Chloride Channel Expression in Cultured Human Fetal RPE Cells: Response to Oxidative Stress

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PURPOSE. The human fetal cell line RPE 28 SV4 has been useful for studies of oxidative stress and apoptosis in retinal pigmented epithelium. This cell model is now assessed in functional investigations of chloride channel activity. The study aims to determine the presence of specific chloride channels, including CFTR and ClC channels, to identify the properties of membrane chloride currents and to assess their modulation by hydrogen peroxide, cAMP, and other agents.

METHODS. Channel expression was determined using RT-PCR and cDNA cloning and biochemical and immunocytochemical methods. Membrane currents were analyzed using whole-cell, patch-clamp techniques.

RESULTS. RT-PCR results confirmed the presence of ClC-5 mRNA, and a full-length clone encoding ClC-3 was isolated from a cDNA library for RPE 28 SV4 cells. Specific staining for CFTR and several ClC channels was detected by immunocytochemistry. Whole-cell chloride currents (under conditions of symmetrical chloride concentrations) averaged 16.9 ± 3.4 pA/pF (at +100 mV; n = 8), showed outward rectification, and had an anion permeability sequence of Cl− > I− > cyclamate. Currents were stimulated by cAMP cocktail (250 μM cAMP, 100 μM IBMX, and 25 μM forskolin) and were inhibited by 1 mM DIDS. The oxidative agent hydrogen peroxide (100 μM) decreased the current by 34% ± 10% (n = 4).

CONCLUSIONS. These findings suggest that RPE 28 SV4 cells possess regulated chloride channels including CFTR and members of the ClC chloride channel family. The inhibition of chloride currents by H2O2 suggests that this cell line may be advantageous for studies of chloride channel modulation by oxidative stress. (Invest Ophthalmol Vis Sci. 2000;41:4247–4255)

The retinal pigment epithelium (RPE) forms a diffusion barrier between the subretinal space and the choroidal blood supply and regulates the transport of fluid, metabolites, and electrolytes between the sensory retina and the serosa. Studies of isolated RPE epithelium and cultured RPE cells indicate that chloride transport is important in several RPE functions including fluid absorption,1,2 volume regulation,3–7 and ligand-regulated ion and fluid transport.8–11

Chloride absorption across human RPE is mediated by entry across the apical membrane via a Na+-K+-2Cl− cotransporter and passive exit through chloride channels in the basolateral membrane.4,12–14 RPE cells also possess a secretory pathway for chloride that is mediated by chloride–bicarbonate exchange across the basolateral membrane and efflux across the apical membrane by apical membrane chloride channels.15

The direction of fluid transport depends on the relative magnitude of the absorptive and secretory chloride fluxes and passively follows the net movement of chloride across the epithelium.

Chloride channels in RPE are regulated by several factors in the vitreous, including ATP and epinephrine.2,11,15 In addition, the environment of the RPE is exposed to high levels of reactive oxygen species such as hydrogen peroxide and superoxides, which are released by immune cells during inflammatory conditions and during normal physiological processes such as phagocytosis.6,17 In addition, there is indirect evidence for involvement of oxidative damage to the RPE in the clinical disorder of age-related macular degeneration (AMD).18 It is presently unknown whether ion channels or transport proteins in RPE are affected by reactive oxygen species. However, in other epithelia recent studies of chloride channels including CFTR19 and other channels20,21 have demonstrated modulation of channel activities by oxidative agents (for review, see Ref. 22).

The present study focuses on the development of a cultured model system for the studies of chloride channel regulation by oxidative agents. Cultured epithelial cells have proven to be advantageous model systems for studies of ion channel regulation for several chloride-transporting epithelia such as the renal distal tubule and colon.25 Cultured human fetal RPE cells (RPE 28 SV4) have recently been used as a model for studies of oxidative damage of mitochondrial DNA, apoptosis, and oxidant induction of glutathione S-transferase expression in RPE cells.24,25 We now extend our investigations of these cells to assess chloride channel expression and regulation.
As a first step in these investigations, we have used immunocytochemical and molecular biological methods to detect the expression of known chloride channels in cultured human fetal RPE cells. Peterson et al.15,20 have reported expression in RPE of CFTR, a chloride channel that is defective in the human genetic disorder cystic fibrosis. In addition, members of the CIC family of voltage-gated chloride channels are also known to be present in retinal neurons and ciliary epithelial cells.27–29 Humans are known to express at least nine members of this gene family, and loss of function mutations of some CIC channels have been linked to specific human genetic diseases.30 For these reasons, we first sought to identify the presence of CFTR and CIC channels in RPE 28 SV4 cells. In a second aspect of these studies, the membrane conductance properties of these cells were measured using whole-cell, patch-clamp methods and the effects of 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid (DIDS), cAMP, and oxidative stress on membrane conductances were determined.

The following issues were addressed: (1) Do cultured human fetal RPE cells express CIC chloride channels? (2) Do these cells show chloride conductances with properties similar to those reported for other native RPE cells? and (3) Do oxidative agents modulate RPE conductance properties?

**METHODS**

**Cell Culture Methods: Human Fetal RPE Cell Line**

SV40 transformed human fetal RPE cells (RPE 28 SV4; Coriell Institute, Camden, NJ) were grown in Eagle’s minimum essential medium (MEM; Sigma Aldrich Corp., St. Louis, MO) supplemented with 1% penicillin and streptomycin and 10% fetal bovine serum (FBS; Hyclone Laboratories, Inc., Logan, UT). The cells were plated at subconfluent density on glass coverslips coated with poly-o-lysine. The cells were incubated in a humidified incubator at 37°C in 95% air and 5% CO₂ overnight or were grown to confluence for a period of 3 to 7 days.

**Immunocytochemistry**

**Antibodies.** Because antibodies were available for CIC-2, CIC-3, and CIC-5, our immunocytochemical studies focused on these CIC channels. Polyclonal antibodies for CIC-2 and CIC-3 were obtained commercially from Alamone Laboratories (Jerusalem, Israel). For CIC-5, the polyclonal antibody C1 was used (for details, see Ref. 31). All these antibodies were raised against residues corresponding to the C-terminal regions of rat CIC proteins. CIC-2 antibody was raised to residues 888 to 906, CIC-3 to residues 593 to 661, and CIC-5 (C1) was raised to residues 570 to 677. Monoclonal CFTR antibody was raised to seven amino acids located at the C-terminus and was obtained from Genzyme (catalog no. 2503–01; Cambridge, MA).

**Assay.** The cells were fixed in chilled (~20°C) methanol for 8 minutes and then washed with sterile filtered phosphate-buffered saline (PBS) solution and incubated overnight at 4°C with primary antibody for CIC-2, CIC-3, CIC-5, or CFTR. Coverslips were then washed in PBS and incubated at room temperature for 1 hour with secondary antibody (Alexa 488 goat anti-rabbit IgG or Alexa 546 goat-anti-mouse IgG; Molecular Probes, Inc., Eugene, OR) followed by a wash in PBS for 1 hour in darkness. Specimens were mounted in Fluorosave (Calbiochem Corp., La Jolla, CA) on glass slides and stored in the dark at 4°C.

**Digital Imaging.** Cells were viewed using a Nikon eclipse E800 epifluorescent microscope equipped with a digital camera and interfaced to a laboratory computer (Micron Electronics, Los Angeles, CA). Images were acquired and visualized using commercially available software (MetaMorph version 4.0B9; MetaSystems, Belmont, MA) and appropriate excitation and emission bandwidths for Texas Red and fluorescein. Pseudocolor processing of images was achieved using software (Adobe Photoshop, San Jose, CA).

**Protein Isolation and Western Blot**

The protocol for Western blot analysis was modified from Chilarron et al.32 RPE cells were washed in Dulbecco’s PBS (Sigma-Aldrich) and homogenized in fractionating medium (FM: 25 mM Tris-HCl and 100 mM mannitol, pH 7.2) with 0.5 mM PMSF. Cells were then lysed by sonication on ice for 15 seconds, and centrifuged for 15 minutes at 10,000 rpm to remove insoluble components. Cell membranes were pelleted at 100,000g and resuspended in FM with 0.5 mM PMSF. For Western blot, 25 μg of protein sample in LDS buffer (NP0007; Novex, Inc., San Diego, CA) was boiled 5 minutes, loaded in a lane of a precast gel (NuPage Bis-Tris Gel 4% to 12%; Novex, Inc.), electrophoresed, and transferred to nitrocellulose membrane (Novex Inc.). The membrane was then blotted overnight with a polyclonal antibody to CIC-5 or CIC-3. For peptide blocked controls, the same incubation protocol was used except that antigenic peptide was added in a 5:1 ratio of peptide to primary antibody.

**Patch-Clamp Recordings of Whole-Cell Currents**

Solutions for patch-clamp experiments are as follows (in mM): bath = 130 tetramethylammonium chloride (TMA-Cl), 2 NaH₂PO₄, 2 calcium cyclamate, 1 MgSO₄, 5 glucose, 10 HEPES; pipette = 130 TMA-Cl, 0.2 calcium cyclamate, 3 MgSO₄, 2 EGTA, 10 HEPES, 3 Na-ATP. The pH of the solutions was 7.4, and the osmolalities were 300 and 270 mOsm/kg H₂O, respectively. Pipettes are constructed from borosilicate glass (Corning 7052, Warner Associates, Inc., Hamden, CT) pulled in two stages to a tip diameter of 1 to 2 μm, (3–6 MΩ) and fire polished. For recordings, the pipette is connected to an Ag-AgCl wire led to the head stage of a patch clamp (Axopatch 200B; Axon Instruments, Foster City, CA) and is positioned next to the cell using a low-drift micromanipulator (PCS-5200; Burleigh, Fishers, NY) under observation with an inverted phase contrast microscope (Zeiss IM, Thornwood, NY). Stimulus control and data acquisition and processing are carried out with a Pentium PC and A/D interface, using commercially available data acquisition and analysis software, (DigitData 1200 and pClamp 6.05 software; Axon Instruments, Inc.). Electrode offset is balanced before forming a gigaseal, and capacitative current is cancelled using circuitry on the amplifier (seal resistances > 5 GΩ). Currents are low-pass Bessel filtered at 5 kHz and digitized at 10 kHz for storage and analysis. Solution changes and drug delivery were achieved by a gravity-drive superfusion system. The bath reference electrode consisted of a 3 M KCl agar bridge led to ground. Solution junction potentials were negligible (<3 mV).

**RT-PCR Methods and Cloning of CIC-3 from RPE cDNA Library**

RT-PCR methods were similar to those previously described in Lindenthal et al.33 Cloning methods were similar to those
previously described in Mo et al. A CDNA library was made from size-selected (2–4 kb) mRNA harvested from continuously cultured human fetal RPE cells. The cDNA library was ligated into the ZAP-Express Lambda vector and subsequently probed at high stringency with a small internal fragment of human ClC-3 made by RT-PCR. Hybridizing clones were plaque-purified using three rounds of selection followed by in vivo excision. The resulting cDNA clones were analyzed using restriction analysis and sequenced using an automated DNA sequencer (model 373; Applied Biosystems; Foster City, CA) and synthetic primers.

**Solutions and Drugs**

Forskolin (FSK), 8-bromo-adenosine 3'-5' cyclic monophosphate (cAMP), hydrogen peroxide, and DIDS were from Sigma-Aldrich. 3-Iosbutyl-1-methyl-xanthine (IBMX) was from Biomol Research Laboratory., Inc. (Plymouth Meeting, PA), and glutathione was from Calbiochem (La Jolla, CA). All drugs were dissolved in DMSO, except cAMP which was dissolved in distilled water.

**Statistics**

Results are presented as mean values and SEMs. Paired t-tests or nonparametric test were used to evaluate statistical significance, as appropriate.

**RESULTS**

Cloning of ClC Chloride Channels in RPE Cells

As an initial step in detecting ClC channels in RPE cells, mRNA was extracted from the cultured fetal RPE cells using methods previously reported by Lindenthal et al., and cDNA was synthesized. In addition, a cDNA library was constructed following methods described by Mo et al. Screening of the library using a 1-kb gene-specific probe for ClC-3, led to the identification of a 2.9-kb clone that was plaque purified and sequenced in two directions. The cDNA sequence (shown in Fig. 1) encoded the entire open reading frame (ORF) and untranslated 5' and 3' regions. The predicted protein contains 818 amino acids and has a predicted molecular weight of 91 kDa. The ORF was identical with that reported for ClC-3 for human lens by Rae and Shepard (Accession no. AF29346, unpublished). Untranslated regions (UTRs) of the nucleotide sequence were not reported by these investigators; however, Borasani et al. reported both UTRs and ORFs for ClC-3 cloned from the human retina. In the present sequence, positions 74 to 432 in the 5' UTR and positions 2884 to 2990 for the 3' UTR were the same as reported by Borasani et al. However, a difference was noted in the ORF. The nucleotide sequence reported by Borasani et al. included six additional base pairs after position 2374, resulting in the insertion of a glutamic acid and phenylalanine (EF) at amino acid position 648 not found in the present sequence or in the previous human lens sequence (Rae JL and Shepard AR, unpublished results).
A “short” isoform of ClC-3 that had a 58 amino acid deletion at the N terminus was previously reported for rat ClC-3 cDNA sequence by Kawasaki et al. Both “long” and short isoforms of ClC-3 have been recently identified in rat hepatocytes by Shimada et al. Using primers generated for gene-specific sequences in the N-terminal domain and RT-PCR, we did not detect the short isoform for ClC-3 in fetal RPE cells. Therefore, the cultured human fetal RPE cells used in this study appear to have only the long isoform.

In additional studies, we used RT-PCR to amplify a second ClC channel. Using human RPE cRNA and gene-specific primers for ClC-5 located within the ORF, 291bp fragment of the expected size was obtained that had a sequence that corresponded to bp 715 to 1006 of human ClC-5 (Accession number NM_00008437). The full-length ClC-5 protein contains 746 amino acids and has a predicted molecular weight of 83 kDa.

To confirm the expression of ClC-3 and ClC-5 channel proteins in RPE, Western blot analyses of human RPE proteins were performed. As shown in Figures 2A and 2B, using polyclonal antibodies to rat ClC-3 or ClC-5, single bands at the predicted molecular weights (~90 and ~80 kDa, respectively) were obtained. Staining was not observed in the presence of blocking peptide (data not shown).

**Chloride Channel Immunocytochemistry**

The expression of chloride channel proteins further assessed using immunofluorescence imaging. Cells were plated at sub-confluent densities, grown overnight, permeabilized, and stained with primary antibodies for ClC-2, ClC-3, ClC-5, or CFTR. The secondary fluorescent antibody was Alexa 546 goat anti-rabbit IgG (for the monoclonal CFTR antibody) or Alexas 594 goat anti-mouse IgG (both from Molecular Probes, Inc.). The primary antibodies available for these studies were raised to the C-terminal domains of the above chloride channel proteins. Because ClC-3 and ClC-5 have some potential sequence homology in this region, we next determined the cross-reactivity of these ClC antibodies. Figure 3 shows brightfield and fluorescence images of RPE cells stained with ClC-3 antibody in presence or absence of blocking peptides. Figure 3 (panel 2) shows ClC-3 antibody staining in the absence of blocking peptides. No fluorescence was evident when cells were stained in the presence of blocking peptide for ClC-3 (panel 4) or in the absence of primary ClC-3 antibody (panel 10). When synthetic peptides for regions of the C-terminal domains of ClC-2 or ClC-5 were present (panels 6 and 8, respectively), the staining for ClC-3 was not significantly affected. Figure 5 shows staining for ClC-5 antibody and the block of staining by ClC-5 antigenic peptide. As in the case of ClC-3 antibody, peptides for ClC-3 and ClC-2 did not block ClC-5 antibody staining (data not shown). These results indicate that the ClC-3 and ClC-5 antibodies specifically stain their respective ClC channels. As shown in Figures 4 and 5, staining was found throughout the cytoplasm and near the perinuclear region for cells stained with antibody for ClC-3 and ClC-5. Similar results were obtained for cells stained with ClC-2 and CFTR (Fig. 6). ClC-2 staining was blocked by ClC-2 peptide (data not shown; note: the CFTR antibody is a monoclonal antibody, and therefore binding of this antibody is likely to be to a single specific epitope.)

**FIGURE 2.** Immunoreaction of antibodies to ClC-3 (A) and ClC-5 (B) to RPE membrane proteins. The ClC-3 antibody recognized an ~90-kDa protein, and the ClC-5 antibody recognized an ~80-kDa protein, with the expected sizes for ClC-3 and ClC-5, respectively.

**FIGURE 3.** RPE cells stained with antibody to ClC-3 (ClC-3 Ab) in the presence or absence of blocking peptides. Left panels: differential interference contrast images; right panels: fluorescence images of the same cells. Panel 2, ClC-3 Ab staining without blocking peptide; panel 4, inhibition of staining in the presence of blocking peptide for ClC-3; panels 6 and 8, the effects of peptides from corresponding regions of the C-terminal domain of ClC-2 (panel 6) and ClC-5 (panel 8). Panel 10 lacks ClC-3 Ab (negative control). The results indicate that ClC-3 Ab is specific for ClC-3 compared with other ClC proteins. Scale bar, 100 μm.
Membrane Conductance Measurements

For electrophysiological recordings, cells were plated on polylysine-coated glass coverslips and incubated overnight (for details, see Methods). Figure 7 shows the average whole-cell current measurements for six cells measured under conditions of symmetrical chloride solutions in the bath and pipette. A moderately outwardly rectifying current at positive holding potentials was observed in all cells. Currents were stable within 2 minutes and did not change over the recording periods (typically 10–30 minutes). In addition, there was no detectable time dependence in the currents (Fig. 7, see insert). Similar results were recently obtained in preliminary studies of primary cultures of adult human RPE (data not shown).

As shown in Figure 8, substitution of chloride in the bathing solution by 130 mM cyclamate led to a decrease in the current as expected for a large chloride to cyclamate permeability ratio. Iodide substitutions of chloride had a similar effect. The reversal potential was not significantly different from zero in symmetrical chloride solutions (−2 ± 1 mV) and shifted to a positive direction after iodide or cyclamate replacement of the bathing solution (8 ± 2 and 13 ± 3 mV, respectively). The permeability ratio was calculated using the modified Goldman-Hodkin-Katz equation:

\[
V_c - V_e = 58 \log \left( \frac{[X^-]_i}{P_{Cl}} + [Cl^-] \right) \left( \frac{[Cl^-]}{[Cl^-]_c} \right)
\]

where \( V_c \) is the reversal potential in the control solution, \( V_e \) is the reversal potential in the experimental solution (containing anion \( X^- \)), \([Cl^-]_i\) is the \( Cl^- \) concentration in the control bathing solution, \([Cl^-]_c\) is the \( Cl^- \) concentration in the experimental solution, \([X^-]_i\) is the substituted anion concentration, and \( P_{Cl} \)
and $P_X$ are the permeabilities for chloride and the substituted anions, respectively. The relative anion permeability sequence calculated from the reversal potential measurements was 1: 0.66 ± 0.14: 0.46 ± 0.18 for Cl⁻:I⁻:cyclamate, respectively. Using the approach of Mo et al., a similar sequence was calculated for the relative anion conductance (Cl⁻:I⁻:cyclamate = 1: 0.63 ± 0.17: 0.47 ± 0.18).

Application of the chloride channel blocker DIDS (1 mM) reduced the whole-cell currents (Fig. 9). The current was inhibited by 39.7% ± 5% at +100 mV and by 37.1% ± 3% at −100 mV ($n = 4$, paired measurements, $t = 5.23; P < 0.05$). Therefore, no voltage sensitivity was apparent for the DIDS block.

Modulation of the Membrane Conductance

To determine whether the membrane conductance is altered by intracellular cAMP, we tested the effects of a cocktail containing 250 μM 8-Br-cAMP, 100 μM IBMX, and 25 μM forskolin. The results are shown in Figure 10. The current was increased by 34% ± 7% at +100 mV and by 31% ± 14% at −100 mV ($n = 4$, paired measurements, $t = 2.09; P < 0.05$) within 3 minutes of the application of the cAMP cocktail. Therefore, the cellular conductance is apparently stimulated by agents that increase cAMP.

As noted above, RPE cells in vivo produce intracellular oxidative agents such as hydrogen peroxide (H₂O₂). For this reason, we next assessed the effects of H₂O₂ on the membrane chloride conductance. As shown in Figure 11A, addition of 100 μM H₂O₂ to the bathing solution reduced the RPE chloride currents over the measured voltage range, with an average decrease on 38% ± 6% at +100 mV and 34% ± 5% at −100 mV ($n = 5$, paired measurements, $t = 4.81; P < 0.01$). The effects were rapid and occurred within 1 minute of exposure. After washout of H₂O₂, the currents typically recovered with 98% of their initial levels within 3 minutes (Fig. 11B).

**DISCUSSION**

The importance of these findings is threefold. First, the results indicate that the cultured human fetal RPE cell line RPE 28 SV4 is capable of expressing several ClC channels and CFTR. Specifically, the results showed that these cells expressed ClC-2, ClC-3, ClC-5, and CFTR. These findings raise the possibility that ClC channels could play a role in RPE cell functions such as fluid and ion transport or cell volume regulation. Perhaps more importantly, the chloride conductance of these cells was modulated by cAMP and DIDS, similar to native RPE cells. Consequently, the RPE 28 SV4 cell line apparently retains some of the major features of chloride transport found in native cells. Lastly, the chloride conductance was inhibited by H₂O₂. These findings raise the possibility that chloride conductances in RPE cells may be impaired during oxidative stress.

**Molecular Identity of Chloride Channels**

The present results provide evidence for ClC channel expression in RPE cells. First, a full-length clone with a sequence identical with human ClC-3 was isolated from our cDNA library for RPE 28 SV4 cells. In addition, a partial clone of human ClC-5 was obtained from this cell line by RT-PCR. The presence of mRNA for these channels, however, does not prove protein expression. For this reason, Western blot analysis was used to confirm the expression of ClC-3 and ClC-5 proteins in these cells. In both cases, stained protein bands of the appropriate
size were detected. To obtain further information about the expression of CFTR and CIC channels, immunocytochemistry was used to detect CIC-2, CIC-3, CIC-5, and CFTR expression. The results of these studies also indicate that several CIC channels and CFTR are expressed in these cells.

Peterson et al.\textsuperscript{15,26} previously reported the expression of CFTR for native human and bovine RPE cells. The present findings confirm that CFTR is also expressed in actively dividing (subconfluent) transformed human fetal RPE cells. The identification of mRNA for CIC-2, CIC-3, and CIC-5 suggests that several CIC channels may also play a role in mediating chloride transport functions in these cells. Work is presently underway to determine the expression and distribution of these channels in the adult human RPE.

In the present study, we isolated a cDNA encoding CIC-3, which has a nucleotide sequence that is identical with that previously reported by Rae and Shepard for CIC-3 from human lens (Accession no. AF29346). This sequence differs slightly from CIC-3 previously cloned from the human retina by Borisani et al.\textsuperscript{9} in the 5' and 3' untranslated regions. In addition, there is a 6-bp deletion in the nucleotide sequence, resulting in the deletion on two amino acids (EF) at positions 648 to 649. It is notable that CIC-3 channels are highly conserved, and rat and human homologues for CIC-3 differ by only two amino acids.\textsuperscript{27} Recently, two isoforms of CIC-3 channel have been identified in the rat, a short form and a long form resulting in the deletion on two amino acids (EF) at positions 648 to 649. It is notable that CIC-3 channels are highly conserved, and rat and human homologues for CIC-3 differ by only two amino acids.\textsuperscript{27} Recently, two isoforms of CIC-3 channel have been identified in the rat, a short form and a long form that contains an additional sequence of 58 amino acids at the N-terminus of the protein.\textsuperscript{36} The predicted amino acid sequence for our human CIC-3 clone had a 99% homology to that previously reported by Rae and Shepard for ClC-3 from human lens.

The present results demonstrated weak outward rectification of the chloride currents for membranes in symmetrical (high) chloride solutions. Previous whole-cell, patch-clamp studies of RPE ells have varied with respect to the degree of outward rectification of the membrane chloride currents and the relative anion selectivity of the chloride conductance. For example, Botchkin and Matthews\textsuperscript{6} reported that swelling-activated chloride currents were outwardly rectifying but only when low intracellular chloride concentrations were used. Hughes and Segawa\textsuperscript{9} reported a linear cAMP-activated chloride conductance in amphibian RPE cells. In contrast, Strauss and coworkers found voltage-dependent chloride currents activated by IP\textsubscript{3} and tyrosine kinase\textsuperscript{44} or by protein kinase C-dependent phosphorylation.\textsuperscript{46} The reasons for these differences are unclear but may involve activation of different chloride channel populations under different conditions of the above studies.

The relative anion selectivity of the chloride conductance measured in the present study was Cl\textsuperscript{−} > I\textsuperscript{−}. This finding is consistent with either CFTR\textsuperscript{52} or CIC channels.\textsuperscript{53} In contrast, Hughes and Segawa\textsuperscript{9} reported that the cAMP-activated chloride conductance was more permeable to iodide than chloride for amphibian RPE cells. Swelling-activated chloride conductances are also typically more permeable for iodide than chloride (for review, see Ref. 53). The anion permeabilities of the cAMP-or
swelling-activated chloride conductances were not evaluated in the present study.

**Modulation and Mediation of RPE Chloride Conductances**

The stimulation of membrane conductances of RPE 28 SV4 cells by cAMP is in agreement with previous reports for native RPE cells by Hughes and coworkers. The nature of the DIDS-sensitive chloride conductance was not identified in the present study. Several candidate chloride channels could mediate this conductance, including ClC-2. DIDS is known to block ClC-2, and this channel is expressed together with cAMP in airway epithelia. Further studies are needed to determine whether ClC channels such as ClC-2 or ClC-3 contribute to this conductance.

**Oxidant Inhibition of Chloride Conductance**

A novel finding of the present study was the reversible inhibition of the cellular chloride conductance by H2O2. Efforts are currently underway to assess whether the effects of this oxidative agent are abolished in the presence of the cytoprotective antioxidants such as glutathione. Reactive oxygen species including H2O2 are known to inhibit a wide variety of transporters in different tissues from a number of species (for review, see Ref. 22). In epithelial cells these agents have been previously shown to inhibit epithelial sodium channel activity and to derange the polarity of renal cells. However, relatively few studies have examined the effects of these agents on chloride channel activity.

The chloride channel from the apical membrane of bovine trachea and the voltage-activated anion-selective channel from the mitochondrial outer membrane have been shown to be regulated by an oxidative-reduction mechanism. Oxidative agents are also thought to affect the permeability properties of other ocular epithelial cells. In studies of cataract formation in the lens, Specter et al. found that exposure to reactive oxygen species for 2 to 16 hours led to epithelial damage, which preceded a loss of transparency. They postulated that the oxidants caused changes in the cellular membrane permeability that led to cell swelling and opacification. The present result suggest that the conductance properties of RPE cells may also be reversibly inhibited by short exposure to oxidative agents, possibly by binding without protein damage. Further studies are needed to confirm whether H2O2 decreases membrane chloride conductances across native human RPE cells and whether chloride and fluid transport are altered by reactive oxygen species. Possible alterations in the chloride transport properties of RPE after oxidative damage could have potential importance in view of studies that have implicated a role for oxidative damage in degenerative diseases of the retina such as AMD.

In summary, the cultured human fetal RPE cell line, RPE 28 SV4, expresses CFTR and ClC chloride channels and retains several of the features of chloride conductances of native RPE cells. In addition, chloride channel activity was reversibly inhibited by hydrogen peroxide in these cells. These findings suggest that RPE 28 SV4 cells may be a useful model system for studies of chloride channel regulation and the effects of oxidative agents on RPE.

**Acknowledgments**

The authors thank Guifang Jin for her advice regarding the care of fetal human RPE cultured cells and the hydrogen peroxide experimental protocols, Brian Kennedy and Steve Weinmann for their comments on an earlier version of this manuscript, and Satish Srivastava for his insightful comments regarding reactive oxygen species.

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