Cx46 hemichannels contribute to the sodium leak conductance in lens fiber cells

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Submitted 19 November 2013; accepted in final form 24 December 2013

Ebihara L, Korzyukov Y, Kothari S, Tong JJ. Cx46 hemichannels contribute to the sodium leak conductance in lens fiber cells. Am J Physiol Cell Physiol 306: C506–C513, 2014. First published December 31, 2013; doi:10.1152/ajpcell.00353.2013.—The lens is proposed to have an internal microcirculation system consisting of continuously circulating ionic fluxes. Gap junctions play an important role in maintaining lens transparency. One of the key components of this system is the sodium leak conductance. Here we investigate the contribution of Cx46 hemichannels to the sodium leak conductance in lens fiber cells. Our results show that Cx46 hemichannels were largely closed at a resting voltage of −60 mV in the presence of millimolar divalent cation concentrations. However, even though the vast majority of these channels were closed at −60 mV, a small, persistent, inward current could still be detected. This current could be blocked by exposure to 1 mM La3+ and was not observed in fiber cells isolated from dKO mouse lenses suggesting that it was due to Cx46 hemichannels. In addition, Cx50−/− fiber cells showed increased open channel noise and a depolarized resting potential compared with dKO fiber cells. Exposure of Cx50−/− fiber cells to La3+ hyperpolarized the resting potential to −58 mV, which is similar to the value of resting potential measured in dKO fiber and significantly reduced the open channel noise. In conclusion, these results suggest that Cx46 hemichannels may contribute to the sodium leak conductance in lens fiber cells.

Connexin; Cx46; lens; hemichannel

According to the fluid circulation system model proposed by Mathias (reviewed in Ref. 15), the lens has an internal circulatory system which plays a critical role in maintaining lens homeostasis. This circulatory system consists of continuously circulating sodium fluxes and associated fluid, which flow into the lens along the narrow extracellular spaces between the fiber cells. As sodium moves toward the center of the lens, it is continuously entering the fiber cells through sodium leak channels. Once inside the fiber cell, sodium flows toward the surface of the lens via an intracellular pathway involving gap junctions. The surface epithelial cells contain large numbers of Na+-K+-ATPases that then pump sodium out of the lens. Gap junctions play an important role in this circulation system. Gap junctions are composed of cell-to-cell channels that allow the flow of monovalent ions and other small molecules between neighboring cells. These channels are formed by the docking of two oligomeric subunits called hemichannels or connexons, each of which is located in the plasma membrane of closely apposed cells. Hemichannels can also exist as large, relatively nonselective ion channels in single plasma membranes (5). Vertebrate gap junctions are composed of a family of proteins called connexins with 21 human and 20 mouse members (31). Three connexins have been identified in the lens: Cx43, Cx50, and Cx46. These connexins show differences in their pattern of distribution within the lens. Cx43 is present only in the epithelium (20), whereas Cx46 is expressed exclusively in the fiber cells (21, 30). Cx50 is expressed in both the epithelium and in fiber cells (4, 24, 29).

Another important component of the lens microcirculation system is the sodium leak conductance. The molecular identity of this conductance is still unclear. However, one potential candidate is the undocked connexin hemichannel (16). There is increasing evidence that connexin hemichannels can open under certain physiological conditions without affecting cell viability. For example, Shahidullah et al. (26) showed that hypertonic stress caused the release of ATP in the porcine lens by a pathway that involves connexin and/or connexin hemichannels. In addition to its role in healthy cells, aberrant hemichannel activity has been linked to several human hereditary disorders such as KIDS syndrome (9, 13, 17, 18) and cataracts (19, 23).

We previously showed that Cx50−/− fiber cells lacking Cx50 exhibited a large, nonselective current that was activated by reducing extracellular divalent cations (7). This current was absent in fiber cells isolated from dKO mouse lenses lacking both Cx50 and Cx46 confirming that it was due to Cx46 hemichannels. Here, whole cell current-clamp recordings of freshly dissociated fiber cells from transgenic mouse lenses were used to investigate the hypothesis that Cx46 hemichannels may contribute to the sodium leak conductance observed in the intact lens. Our results suggest that Cx46 channels make a significant contribution to the resting membrane conductance of peripheral fiber cells even in the presence of extracellular divalent cations.

Materials and methods

Transgenic mice. Cx50−/− mice were a generous gift from TW White. dKO mice were generated as previously described by Ebihara et al. (7). All of the mice were in a C57 genetic background. All animal husbandry and experimental procedures were approved by the Rosalind Franklin University Animal Care and Use Committee and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Dissociation of differentiating lens fiber cells. Mice (4–8 wk old) were killed by CO2 asphyxiation and cervical dislocation. The lenses were dissected free from the extracted eyes and placed in M199 with Earle’s balanced salts for 30 min. Typically, four lenses were used for each dissociation. We observed that it was important to keep the lenses continuously submerged in fluid during and following removal of Earle’s balanced salts.

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Electrophysiological recording and analysis. Membrane currents were recorded in dissociated fiber cells using the whole cell patch-clamp technique as previously described by Ebihara et al. (7). The resistance of the patch pipettes was 5 to 10 MΩ when filled with standard internal solution. The internal solution contained the following (in mM): 140 CsCl or 140 KCl, 10 EGTA, 2 MgATP, 3 Na2ATP, 10 HEPES-Na, pH 7.4, and osmolarity 310–320 mosM. The standard extracellular solution was Na-glucuronate Ringer that contained the following (in mM): 150 Na-glucuronate, 4.7 KCl, 2 MgCl2, 5 glucose, 5 HEPES, pH 7.4, and osmolarity 310–320 mosM. The osmolarity was measured using a freezing point micro-osmometer (5004 Micro-osmette; Precision Systems, Natick, MA). All the membrane potentials in the graphs were corrected for liquid-junction potentials after the experiment using the “junction potential calculator” interface (Clampex version 10.2; Molecular Devices). The voltage-clamp protocols were not corrected for the liquid junction potential. They represent the command potentials that were applied to the patch pipette. Measurements of cell membrane parameters in response to 5-mV voltage-clamp pulses from −60 mV were performed using the Membrane test protocol in Clampex immediately following patch rupture. The data are given as means ± SE. N represents the number of fiber cells interrogated. Data were analyzed for their statistical significance using the unpaired Student’s t-test or the one-sample t-test.

Table 1. Membrane properties of isolated fiber cells from Cx50−/− and dKO mouse

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cm, pF</th>
<th>Rm, MΩ</th>
<th>Raccess, MΩ</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx50−/−</td>
<td>66.45 ± 3.39</td>
<td>448.00 ± 36.98*</td>
<td>17.88 ± 1.16</td>
<td>29</td>
</tr>
<tr>
<td>dKO</td>
<td>73.43 ± 10.1</td>
<td>712.08 ± 79.52</td>
<td>15.79 ± 0.97</td>
<td>12</td>
</tr>
</tbody>
</table>

Data represent means ± SE. Membrane properties of fiber cells were measured immediately following patch rupture at −60 mV in the presence of 0 Ca2+ and 2 mM Mg2+. Cm, membrane capacitance; Rm, membrane resistance; Raccess, access resistance; dKO, mouse lenses lacking both Cx50 and Cx46. *P < 0.001, compared with dKO cells by Student’s t-test.

from the eye to avoid damage to peripheral fiber cells. The lenses were then transferred to dissociation buffer (DB; in mM: 170 Na-gluconate, 4.7 KCl, 5 glucose, and 5 HEPES, pH 7.4). Adherent nonlens material was carefully removed from the lens with a pair of forceps. A posterior tear was made in the lens capsule, and the capsule was removed. The epithelial cells and strands of elongating fiber cells remained attached to the capsule. The capsule and adherent epithelial and fiber cells were then transferred to dissociation buffer (DB; in mM: 170 Na-gluconate, 4.7 KCl, 2 MgCl2, 5 glucose, and 5 HEPES, pH 7.4, and osmolarity 310–320 mosM. The osmolarity was measured using a freezing point micro-osmometer (5004 Micro-osmette; Precision Systems, Natick, MA). All the membrane potentials in the graphs were corrected for liquid-junction potentials after the experiment using the “junction potential calculator” interface (Clampex version 10.2; Molecular Devices). The voltage-clamp protocols were not corrected for the liquid junction potential. They represent the command potentials that were applied to the patch pipette. Measurements of cell membrane parameters in response to 5-mV voltage-clamp pulses from −60 mV were performed using the Membrane test protocol in Clampex immediately following patch rupture. The data are given as means ± SE. N represents the number of fiber cells interrogated. Data were analyzed for their statistical significance using the unpaired Student’s t-test or the one-sample t-test.

Fig. 1. Effect of changing external calcium on membrane currents recorded from a Cx50−/− fiber cell using the whole cell patch-clamp technique. A: families of current traces were recorded from the fiber cell shown in B in the presence of 0 Ca2+ and 2 mM Mg2+ (left), after exposure to 1 mM Ca2+ and 1 mM Mg2+ (middle), and finally after exposure to 1 mM Ca2+, 1 mM Mg2+, and 1 mM La3+ (right). A series of depolarizing steps was applied in 10 mV increments between −60 and 80 mV from a holding potential of −60 mV. The intracellular pipette solution in these whole cell patch-clamp experiments contained 140 mM CsCl to block potassium currents. Dashed line represents zero current. Scale bar = 100 µm. C: current-voltage (I–V) relations obtained from the data shown in A. The current was measured at the end of the 8-s pulse and plotted as a function of voltage after correction for liquid junction potential. D: bar graph summarizing the La3+–sensitive current data at the holding potential in the presence of the indicated concentrations of Mg2+ and Ca2+. Data are means ± SE. Number of cells indicated by the values in parentheses. *P < 0.002, compared with the hypothesized population mean of 0.0 using the one-sample t-test.
Fig. 2. La\(^{3+}\) blocks the Cx46 hemichannel current but not the potassium current. A: currents before and after the application of La\(^{3+}\) (1 mM) and the subtracted (La\(^{3+}\)-sensitive) component recorded from a Cx50\(^{-/-}\) fiber cell. Dashed line represents zero current level. B: I–V relations obtained from the data shown in A. The current was measured at the end of the 8-second pulse and plotted as a function of voltage after correction for liquid junction potential. C: bar graph summarizing the La\(^{3+}\)-sensitive current data at −60 mV in the presence of the indicated concentrations of Mg\(^{2+}\) and Ca\(^{2+}\). Data are means ± SE. Number of cells indicated by the values in parentheses. *P < 0.005, compared with the hypothesized population mean of 0.0 using the one-sample t-test.

RESULTS

Our results are based on data from 29 Cx50\(^{-/-}\) fiber cells and 13 dKO fiber cells lacking both Cx50 and Cx46 that had capacitances that ranged from 21.4 to 123 pF, which corresponded to cell lengths between 85 and 531 μm (Table 1). There was a linear relationship between cell length and cell capacitance (data not shown). All of the fiber cells had nuclei indicating that they were isolated from the outer cortex. The absence of Cx50 and Cx46 in the dKO fibers was confirmed by immunolocalization (7).

We used the whole cell patch-clamp technique to study the effects of divalent cations on the nonselective cation current in Cx50\(^{-/-}\) fiber cells that we previously identified as the Cx46 hemichannel current based on a combination of patch-clamp and dye uptake experiments in Cx50\(^{-/-}\) and dKO fiber cells (7). Figure 1A shows a representative family of current traces and corresponding steady-state current-voltage (I–V) relationship recorded from a Cx50\(^{-/-}\) fiber in the presence of zero-added Ca\(^{2+}\) (0 Ca\(^{2+}\)) and 2 mM Mg\(^{2+}\). The pipette solution contained CsCl internal solution to prevent contamination by potassium currents. Application of depolarizing voltage-clamp steps from a holding potential of −60 mV elicited a slowly activating current that reversed polarity from inward to outward at −10 mV. The threshold for activation of the current was approximately −50 mV. Following repolarization to −60 mV, the current closed to a value close to zero over a time course of several hundred milliseconds. Superfusion of the fiber cell with a solution containing 1 mM Ca\(^{2+}\) and 1 mM Mg\(^{2+}\) resulted in a significant reduction in the size of the hemichannel current (Fig. 1B). Both the inward current and the outward current were reduced. Another effect of changing external calcium was to alter the kinetics of activation and deactivation. Increasing calcium caused a slowing of activation and an acceleration of deactivation. These effects were very

Fig. 3. Effect of La\(^{3+}\) on the resting membrane potential of Cx50\(^{-/-}\) fiber cells. A: representative current responses to a voltage-clamp ramp protocol in the presence of 0 Ca\(^{2+}\), 2 Mg\(^{2+}\) (black current trace); 1 Ca\(^{2+}\), 1 Mg\(^{2+}\) (red current trace); or 1 Ca\(^{2+}\), 1 Mg\(^{2+}\), 1 La\(^{3+}\) (blue current trace). Smooth lines are exponential fits of the current response to determine the resting potential. B: plot summarizing the resting potential data determined using the voltage-ramp protocol shown in A after correction for liquid junction potential. Data are means ± SE (n = 12). *P < 0.001, compared with fiber cells treated with 1 mM La\(^{3+}\) by student’s t-test.
similar to those previously described in *Xenopus* oocytes expressing rat Cx46 hemichannels (6, 22, 27).

To determine if the persistent inward current observed at negative membrane potentials was due to Cx46 hemichannels, we used the nonspecific hemichannel blocker, La$^{3+}$ (3, 11). Application of 1 mM La$^{3+}$ completely blocked the voltage- and time-dependent component of the current (Fig. 1C). It also reduced the inward holding current and baseline current fluctuations at −60 mV. By subtracting the current traces recorded in the presence of La$^{3+}$ from the control current traces, La$^{3+}$-sensitive currents could be isolated. The amplitude of the La$^{3+}$-sensitive inward holding current measured in the presence of 1 mM Ca$^{2+}$ and 1 mM Mg$^{2+}$ ranged between −66 and −12 pA ($n = 6$). These results suggest that a small number of Cx46 hemichannels are active and contribute to the holding current and baseline current fluctuations at −60 mV even in the presence of normal concentrations of divalent cations.

To evaluate the relative contribution of potassium conductances and hemichannel conductances to the overall membrane conductance, we performed similar experiments using potassium chloride in the patch pipette. Application of 1 mM La$^{3+}$ caused complete block of the Cx46 hemichannel current without appearing to affect the delayed rectifying K$^{+}$ current indicating that its effects were reasonably specific for connexin hemichannels (Fig. 2). A similar effect was observed when we used 250 μM Gd$^{3+}$ instead of 1 mM La$^{3+}$ (data not shown).

To investigate the effect of hemichannel conductances on the resting potential of the fiber cells, current recordings were obtained in response to 2-s voltage-clamp ramps from −80 to −10 mV from a holding potential of −60 mV, first in the presence of various concentrations of divalent cations and then after block of the hemichannel currents by La$^{3+}$. Reversal potentials were measured by fitting the ramp currents to single exponentials and determining the zero crossing potential as illustrated in Fig. 3A. The results of these experiments are summarized in Fig. 3B. In the presence of 0 Ca$^{2+}$ and 2 mM Mg$^{2+}$, the mean resting potential was −39.7 ± 3.5 (n = 12). Perfusion with solutions containing 1 mM Ca$^{2+}$ and 1 mM Mg$^{2+}$ resulted in little or no change in resting potential. Subsequent perfusion with Na-glucuronate Ringer containing 1 mM Ca$^{2+}$ and 1 mM Mg$^{2+}$ and 1 mM La$^{3+}$ resulted in a significant shift in the resting potential toward the potassium equilibrium potential. The mean resting potential in the presence of La$^{3+}$ was −58.1 ± 1.6 mV (n = 12). The deviation of the resting potential from the potassium equilibrium potential in the presence of La$^{3+}$ can be accounted for by the pipette leak conductance. The resting membrane potential of the fiber cells did not depend on cell length. Fiber cells ranging from 50 to

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![Fig. 4](http://ajpcell.physiology.org/) Cx50$^{-/-}$ fibers show a much larger increase in inward holding current in response to removal of external calcium than mouse lenses lacking both Cx50 and Cx46 (dKO) fibers. A: representative currents, obtained from a Cx50$^{-/-}$ fiber in response to changing external divalent cation concentrations to the indicated values. The fiber was continuously held at a holding potential of −60 mV in the presence of different concentrations of Mg$^{2+}$ and Ca$^{2+}$ for the experiment shown in A. B: bar graph summarizing the holding current data at −60 mV in the presence of different concentrations of Mg$^{2+}$ and Ca$^{2+}$ for the experiment shown in A. C: representative current response obtained from a dKO fiber at −60 mV in response to changing external divalent cations concentrations to the indicated values. D: bar graph summarizing the holding current data at −60 mV in the presence of different concentrations of Mg$^{2+}$ and Ca$^{2+}$ for the experiment shown in C.
600 μm showed very similar values for resting membrane potential. These results suggest that both potassium conductances and connexin hemichannel conductances contribute to the resting conductance of the peripheral fiber cells under physiological conditions.

Genetic ablation of Cx46 reduces the inward holding current and membrane noise under resting conditions. To further examine the contribution of Cx46 hemichannels to the resting membrane conductance, we applied different concentrations of external divalent cations to fiber cells isolated from Cx50−/− and dKO mouse lenses and compared the effect that this had on the holding current measured at −60 mV. For these experiments, pipettes were filled with CsCl internal solution to minimize contamination by potassium channels. Figure 4A shows a representative example of an experiment performed on long fiber cell isolated from a Cx50−/− mouse lens that was continuously held at a holding potential of −60 mV. The bath solution initially contained 0 Ca2+ and 2 mM Mg2+. The fiber cell was then superfused with Na-glucor Mepic Ringer containing no added divalent cations, which resulted a large increase in the inward holding current at −60 mV. This increase in holding current could be completely reversed by reperfusing the cell with Na-glucor Mepic Ringer containing 2 mM Mg2+. Subsequent perfusion with Na-glucor Mepic Ringer containing 1 Ca2+ and 1 mM Mg2+ caused a slight decrease in holding current.

Figure 4B shows a representative example of an experiment performed on a fiber cell isolated from a dKO mouse lens using the protocol described above. Removal of external divalent cations caused a small but reproducible increase in the holding current at −60 mV that could be reversed by perfusing the cell with Na-glucor Mepic Ringer containing 0 Ca2+ and 2 mM Mg2+. Subsequent perfusion with Na-glucor Mepic Ringer containing 1 or 2 mM Ca2+ and 1 mM Mg2+ caused little or no change in the holding current. A population comparison of the holding current between Cx50−/− and dKO fibers (Fig. 5A) showed that in the absence of divalent cations, the inward holding current was ~18 times larger in Cx50−/− cells than it was in dKO cells. A small but significant increase in inward holding current was also observed in the Cx50−/− cells in the presence of divalent cations. Cx50−/− fibers also showed a significantly increased level of baseline current fluctuations as illustrated in Fig. 5B, which shows sample current traces for representative fiber cells isolated from Cx50−/− and dKO lenses in the presence of 0 Ca2+ and 2 mM Mg2+. A population comparison of the current fluctuations showed an increase in mean current fluctuations in Cx50−/− fibers in the presence of divalent cations. These experiments suggest that although Cx46 hemichannels occurred less frequently in the presence of divalent cations, they were clearly present even at −60 mV.

**Effect of La3+ on membrane conductances in dKO fibers.** We were concerned that La3+ might be affecting other membrane channels in addition to connexin hemichannels. To test for this possibility, we examined the effect of La3+ on whole cell membrane currents in dKO fibers. Figure 6 shows whole cell currents recorded from a long, dKO fiber cell with KCl solution in the pipette. Under these conditions, the whole cell recording showed a predominant, delayed rectifying potassium current but lacked any indication of a connexin hemichannel current. Application of 1 mM La3+ had little or no effect on the membrane currents at potentials more negative than −20 mV. However, at more positive potentials, La3+ blocked a small, slowly activating inward current that appeared to reverse polarity at large, positive potentials. The voltage-gating properties of this current resembled those of the L-type calcium

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**Fig. 5.** dKO fibers show reduced inward holding current and basal current fluctuations at −60 mV. A: population response of holding current between Cx50−/− (black bars) and dKO fibers (gray bars). Data are means ± SE. Results are based on data obtained from 6 Cx50−/− fibers and 8 dKO fibers. *P < 0.001, compared with dKO by Student’s t-test. **P < 0.01, compared with dKO by Student’s t-test. B: sample current traces for representative fiber cells isolated from Cx50−/− and dKO lenses at −60 mV in the presence of 0 extracellular Ca2+ concentration and 2 mM extracellular Mg2+ concentration. C: population comparison of the basal current fluctuations at −60 mV showed an increase in mean current fluctuations in Cx50−/− fibers relative to dKO fibers in the presence of divalent cations. The size of the current fluctuations about the mean was quantified by calculating the standard deviation of a 1.5-s segment of the current trace. *P < 0.001, compared with dKO by unpaired Student’s t-test.
current, which is blocked by μM concentrations of La$^{3+}$ (2, 12). Figure 6 summarizes the effect of La$^{3+}$ on the amplitude of the holding current at $-60$ mV. No significant change in holding current was observed following application of La$^{3+}$ in the presence of 1 mM $[\text{Ca}^{2+}]_o$. Furthermore, voltage-clamp ramp experiments showed that La$^{3+}$ had no effect on the resting potential of the dKO fiber cells (Fig. 6).

**DISCUSSION**

In this study, we addressed two questions. First, do Cx46 hemichannels contribute to the resting membrane conductance in Cx50/−/− fiber cells? Second, can any additional sodium selective or nonselective cation conductances be detected by patch-clamp methods? Our results suggest that there are two main cation channels that contribute to the resting membrane conductance of the Cx50/−/− fiber cells: potassium channels and Cx46 hemichannels. In the presence of normal concentrations of divalent cations, blocking Cx46 hemichannels using the nonspecific hemichannel blocker, La$^{3+}$, resulted in a 20–30 pA reduction in the inward holding current at $-60$ mV (corresponding to 1 or 2 hemichannels) and a negative shift in the resting membrane potential toward the potassium equilibrium potential. A similar shift in the resting membrane potential was observed when connexin hemichannels were genetically deleted. Since the input conductance of fiber cells lacking connexin hemichannels was $\sim 1,400$ pS, we would predict that if even 1 Cx46 hemichannel was open at $-60$ mV, it should be sufficient to cause an increase in input conductance and result in significant shift in the resting membrane potential as observed experimentally. In addition, we consistently observed that Cx50/−/− fiber cells exhibited increased open channel noise at negative potentials compared with dKO fiber cells. This increase in open channel noise disappeared when the Cx46 hemichannel current ran down during prolonged patch-clamp experiments (data not shown) and was absent in Cx50/−/− fibers treated with La$^{3+}$ suggesting that it was due to the reopening of Cx46 hemichannels.

It has been proposed that a better candidate for the sodium leak conductance is a Gd$^{3+}$-insensitive, La$^{3+}$-sensitive conductance that is activated by cell shrinkage (10). However, we did not observe a significant La$^{3+}$-sensitive, cation current in dKO mouse fibers at potentials more negative than $-20$ mV in

Fig. 6. La$^{3+}$-sensitive currents in dKO fibers. A: currents before and after the application of La$^{3+}$ (1 mM) and the subtracted (La$^{3+}$-sensitive) component recorded from the dKO fiber cell shown in B. Dashed line represents zero current level. Scale bar = 100 μm. The internal pipette solution contained 140 mM KCl. The bath solution initially contained zero-added calcium and 2 mM Mg$^{2+}$. C: $I-V$ relations obtained from the data shown in A. The current was measured at the end of the 8-s pulse and plotted as a function of voltage after correction for liquid junction potential. D: bar graph summarizing the La$^{3+}$-sensitive current data at $-60$ mV in the presence of 0 Ca$^{2+}$, 2 Mg$^{2+}$ or 1 Ca$^{2+}$, 1 Mg$^{2+}$. Data are means ± SE. Number of cells indicated by the values in parentheses. *P < 0.01, compared with the hypothesized population mean of 0.0 using the one-sample $t$-test. E: plot summarizing the resting potential data after correction for liquid junction potential. Data are means ± SE ($n = 5$).
the presence of millimolar Ca\textsuperscript{2+}. Furthermore, the resting potential in the dKO fibers was insensitive to external divalent cations or La\textsuperscript{3+} suggesting that the effects of these agents in Cx50\textsuperscript{–/–} fibers were primarily due to their blocking actions on Cx46 hemichannels. In addition, we did not observe cell shrinkage when dKO fibers were bathed in isotonic Na-gluconate Ringer. One source of variation between these studies could arise from species variations or alterations in the pattern of gene expression in transgenic mouse lenses lacking lens fiber connexins. Although we cannot completely rule out this possibility, the peripheral fiber cells isolated from the Cx50\textsuperscript{–/–} and dKO fibers appeared to be remarkably normal in terms of their morphology in agreement with findings previously reported by Xia et al. (32) and White et al. (30). Furthermore, we did not detect any obvious functional differences between the dKO fibers and the Cx50\textsuperscript{–/–} fibers except for the loss of connexin hemichannels.

Another potential source of variability comes from the methodology used to dissociate the fiber cells. In the studies reported by Gunning et al. (10), peripheral fiber cells were studied in the presence of the nonselective cation channel blocker Gd\textsuperscript{3+} to prevent fiber cell vesiculation and death. In contrast, we used lenses from transgenic mice that either lacked Cx50 or both Cx46 and Cx50 to reduce the size of the nonselective cation current activated during the dissociation process (7). Following cell dissociation, the fiber cells were maintained in Na-glucorate Ringer containing zero added cations or La\textsuperscript{3+} to prevent cell swelling and calcium loading. We also made several modifications in our dissociation protocol to improve the yield of healthy fiber cells. Instead of incubating the capsule in collagenase at 37°C, we incubated the capsule and adherent fiber cells in low concentrations of collagenase and protease at room temperature for 15–20 min and then mechanically dislodged the fiber cells from the capsule by tituration. The main advantage of our approach is that it allows us to study connexin hemichannels, other mechanosensitive channels and calcium channels, all of which are blocked by Gd\textsuperscript{3+} (1, 2, 8, 14, 28).

In conclusion, our results suggest that Cx46 hemichannels may play an important role in the lens internal circulation system by allowing the entry of sodium from the extracellular space into the lens fiber cells. Much remains to be learned about how Cx46 hemichannels are regulated in the lens under both physiological and pathophysiological conditions. It is likely that many of the same factors that have been shown to regulate connexin hemichannels in other tissues (25) also regulate Cx46 hemichannels in the lens.

REFERENCES


