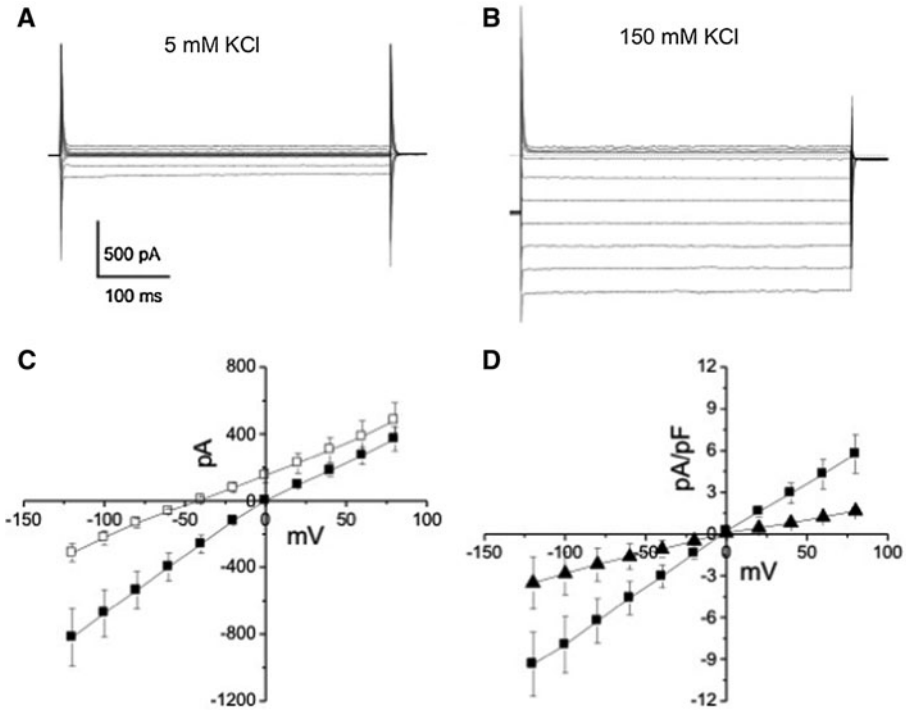


**Fig. 6** CTM and GTM cells express an inwardly rectifying $K^+$ current. **a** Example of inward $K^+$ currents elicited in a CTM cell perfused with a physiological solution containing 5 mM $K^+$. The membrane was stepped from −120 to + 80 mV from a holding potential of −50 mV. **b** The same cell shown in **a** was perfused with a solution containing 150 mM $K^+$. *Dashed line* is the zero current level. **c** Average current–voltage relationship of CTM cell inward $K^+$ current in 5 mM (*open squares*, $n = 7$) and 150 mM (*filled squares*, $n = 7$) $K^+$. Note the shift in reversal potential from ∼−50 to 0 mV when extracellular $K^+$ is increased to 150 mM. **d** Average current–voltage relationships normalized by the cell capacitance of the inward $K^+$ current in CTM (*filled squares*, $n = 8$) and GTM (*filled triangles*, $n = 8$) cells perfused with 150 mM $K^+$

higher concentrations in GTM versus CTM cells. One candidate molecule is cAMP, which is known to inhibit ICl swell (Voets et al. 1996). cAMP is known to also inhibit inwardly rectifying $K^+$ currents in TM cells (Llobet et al. 2001). Coincidently, we found not only that a lower percentage of GTM cells expressed the inwardly rectifying $K^+$ current but also that its amplitude was smaller compared to that of CTM cells. Further experiments are needed to establish if cAMP levels are higher in GTM versus CTM cells and/or whether ICl swell becomes activated in GTM cells later during the patch-clamp recording, when intracellular components have been diluted through the pipette. However, regardless of the basis of the difference in ICl swell and inwardly rectifying $K^+$ current between GTM and CTM cells, our data suggest that such differences need to be taken into account when considering the possible mechanisms underlying the development of glaucoma.

We found that both CTM and GTM cells express an outwardly rectifying $K^+$ current. Previous work by Soto and colleagues (2004) showed that TM cells are endowed with an outwardly rectifying, $Ca^{2+}$-activated $K^+$ current (BK) that is activated by osmotic shock with hypotonic solution. Soto and colleagues showed that inhibition of the BK current reduced RVD and aqueous humor outflow. The BK current might indeed be the major pathway for the $K^+$ efflux needed for RVD.

In summary, our findings show that the basic electrophysiological properties of glaucoma TM cells differ from those of normal cells. The ionic channels expressed are the same, but the current amplitudes and activities of the channels at baseline are different, suggesting that the number of channels and/or the channel regulation of glaucoma TM cells are different from those of normal cells. Our work suggests consideration of such differences in basic electrophysiological properties when weighing factors that might influence the development of glaucoma.

**Acknowledgments** This work was supported by American Cancer Society (RGS-09-043-01-DDC) and NIH (RO1NS070969) Grants to L. B. and by Grant EY016112 and an RPB career award to S. K. B.

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