Stimulation of Aquaporin-Mediated Fluid Transport by Cyclic GMP in Human Retinal Pigment Epithelium In Vitro

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PURPOSE. The retinal pigment epithelium (RPE) expresses aquaporin-1 (AQP1) and components of the natriuretic peptide signaling pathway. We hypothesized that stimulation of the natriuretic signaling pathway in RPE with atrial natriuretic peptide (ANP) and with membrane-permeable analogs of cGMP would induce a net apical-to-basal transport of fluid.

METHODS. The hypothesis was tested using human RPE cultures that retain properties seen in vivo. Confluent monolayers were treated with ANP or membrane-permeable cGMP analogs in the presence of anantin, H-8, and an AQP1 inhibitor, AqB013. Fluid movement from the apical to basal chambers was measured by weight and used to calculate net fluid transport.

RESULTS. Our results demonstrated a 40% increase in net apical-to-basal fluid transport by ANP (5 μ M) that was inhibited completely by the ANP receptor antagonist anantin and a 60% increase in net apical-to-basal fluid transport in response to the extracellularly applied membrane-permeable cGMP analog pCPT-cGMP (50 μ M), which was not affected by the protein kinase G inhibitor H-8. The aquaporin antagonist AqB013 (20 μ M) inhibited the cGMP-stimulated RPE fluid flux.

CONCLUSIONS. The effect of cGMP is consistent with an enhancement of the net fluid flux in RPE mediated by AQP1 channels. Pharmacologic activation of cGMP signaling and concomitant stimulation of fluid uptake from the subretinal space could offer insights into a new approach to treating or reducing the risk of retinal detachment. (*Invest Ophthalmol Vis Sci.* 2012;53:2127–2132) DOI:10.1167/iovs.11-8471

The retina is the multilayered neural network in the eye that transforms light information into neural impulses, which travel through the optic nerve to the visual centers of the brain. The retinal pigment epithelium (RPE) is a protective barrier in the back of the eye that absorbs stray photons, provides trophic and metabolic support of sensory retinal cells, and is an essential component of the blood-retinal barrier. The atrial natriuretic peptide (ANP) receptor natively expressed in

RPE and other tissues is a guanylate cyclase that increases intracellular cGMP in response to peptide binding.^{1,2} ANP is involved in the regulation of fluid movement in tissues including heart, kidney, and brain.^{3–5} Evidence suggests local sources of ANP also are located within the eye.^{2,6}

The water channel aquaporin-1 (AQP1) is expressed in RPE and a number of other fluid-transporting tissues.^{7,8} Changes in levels of expression and trafficking of aquaporin channel proteins in conditions of dehydration or edema have been demonstrated to be important for modulation of water transport capacity.^{9,10} The concept that AQP1 channels enable water flux out of the subretinal space was supported by work that showed a reduction of fluid transport in cultured RPE after treatment with AQP1-specific antisense RNA.¹¹ Investigation of detailed physiologic roles of aquaporins in dynamic regulation of fluid transport has been slowed by the lack of pharmacologic agents that directly block AQP channels.¹² The recent discovery of an effective pharmacologic inhibitor of AQP1 and AQP4 channels, AqB013,¹³ has opened new opportunities to test for direct roles of water channels in physiologic conditions.

The potential translational relevance is in defining possible factors that contribute to subretinal edema formation and risk of retinal detachment. Impairing fluid clearance from retinal tissue promotes retinal edema formation.¹⁴ A decrease in cGMP content has been linked with conditions of retinal detachment in animal models¹⁵ and in clinical studies.¹⁶ Data presented here show that ANP and cGMP stimulate apical-to-basal fluid movement in confluent monolayers of differentiated human retinal pigment epithelium in vitro and indicate that the cGMP-activated response is independent of protein kinase G (PKG). Effects of a pharmacologic AQP blocker, AqB013, indicate that water channel activity of AQP1 is necessary for the effect of cGMP on retinal fluid homeostasis. Characterizing the molecular mechanisms that control fluid transport across human RPE is important for understanding processes that contribute to normal RPE physiology and pathophysiology in conditions such as retinal detachment, which, if left untreated, ultimately results in blindness.¹⁷

MATERIALS AND METHODS

Human Retinal Pigment Epithelium Cell Culture Preparation

Retinal pigment epithelium from a human tissue donor was prepared using precisely optimized culture methods, reported previously to enable differentiation of properties comparable to those seen in vivo, including establishment of polarity, transepithelial electrical resistance, and pigmentation.¹⁸ In brief, newly proliferated cells were collected every 3 to 4 days from primary tissue maintained in low calcium medium over a period of approximately 4 weeks and frozen in culture medium supplemented with serum and dimethyl sulfoxide (DMSO; 10% DMSO and 10% fetal bovine serum, v/v in Dulbecco's modified Eagle's medium). Cells were thawed, combined, plated at ~300,000

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cells per well on filters (12 mm Millicell HA; Millipore, Billerica, MA) coated with laminin (~1 to 2 μ g; Sigma-Aldrich Cat#L2020) and cultured in a CO₂-controlled 37°C incubator for 8 to 10 weeks. Transepithelial resistances measured using a volt-ohm meter (World Precision Instruments, Sarasota, FL) were monitored to confirm integrity of the confluent monolayer at the start of the 2-day experiments. Filters with RPE-cultured cells had a mean net resistance of >500 Ohm cm², calculated from the measured total resistance minus the average resistance of cell-free filters in media. Use of human tissue was done in accord with the Declaration of Helsinki ethical guidelines.

Measurement of Fluid Displacement Rates in RPE Cultures

The rates of net fluid transport by RPE cell layers at 37°C in filter chambers were determined from the change in mass of the fluid present in the apical chamber, sampled at 2-hour intervals. Baseline fluid transport assays confirmed cell viability and set the control value of transport for paired comparisons done on the same day. The initial volume in the apical chamber, replaced at the start of each 2-hour treatment interval, was 150 μ L isotonic (300 mOsm) saline with or without pharmacologic agents as specified. The basolateral chamber contained 1 mL of isotonic saline. Rates were calculated as volume per hour based on the mass of apical chamber fluid content converted to microliters and divided by 0.6 to account for the area of the Millicell filter, yielding units of μ L/(h cm²). Data are summarized as total transport rates or as net transport rates (treatment minus baseline), as indicated. Statistically significant differences in fluid transport rates between treatments and paired baseline control data were analyzed using ANOVA for multiple comparisons, and post hoc paired Student's *t*-test, with significance set at *P* < 0.05.

Agents used were 8-bromo-cyclic guanosine monophosphate (8-Br-cGMP; Sigma, St. Louis, MO, #B1381); 8-(4-chlorophenylthio) guanosine-3', 5'-cyclic monophosphate (8-pCPT-cGMP, Biolog, Life Science Institute, Bremen, Germany, #C009); atrial natriuretic peptide (ANP; Sigma, St. Louis, MO, #A1663); ANP receptor antagonist, anantin (Sigma, St. Louis, MO, #A4316); PKG inhibitor, H-8 (N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride; Calbiochem, San Diego, CA, #371,958); and aquaporin inhibitor, AqB013 (synthesized by Dr Gary Flynn, Spacefill Enterprises LLC, Tucson AZ) prepared as a 10 mM stock solution in DMSO and used at a final concentration of 20 μ M (final DMSO concentration 0.2% v/v). For AqB013 experiments, baseline data were collected with the equivalent concentration of DMSO alone as the vehicle control. For experiments with 20 mM 8-Br-cGMP, the saline NaCl concentration was adjusted to maintain isotonicity.

Western Blot Analysis

Proteins collected from RPE culture lysates in Laemmli buffer¹⁹ were run on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels (0.3 mA for 1 h), and transferred to nitrocellulose (100 mV for 90 minutes). Following transfer, nitrocellulose blots were incubated 1 h in blocking buffer TBST (100 mM Tris, 137 mM NaCl, 2.7 mM KCl, pH 7.4, with 2% Tween-20 and 5% nonfat dry milk), and then 16 hours at 4°C in blocking buffer containing 300 ng/mL anti-AQP1 (affinity-purified polyclonal rabbit anti-AQP1 antibodies targeted against the carboxyl terminal domain of AQP1 as previously described.^{20,21} Blots were rinsed (4 times at 15 minutes each) in TBST, incubated 1 hour in blocking buffer with goat anti-rabbit IgG conjugated to horseradish peroxidase (Santa Cruz, CA) at a dilution of 1:5000, and rinsed again as before. Protein antibody complexes were visualized by chemiluminescence (HyGlo; Denville Scientific, Metuchen, NJ, #RPN2106 and RPN2135) recorded on x-ray film (Genesee, CA).

PKG Activity Assay

Confirmation that H-8 used in the RPE transport studies was effective in blocking PKG activity was assessed using a Cyclax cGMP-dependent protein kinase Assay Kit (MBL International, Woburn, MA, #CY-1161), as per the manufacturer's guidelines. Briefly, the purified catalytic domain of PKG was incubated with 125 μ M ATP for 30 minutes in the presence or absence of H-8, and enzymatic activity was determined by phosphorylation of a peptide substrate using an anti-phospho-amino acid antibody provided with the kit. Colorimetric changes were measured with a Molecular Devices 96-well plate reader (450 nm).

Human AQP1 Channel Expression in *Xenopus* Oocytes

Unfertilized oocytes of *Xenopus laevis* were obtained by partial ovariectomy, treated with collagenase in nominally Ca²⁺-free saline, transferred to standard isotonic saline (ND96), and injected with cRNA transcribed in vitro from linearized human AQP1 cDNA, by methods described previously.^{22,23} Swelling rates were assessed by video microscopy in 50% hypotonic saline, with images captured at 2-second intervals for 40 seconds. Oocytes were immediately transferred to isotonic saline for 1.5 to 2 hours with AqB013 at 0, 20, or 50 μ M and then subjected to a second swelling assay in 50% hypotonic saline. Rates of swelling were determined from the slope of the linear fit of the relative area as a function of time in hypotonic saline. Animal use was in accord with the Association for Assessment and Accreditation of Laboratory Animal Care International guidelines, with protocols approved by the University of Adelaide Animal Ethics committee.

RESULTS

Validation of the Human Retinal Pigment Epithelial Culture Model

The pharmacologic dissection of RPE fluid transport was facilitated by the use of an in vitro model that retains distinctive in vivo features. Methods for establishing and maintaining human RPE cultures were optimized empirically¹⁸ to achieve characteristic properties of RPE, including hexagonal cell morphology, high transepithelial resistance plus correct expression, and localization of marker proteins such as the Na⁺-K⁺-ATPase pump.²⁴ Cultured RPE cells generated pigment granules characteristic of the differentiated phenotype (Fig. 1a). Expression of aquaporin-1 protein in cultured RPE¹¹ was confirmed by Western blot analysis of cell lysates of cultured RPE, probed with anti-AQP1 antibodies (Fig. 1b). The signals in cultured RPE were comparable with those seen in bovine trabecular meshwork and mouse kidney, which served as positive controls.^{25,26} Variations in intensity of the heavier band are likely to reflect differences in glycosylation patterns across tissues and species.

Differences in baseline fluid transfer rates did not result from a passive leak across the barrier cell layer (Figs. 1c, 1d). A plot of the transepithelial resistance against the baseline flux rate for the same culture showed no correlation within any of the control groups (isotonic saline with serum, with DMSO, and serum-free), in that none of the slopes of the linear regression fits were significantly different from zero. The mean transepithelial resistance values ($\pm$ SEM, as Ω cm²) were 591 $\pm$ 16 (*n* = 48) for isotonic saline with serum, 523 $\pm$ 12 (*n* = 18) for isotonic with 0.2% (v/v) DMSO, and 562 $\pm$ 16 (*n* = 41) for serum-free isotonic saline.

Stimulation of RPE Fluid Transfer by cGMP

Significant increases in the apical-to-basolateral fluid transfer rate in human RPE cultures were induced by endogenous and

FIGURE 1. Properties of human retinal pigment epithelial cells in culture. (a) Development of pigmentation in RPE cultures by 9 weeks in vitro, a characteristic feature of the differentiated cell morphology. (b) Western blot showing expression of AQP1 protein in the human RPE (hRPE) cultures. Other tissues known to express AQP1, included as positive controls, were bovine trabecular meshwork (bTM) and mouse kidney (mKid). Monomer subunits of AQP1 run at 28 kD as expected; higher-molecular-weight bands are glycosylated AQP1. (c) Scatter plot showing the lack of correlation between baseline apical-to-basolateral fluid transfer rate ($\mu\text{L}/\text{h cm}^2$) and transepithelial membrane resistance measured for the same RPE culture. The slope of the fit line was not significantly different from zero, indicating that leak across the barrier did not account for differences in fluid transport function between RPE samples. (d) Scatter plot showing the consistent enhancement of transport rates as compared with baseline rates in the same culture after treatment with 5 μM ANP (applied to the apical side) or the membrane-permeable cGMP analog at 50 μM (8p-CPT-cGMP), as indicated by slope values >1.0 . Fits of data by linear regression (GraphPad Prism) gave slope values of 0.40 for ANP and 0.62 for 8p-CPT-cGMP. The reference line (dotted) shows the values expected if there was no difference between the baseline and the treatment flux rates. Most of the points fall above the reference line, indicating a consistent stimulatory effect across samples regardless of baseline starting values for fluid transport.

exogenous stimulation of cGMP signaling over a broad range of baseline transport rate values (Fig. 1d), as seen in the data for total fluid flux rates at baseline (x-axis) plotted against the flux rate in the same filter culture after treatment with ANP or 8p-CPT-cGMP (y-axis). The dotted reference line shows unity, representing the expected position of data points if there were no difference between baseline and treated rates of fluid transfer. Most data points are above the reference line, illustrating the reproducible stimulatory effect of cGMP treatment across samples. ANP significantly increased the fluid transfer rate by 40% and 8pCPT-cGMP by 60% as compared with baseline control rates (Fig. 2a). Statistically significant increases in fluid flux rates were observed for 5 μM ANP and for 50 μM 8p-CPT-cGMP, applied extracellularly. The mean total transport rate ($\pm\text{SEM}$) for ANP-treated cells was $7.5 \pm 0.5 \mu\text{L}/\text{h cm}^2$ ($n = 11$), and the paired baseline control was 5.4 ± 0.6 ($n = 11$). The mean total transport rate ($\pm\text{SEM}$) for cells exposed to 8p-CPT-cGMP was $11.1 \pm 0.9 \mu\text{L}/\text{h cm}^2$ ($n = 10$), and the paired baseline control was 7.0 ± 0.8 ($n = 10$). A consistent stimulation of total fluid transport rate also was seen with extracellular 20 mM 8Br-cGMP (Fig. 2b). The mean stimulation by 8Br-cGMP was 86% over the baseline transport rates. The plot of control and corresponding experimental fluid shift values for each monolayer tested with 8-Br-cGMP demonstrates the consistency of this treatment across individual primary

FIGURE 2. Stimulation of apical-to-basolateral fluid transfer rates by cGMP-dependent signaling. (a) Box plot summary of fluid transfer rates before (control baseline) and after treatment with 5 μM ANP or 50 μM 8p-CPT-cGMP (CPT). Boxes show 50% of the data. The horizontal bar indicates the median value, and the error bars illustrate the full range of data points in each group; n values were 11 for the ANP and 10 for the 8p-CPT-cGMP treatment groups. Statistically significant differences were assessed by paired t -test within groups and unpaired t -test between groups and are indicated as $**P < 0.002$ and NS (not significant). (b) Compiled data from three experiments showing a consistent increase in total fluid transport in response to 8Br-cGMP (20 mM). Lines connect data from the same filter culture.

cultures (Fig. 2b). The concentration of 20 mM of 8-Br-cGMP was comparable with that used in studies of perfused rabbit eye.²⁷ Given that the membrane is a barrier to drug delivery, the effective intracellular doses are expected to be lower. The apparent order of potency (8p-CPT-cGMP $>$ 8Br-cGMP) observed here is consistent with known differences in membrane permeability.²⁸ The stimulatory effect of 8pCPT-cGMP on RPE fluid transport was dose dependent based on the average response at each dose (Fig. 3), with a half-maximal activation dose (EC_{50}) at approximately 102 μM , applied extracellularly.

Pharmacologic Block of Human AQP1 Channels

The dose-dependent block of fluid flux by an aquaporin channel blocker, AqB013, in *Xenopus* oocytes expressing

FIGURE 3. Dose-dependent stimulation of net fluid transfer by 8p-CPT-cGMP applied extracellularly. Net rates were calculated as the baseline value subtracted from the value measured during the treatment with 8p-CPT-cGMP at the dose indicated. Data show mean $\pm$ SEM; n values were 3 to 6 per data point. Data plotted as a function of dose were fit with the equation: Net rate = $(\text{Max rate} \times C)/(\text{EC}_{50} + C)$ (GraphPad Prism), where C is concentration. Fitting yielded an estimated concentration of approximately 100 μM for half-maximal activation (EC_{50}) of net fluid transfer stimulation by 8p-CPT-cGMP.

FIGURE 4. Dose-dependent block by the aquaporin blocker AqB013 of osmotic water permeability in human AQP1 channels expressed in *Xenopus* oocytes. (a) The box plot shows compiled data for mean swelling rates (% S2/S1) for AQP1-expressing oocytes after treatment with AqB013 (1.5 to 2 hours, at 0, 20, or 50 μM). The second swelling rate after treatment (S2) was standardized to the initial swelling rate before treatment (S1) in the same oocyte. Mean values for (% S2/S1; $\pm$ SEM) were 105 ± 4.3 in untreated AQP1-expressing oocytes (0 μM), 38.1 ± 6.8 at 20 μM , and 15.9 ± 2.4 at 50 μM AqB013. *n* values are shown in italics above the x-axis. (b) Chemical structure of the aquaporin antagonist AqB013.

human AQP1 is illustrated in Figure 4. Oocytes expressing human AQP1 channels showed approximately 70% inhibition of osmotic water permeability (monitored by cell swelling) after treatment with AqB013 at 20 μM and approximately 85% inhibition after treatment with 50 μM AqB013, as compared with AQP1-expressing oocytes not treated with the blocker. Control oocytes (not injected with AQP1 cRNA) showed no appreciable osmotic water permeability in identical swelling assays and no difference after treatment with 20 μM AqB013.¹³ Data were standardized by dividing the second swelling rate (S2) by the first swelling rate (S1) measured for the same oocyte and expressed as a percentage (% S2/S1). This method allows each oocyte to serve as its own control, minimizing the effects of differences in AQP protein expression between oocytes.

Pharmacologic Analysis of the Stimulatory Effect of cGMP on RPE Fluid Transport

Anantin (50 μM), a selective antagonist of natriuretic peptide receptor guanylate cyclase activity,²⁹ opposed the stimulatory effect of 5 μM ANP on the net fluid transfer out of the apical chamber (Fig. 5). These results indicated that ANP signaling acts through a receptor-mediated cGMP signaling pathway to increase apical fluid displacement in human RPE cultures. The AQP1 inhibitor, AqB013 (20 μM), blocked the stimulatory effect of the cGMP analog 8p-CPT-cGMP on net fluid transfer in RPE, suggesting that cGMP-induced fluid transport is dependent on the presence of functional AQP1 channels. The net fluid transport values described in Figure 5 are comparable with the total transport values minus baseline values shown in Figure 2, showing consistency between experiments. The protein kinase inhibitor H-8 (100 μM) failed to block the stimulatory effect of 8p-CPT-cGMP on net fluid transfer, indicating that cGMP-induced fluid movement occurs independently of PKG and potentially protein kinase A (PKA) activity as well. Activity of the H-8 agent was confirmed separately in a standard assay of PKG enzymatic activity, in which PKG phosphorylation of a commercial peptide substrate was assessed using ATP and the purified catalytic domain of

FIGURE 5. Summary of effects of pharmacologic agents alone and in combination on the net apical-to-basolateral fluid transfer rates in human RPE cultures. Net rates are the difference between the fluid transfer rate with treatment, referenced to the corresponding baseline rate measured in the same RPE filter culture immediately prior to the treatment. Significant inhibition of the stimulatory effect of 5 μM ANP was observed with the ANP receptor antagonist, anantin (50 μM). Significant inhibition of the stimulatory effect of 8pCPT-cGMP (CPT) was observed with the AQP1 blocker AqB013 (20 μM). The PKG inhibitor, H-8 (100 μM), did not interfere with 8pCPT-cGMP-induced stimulation. Statistically significant differences were assessed by ANOVA and post-hoc *t*-tests: **P* < 0.05 and NS (not significant). *n* values (italics) are indicated above the x-axis.

PKG, with and without H-8 present. Results of the assay showed that H-8 blocked PKG activity by 87%, based on the optical density values of 1.74 ± 0.20 (mean $\pm$ SD, *n* = 3) for control and 0.23 ± 0.13 (paired; *t*-test, *P* < 0.05) with H-8 present. These data demonstrated that the observed lack of effect of H-8 on cGMP-stimulated fluid transport in RPE cultures did not result from a lack of potency of the H-8 compound.

DISCUSSION

Results presented here show that cGMP increases fluid displacement from the apical to the basolateral side of cultured human retinal pigment epithelium and that the stimulatory effect depends on the presence of functional AQP1 channels. Increased fluid transport triggered by endogenous atrial natriuretic peptide receptors was mimicked by the extracellular application of membrane-permeable analogs of cGMP. The response was not altered in the presence of H-8, an inhibitor of protein kinases including PKG, suggesting that cGMP is binding directly to target channels or transporters, rather than working indirectly through kinase-mediated phosphorylation. The effect of atrial natriuretic peptide was reversed by the selective receptor antagonist anantin. The stimulatory effect of the cGMP analog 8p-CPT-cGMP on net fluid transfer was reduced by the AQP antagonist AqB013.

Natriuretic peptide receptors have been detected in human retina and RPE.^{1,2} ANP significantly increases intracellular cGMP levels in human RPE, whereas the nitric oxide donor Na-nitroprusside (an activator of soluble guanylyl cyclase) is less effective.^{30,31} In the rabbit RPE in culture, the application of ANP increases chloride uptake,³² an effect that is reduced by inhibition of PKG by KT5823. Recent investigation of interferon-gamma signaling via cyclic nucleotides cGMP and

cAMP in human RPE demonstrated an increase in fluid transport that was reduced in the presence of either PKA inhibitor H-89 or inhibition of CFTR at the basal membrane.³³ We observed similar responses in human RPE upon treatment with ANP and cGMP, although in our study, the addition of H-8 (which blocks PKG and PKA³⁴) did not affect cGMP-stimulated fluid transport. The combined data indicate that cyclic nucleotides in the RPE can regulate fluid flux by altering the activity of multiple pathways via kinases as well as second messengers, modulating an array of channels and transporters, with experimental differences potentially indicative of the effects of tissue preparation, age, or environmental factors.

Potassium and sodium channels serve as ion conduction pathways for baseline RPE fluid absorption.^{35–37} An important contributor to the net apical-to-basolateral flow of salt and water across the RPE is the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter,^{38,39} a primary route for chloride uptake that contributes to the transepithelial driving force for water.⁴⁰ The $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter appears to be regulated mainly by PKA.⁴¹ Inhibition of PKG with KT5823 did not reduce $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter activity in ciliary epithelial tissues following stimulation with isoproterenol.⁴² Conversely, activation of PKG by cGMP inhibited the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter in rat atrial myocytes.⁴³ The effects of cGMP in our study, seen as a stimulation of net fluid transport, would not be consistent with a downregulation of cotransporter activity, suggesting additional targets are involved in the stimulatory response.

The AQP blocker AqB013¹³ is effective at inhibiting aquaporin-mediated osmotic water fluxes in the *Xenopus* oocyte expression system; 70% AQP1 block was seen at 20 μM AqB013 (Fig. 4). The stimulation of fluid transport by 8p-CPT-cGMP was significantly reduced by the concomitant block of water transport in AQP1 channels with AqB013, indicating an essential role for AQP1 in the enhanced fluid transport response to cGMP. The current data are in agreement with previous studies demonstrating the importance of AQP1 in RPE fluid transport as determined by siRNA treatment and adds a more immediate potential mode of translational therapy to the current strategies for treating diseases associated with retinal detachment.

The results indicate that AQP1 channels become rate-limiting factors in the regulation of transepithelial fluid transport if water channel activity is substantially compromised, suggesting other transporter and leak pathways for water flow are insufficient for maximally efficient fluid handling in RPE. The role of the water channels in the cGMP response is expected to be primarily an osmotically-driven process that is occurring in parallel to cGMP-regulated salt transport through pumps, transporters, and ion channels. However, the results also are consistent with an additional role for cGMP signaling pathways, including the direct gating of AQP1 channels.^{22,23} In the choroid plexus, ANP slows fluid secretion and decreases the rate of production of cerebral spinal fluid.⁵ This downregulation of choroid plexus fluid transport by ANP is influenced by cGMP-mediated activation of a nonselective cation conductance associated with AQP1 channels.^{41,44,45} If cation channel activity were confirmed for AQP1 channels in the RPE, the equivalent mechanism would be consistent with an observed promotion of fluid absorption and enhanced clearance of fluid from the subretinal space in response to cGMP.⁸

The attachment of the retina to the posterior eye depends on extracellular interactions between retina and RPE,⁴⁶ pressure differences between the vitreous and the choroid regions,⁴⁷ and transport processes that reduce the fluid volume of the subretinal space.^{48–50} Regulation of subretinal volume is thought to be essential in preventing retinal edema formation and rhegmatogenous retinal detachment.⁵¹ Low levels of cGMP

have been correlated with cases of retinal detachment,^{15,16} and thus, decreased cGMP signaling could contribute to the risk of retinal detachment. Current therapies use vitreal pressure to tamponade the retina and laser, thermal, and cryogenic methods for physically reattaching the retina to the RPE.⁵² Pharmacologic approaches to reducing edema, such as targeting cGMP levels in RPE, could increase the success of retinal reattachment surgeries and reduce the risk of retinal detachment as an adverse consequence of traumatic injury or postcataract surgery. Cataract is the leading cause of blindness in the world; the risk of retinal detachment increases more than fivefold in patients within 10 years after cataract surgery.⁵³ With an aging population, the discovery of pharmacologic tools for maintaining retinal health and visual function is a compelling goal.

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