Polycystin-2 Expression and Function in Adult Mouse Lacrimal Acinar Cells

Simon Kaja, Jill D. Hilgenberg, Volodymyr Rybalchenko, Wanda E. Medina-Ortiz, Elaine V. Gregg, and Peter Koulen

Purpose. Lacrimal glands regulate the production and secretion of tear fluid. Dysfunction of lacrimal gland acinar cells can ultimately result in ocular surface disorders, such as dry eye disease. Ca^{2+} homeostasis is tightly regulated in the cellular environment, and secretion from the acinar cells of the lacrimal gland is regulated by both cholinergic and adrenergic stimuli, which both result in changes in the cytosolic Ca^{2+} concentration. We have previously described the detailed intracellular distribution of inositol-1,4,5-trisphosphate receptors (IP_3Rs), and ryanodine receptors (RyRs) in lacrimal acinar cells, however, little is known regarding the expression and distribution of the third major class of intracellular Ca^{2+} release channels, transient receptor potential polycystin family (TRPP) channels.

Methods. Studies were performed in adult lacrimal gland tissue of Swiss-Webster mice. Expression, localization, and intracellular distribution of TRPP Ca^{2+} channels were investigated using immunocytochemistry, immunohistochemistry, and electron microscopy. The biophysical properties of single polycystin-2 channels were investigated using a planar lipid bilayer electrophysiology system.

Results. All channel-forming isoforms of TRPP channels (polycystin-2, polycystin-L, and polycystin-2L2) were expressed in adult mouse lacrimal gland. Subcellular analysis of immunogold labeling revealed strongest polycystin-2 expression on the membranes of the endoplasmic reticulum, Golgi, and nucleus. Biophysical properties of lacrimal gland polycystin-2 channels were similar to those described for other tissues.

Conclusions. The expression of TRPP channels in lacrimal acinar cells suggests a functional role of the proteins in the regulation of lacrimal fluid secretion under physiological and disease conditions, and provides the basis for future studies focusing on physiology and pharmacology. (*Invest Ophthalmol Vis Sci.* 2011;52:5605–5611) DOI:10.1167/iovs.10-7114

From the [1]Vision Research Center and Departments of [2]Ophthalmology and [3]Basic Medical Science, University of Missouri-Kansas City, School of Medicine, Kansas City, Missouri.

Supported in part by a Fight for Sight grant-in-aid (SK); Grants EY014227 and EY015672 from NIH/NEI (PK); Grants RR022570 and RR027093 from NIH/NCRR (PK); Grants AG010485, AG022550 and AG027956 from NIH/NIA (PK); and the Felix and Carmen Sabates Missouri Endowed Chair in Vision Research (PK).

Submitted for publication December 21, 2010; revised February 14 and March 25, 2011; accepted April 5, 2011.

Disclosure: **S. Kaja**, None; **J.D. Hilgenberg**, None; **V. Rybalchenko**, None; **W.E. Medina-Ortiz**, None; **E.V. Gregg**, None; **P. Koulen**, None

Corresponding author: Simon Kaja, University of Missouri-Kansas City, School of Medicine, Vision Research Center, 2411 Holmes St., Kansas City, MO 64108; kajas@umkc.edu.

Exocytosis from the lacrimal gland is tightly regulated.[1,2] Stress, aging, and genetic predisposition can contribute to lacrimal gland dysfunction resulting in lack of or inadequate tear fluid secretion, as manifested for instance in dry eye disease and Sjögren's syndrome.[3] As a result of an aging society, the annual societal cost associated with dry eye disease is an estimated $55.4 million.[4] A better understanding of the basic principles regulating lacrimal gland function is critical to the development of novel therapies for dry eye disease.

The intracellular Ca^{2+} concentration in a cell is tightly controlled and local and temporal increases govern a large number of cellular processes,[5,6] including tear secretion.[2]

Ca^{2+} can enter the intracellular environment of the cell either from the extracellular space through voltage-, ligand-, store-, or second messenger-activated Ca^{2+} channels,[5,6] or from intracellular stores such as the endoplasmic reticulum (ER). Release of Ca^{2+} from intracellular stores can be mediated by three main groups of intracellular Ca^{2+} channels: inositol-1,4,5-trisphosphate receptors (IP_3Rs), ryanodine receptors (RyRs), and polycystin family transient receptor potential (TRPP) channels. The group of IP_3Rs consists of three subtypes; all are ligand-gated ion channels, regulated by both their endogenous ligand IP_3 and Ca^{2+}.[6,7] The three known isoforms of RyRs share high sequence homology with IP_3Rs and form tetrameric complexes.[6] TRPPs comprise eight proteins, three of which have been shown to be ion channels: polycystin-2, polycystin-like (PCL), and polycystin-2L2.[8–10] In addition, mechanisms that control Ca^{2+} uptake into intracellular stores or extrusion of Ca^{2+} into the extracellular space contribute critically to Ca^{2+} homeostasis and the shape of Ca^{2+} transients.[5,6]

Mechanistically, lacrimal fluid secretion is controlled by differential modulation of the intracellular Ca^{2+} concentration through two distinct pathways involving both cholinergic and adrenergic stimuli.[1,2] Experimental evidence suggests that cholinergic stimuli activate an intracellular pathway dependent on intracellular Ca^{2+} release from IP_3Rs,[11] whereas β-adrenergic stimulation results in activation of RyRs.[11–13] We have previously described the expression of both IP_3R and RyR isoforms in mouse lacrimal gland acinar cells[14] and been able to correlate their differential distribution patterns with their proposed functions in lacrimal acinar cells.[14]

However, to date there is no knowledge as to whether the intracellular Ca^{2+} channels of the TRPP group of channels are expressed in the lacrimal gland, and/or functionally contribute to tear secretion. Given their expression patterns and role in Ca^{2+} signaling in other tissues, we hypothesized that TRPP Ca^{2+} channels are functionally expressed in the acinar cells of the mouse lacrimal gland.

We here for the first time describe the expression and differential subcellular distribution of all three TRPP channels, polycystin-2, PCL, and polycystin-2L2, in lacrimal aci-

nar cells. Furthermore, we isolated polycystin-2 channels from adult lacrimal gland and identified their biophysical properties.

Materials and Methods

Animals

Male adult albino Swiss-Webster mice (2 months of age) were euthanatized by CO_2 overexposure. All experiments described in the present study were carried out in accordance with the National Institutes of Health, institutional guidelines and in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Antibodies

Rabbit polyclonal antibodies raised against polycystin-2 (PC2), polycystin-L (PCL), and polycystin-2L2 were validated previously[15] and are available from Millipore (Temecula, CA; AB9088, AB9084, and AB9086 respectively. Antibodies were used at 1:1000 dilution for both immunocytochemistry and immunohistochemistry. Secondary AlexaFluor 488- and AlexaFluor 594-conjugated goat anti-rabbit antibodies (Invitrogen, Carlsbad, CA) were used for immunofluorescence detection. The 15-nm gold-conjugated goat anti-rabbit antibody for electron microscopy (EM) (Aurion Immuno Gold Reagent; Electron Microscopy Sciences, Hatfield, PA) was used at a dilution of 1:30.

Isolation of Adult Acinar Cells of the Lacrimal Gland

Isolation of lacrimal acinar cells was essentially as described previously.[14] Individual lacrimal glands were dissected out and enzymatically dissociated for 10 minutes in 0.25% trypsin at 37°C. After centrifugation (2 minutes at 250*g*), tissue was incubated for 5 minutes with soybean trypsin inhibitor (STI; 0.2 mg/mL in 0.5% BSA, 2.5 mM EGTA/Dulbecco's Modified Eagle's Medium [DMEM] without Na pyruvate), subsequently with collagenase solution for 10 minutes (0.4 mg/mL in 0.5% BSA/DMEM without Na pyruvate), and mechanically dissociated by trituration. The cell pellet was washed with media (10% bovine growth serum [BGS], 1% penicillin/streptomycin in DMEM, at 37°C). Cells were resuspended in 10 mL media and plated on poly-d-lysin/laminin coated glass coverslips at a density of 20,000 cells per coverslip. Cells were allowed to attach for 2 hours in a humidified atmosphere of 95% air, 5% CO_2 at 37°C.

Immunocytochemistry

Attached cells were fixed for 20 minutes in 4% paraformaldehyde (PFA) in PBS at room temperature (RT), followed by three washes with PBS. After 1 hour block at RT (10% normal goat serum [NGS], 1% BSA and 0.05% Triton X-100 in PBS), cells were incubated overnight with primary antibody (diluted in 3% NGS, 1% BSA, and 0.05% Triton X-100 in PBS) in a humidified chamber, protected from light and at 4°C. Cells were washed three times with PBS and incubated with secondary antibody for 1 hour at RT, in a humidified chamber and protected from light. After washing, coverslips were mounted on slides (ProLong Antifade; Invitrogen). Negative controls consisted of the omission of primary antibody from the incubation steps. Three separate experiments using tissue from different animals were performed.

Immunohistochemistry

For immunohistochemical analysis, adult lacrimal glands were fixed with 4% PFA in PBS for 30 minutes at RT, then cryoprotected by sequential immersion in 10%, 20%, and 30% sucrose in PBS. Tissue was mounted (OCT Compound; Sakura Finetek USA., Torrance, CA) and sectioned at 12 μm thickness on a cryostat (Leica Microsystems, Bannockburn, IL). Sections were collected on silane coated slides (Mercedes Medical, Sarasota, FL), allowed to dry for 30 minutes at RT and stored at −80°C until used in immunohistochemistry. Immunostaining was performed as described above.

Electron Microscopy (EM) Analysis

Mouse lacrimal glands were immersion-fixed in PBS containing 4% PFA and 0.5% glutaraldehyde for 5 hours at 4°C. After rinsing in PBS, the tissue was dehydrated in a graded series of ethanol (10%, 30%, 50%, 70%, 95%, and 100%) followed by infiltration (Lowicryl K4M; Electron Microscopy Sciences, Hatfield, PA) according to the manufacturer's recommendation (except that all steps were performed at 4°C and the initiator C was replaced by the same amount of benzoin ethylether). The blocks were UV polymerized starting at 4°C, and slowly raising the temperature to 16°C over a period of 24 hours UV polymerization was then continued at RT for a total of 5 days. Tissue blocks were sectioned on an ultramicrotome (Reichert Ultracut S; Leica Microsystems, Deerfield, IL) at 70 to 100 nm thickness and were collected onto carbon-coated copper 200-mesh grids (Formvar; Ted Pella Inc., Redding, CA). Immunostaining was performed on 20 to 30 μL droplets on Parafilm by placing the grids section side down onto the solution droplets. To quench free aldehydes, the sections were first incubated three times for 5 minutes with 0.05 M glycine in PBS. To block nonspecific antibody binding, the sections were then incubated for 15 minutes in incubation buffer I (150 mM NaCl, 1% BSA, 1% cold water fish skin gelatin, and 5% NGS in PBS). Primary antibodies and the gold complex were incubated for 1 hour each. Grids were rinsed six times for 5 minutes each in incubation buffer II (same as incubation buffer I but containing 0.1% Aurion BSA-c instead of BSA) between each incubation step. After the gold-complex incubation, grids were rinsed three times in incubation buffer II, fixed in 2% glutaraldehyde in PBS for 20 minutes, rinsed two times (5 minutes each) in PBS, further rinsed in distilled water five times (5 minutes each), and counterstained with aqueous saturated uranyl acetate for 10 minutes and Sato's lead citrate stain for 2 minutes (both from Polysciences, Inc., Warrington, PA). Control grids were exposed to normal rabbit serum or incubation buffer with the addition of normal goat serum instead of the primary antibody. Grids were viewed on an electron microscope (Zeiss EM 910; Carl Zeiss NTS, LLC, Thornwood, NY) at 100 kV accelerating voltage. Pictures were recorded on electron image films (Kodak SO-163; Electron Microscopy Sciences, Hatfield, PA), and processed (Mohr Pro 8 film/paper processor; Ted Pella Inc., Redding, CA). Negatives were scanned to produce digital image files. For analysis, we performed three separate experiments. Images were chosen randomly and 20 fields were analyzed per data point, as described by us previously.[14]

Single Channel Recordings

Single channel recordings of polycystin-2 were conducted essentially as previously described by us.[9] Lacrimal glands from 10 animals were pooled for ER preparations. Vesicles enriched in ER membranes obtained from lacrimal glands were fused to lipid bilayers containing phosphatidylethanolamine and phosphatidylserine (3:1 wt/wt; Avanti Polar Lipids, Alabaster, AL) dissolved in decane (40 mg lipid mL^{-1}). Fusion was facilitated by the establishment of a potassium chloride gradient (600 mM potassium chloride on the cytosolic side (cis side), 0 mM potassium chloride on the trans side). Cis side buffer contained 250 mM HEPES-Tris at pH 7.35, whereas trans-side buffer contained 250 mM HEPES supplemented with either 55 mM $Ba(OH)_2$, 55 mM $Ca(OH)_2$, or 55 mM $Mg(OH)_2$, at pH 7.35. Experiments were recorded under voltage-clamp conditions. Ca^{2+} flux from the ER lumen into the cytoplasm was defined as an inward current, shown as downward deflections. Data were filtered at 1 kHz and digitized at 3 kHz and analyzed (pClamp software version 9; Molecular Devices, Burlingame, CA). The free Ca^{2+} concentration on the cis side of the protein was adjusted as described previously.[16] Voltage-dependence of the polycystin-2 current was assessed in every experiment; most channels were

recorded by applying a holding potential of −3 to −5 mV. Three separate experiments were performed; the mean value for every experiment was derived from 5 to 8 bilayers measured per experimental condition.

Results

Distribution and Subcellular Localization of Polycystins in Mouse Lacrimal Gland Acinar Cells

To obtain an initial assessment of the distribution polycystin proteins in adult mouse lacrimal gland we performed an immunohistochemical analysis. Cryosections of adult lacrimal gland tissue showed strong specific immunoreactivity for polycystin-2, PCL and polycystin-2L2 (Fig. 1). All isoforms were expressed in acinar cells of ducts as well as surrounding tissue, especially cells of the caruncula lachrymalis. Omission of primary antibody (control) confirmed the specificity of immunostaining.

Similarly, all three polycystin isoforms were expressed in freshly isolated lacrimal acinar cell preparations (Fig. 2). Whereas polycystin-2 is distributed throughout the cell body, PCL and polycystin-2L2 show lighter cell body labeling yet distinct puncta indicative of plasma membrane expression (Fig. 2).

Our quantitative subcellular analysis using EM revealed highest expression of polycystin isoforms in the membranes of the ER/Golgi apparatus and in the membranes of the nuclear envelope (Fig. 3A). Low level expression was found in mitochondria and secretory vesicles, with even lower numbers at the plasma membrane (Fig. 3A). Quantitation is based on analysis of EM micrographs from three separate lacrimal glands per antibody (Fig. 3B).

Functional and Biophysical Characterization of Polycystin-2 Isolated from Adult Mouse Lacrimal Gland

With polycystin-2 primarily expressed on the membrane system of intracellular Ca^{2+} stores, namely the ER contiguous with the nuclear envelope and the Golgi apparatus, we next determined if polycystin-2 forms functional intracellular Ca^{2+} channels when isolated from lacrimal gland tissue. ER microsomes were prepared from adult mouse lacrimal gland tissue, reconstituted into planar bilayer lipid membranes, and single channel activity of native polycystin-2 channels was measured in the presence and absence of physiological and pharmacological modulators (Fig. 4). To exclude the contribution of other intracellular Ca^{2+} channels recordings were performed in the absence of the IP_3 receptor agonist IP_3,[5,9] and after adding the ryanodine receptor inhibitors ruthenium red (20 μM) and ryanodine (10 μM).

Polycystin-2 from lacrimal glands displayed strong a voltage-dependent open probability and activity, as well as inhibition by cytoplasmic Mg^{2+} (3 mM and 10 mM) and a bimodal dependence on cytoplasmic Ca^{2+} concentrations (Figs. 4A and 4B). Specifically, the slope conductance of lacrimal gland polycystin-2 was determined as 114 pS with Ba^{2+} as the current carrier and an ion selectivity of 89%, 85%, 77% of the Ba^{2+} conductance for Sr^{2+}, Ca^{2+}, and Mg^{2+}, respectively. Polycystin-2 from adult mouse lacrimal

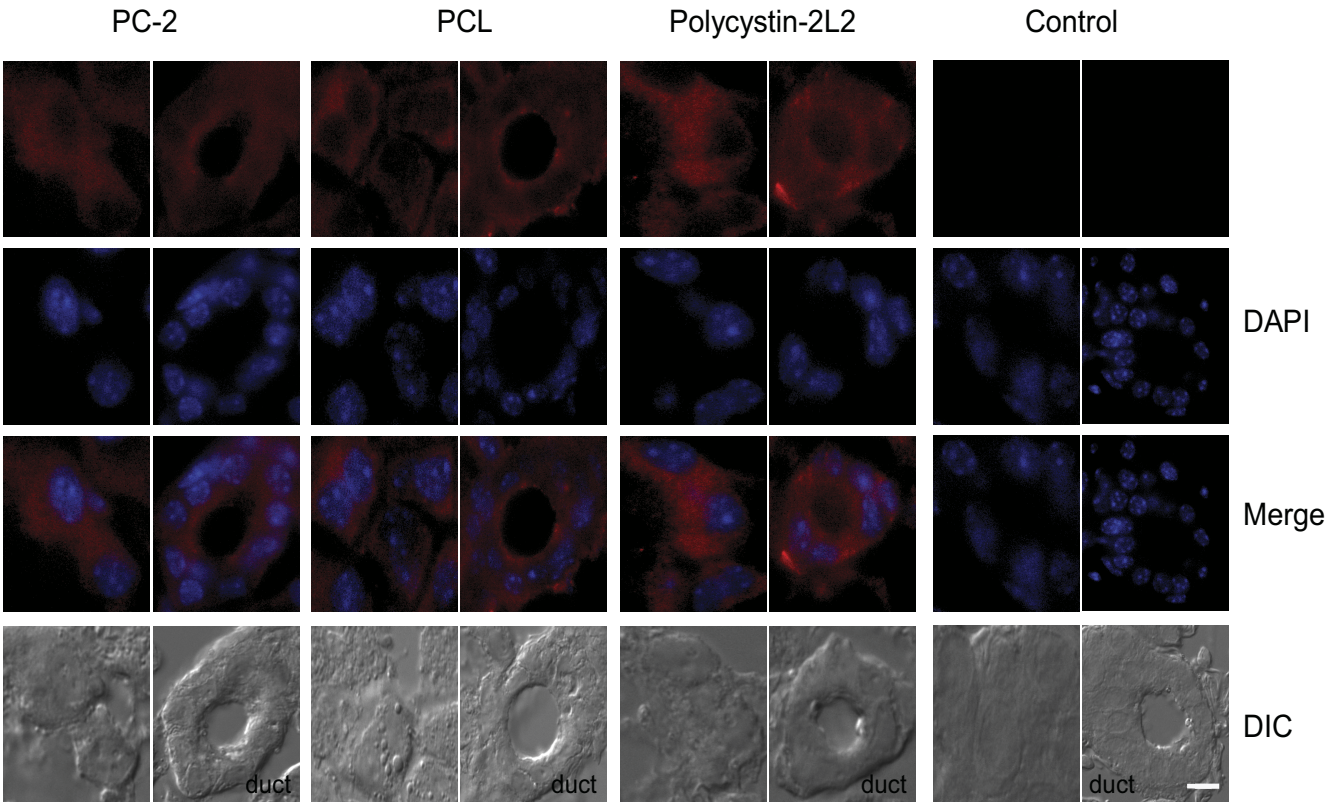

Figure 1. Lacrimal gland ducts express polycystin-2 Ca^{2+} channels. Immunohistochemistry on sections of adult mouse lacrimal gland shows specific immunoreactivity for polycystin-2, PCL, and polycystin-2L2 in the ducts. Polycystins were detected using AlexaFluor 594-conjugated secondary antibody (Invitrogen), DAPI was used to label nuclei. Primary antibody was omitted in the control condition. Representative images are shown. Scale bar, 10 μm.

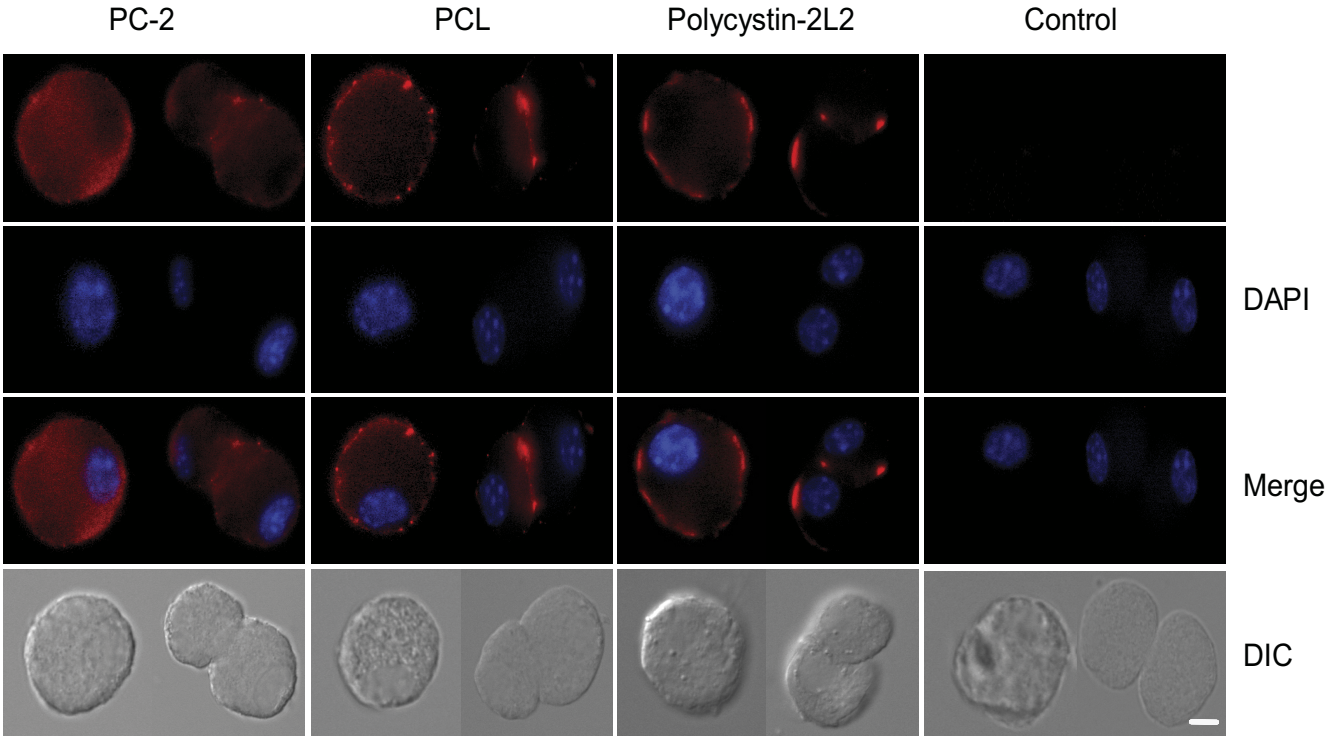

FIGURE 2. Polycystins are expressed in freshly isolated acinar cell preparations of the lacrimal gland. Representative images of polycystin-2, PCL, and polycystin-2L2 specific immunoreactivity in freshly isolated acinar cell preparations of adult mouse lacrimal gland are shown. All three isoforms yielded strong cytosolic immunoreactivity, while PCL and polycystin-2L2 also showed strong plasma membrane labeling. Primary antibody was omitted in the control condition. Representative images are shown. Scale bar, 10 μm.

gland tissue was Ca^{2+}-dependent and was activated differentially by a range of physiological cytosolic Ca^{2+} concentrations (pCa 7–3; Fig. 4A) and inactivated at higher cytosolic Ca^{2+} concentrations (Fig. 4A). Physiological and pharmacological modulators of other known ER calcium-release channels, namely of IP_3Rs and RyRs (10 μM cyclic adenosine diphosphate ribose; 10 mM caffeine; 10 μM ryanodine; 10 μM ruthenium red, 1 μM IP_3; 1 μM Xestospongine C; 50 μg/mL heparin), did not have an effect on activity or biophysical properties of single-channel lacrimal gland polycystin-2. In contrast, Mg^{2+}, when added to the cytosolic side of the channel, reduced single-channel open probability to inactivity while biophysical hallmarks of the channel (current, conductance, and voltage dependence of the channel activity; Fig. 4B) were not affected.

DISCUSSION

We here describe the expression, subcellular localization and functional properties of polycystin-2 channels in the adult mouse lacrimal gland.

The lacrimal gland is a component of the 'lacrimal functional unit,' consisting of lacrimal glands, ocular surface, lids, and meibomian glands, as well as the interconnecting neural reflex loops.[17] As such, the lacrimal gland serves a critical role in tear production and secretion; dysfunction of lacrimal acinar cells is a pathophysiological mechanism underlying dry eye disease.[1,17] Intracellular Ca^{2+} signaling pathways are pivotal in the response of lacrimal acinar cells to neuronal stimuli,[2] and dysregulated Ca^{2+} signaling has been identified in diseases of the lacrimal gland.[18] However, little is known about the molecular identity of the intracellular Ca^{2+} channels involved. We have previously identified IP_3Rs and RyRs in lacrimal acinar cells[14] and proposed the existence of distinct pathways selectively using IP_3Rs and RyRs in response to cholinergic or β-adrenergic stimulation,[2,14] respectively. Our discovery of polycystin-2 expression in the lacrimal gland adds to the complexity of the role of Ca^{2+} signaling in tear secretion and provides potential new targets for pharmaceutical intervention for dry eye disease and other diseases affecting structure and function of lacrimal glands.

Using immunohistochemistry on sections of mouse adult lacrimal gland and immunocytochemistry on acutely isolated lacrimal acinar cells, we show significant expression of all three isoforms of polycystin-2 channels of the TRPP family, polycystin-2, PCL, and polycystin-2L2. The diffuse cytosolic pattern of immunoreactivity and the high expression on the membranes of the ER/Golgi (and to a lesser extent to the membranes of the nuclear envelope) is similar to that of other intracellular Ca^{2+} channels, specifically IP_3Rs and RyRs.[14] PCL and polycystin-2L2 show additional immunoreactivity at the plasma membrane, consistent with their role as plasma membrane Ca^{2+} channels.[8,10,19–21] In contrast, polycystin-2 is the only intracellular Ca^{2+} channel featuring a noncanonical ER retention sequence[9] and accordingly does not show plasma membrane expression in lacrimal gland ducts or freshly isolated lacrimal acinar cell preparations.

To further characterize polycystin-2 in the lacrimal gland, we performed a detailed electrophysiological analysis of polycystin-2 channels using the lipid planar bilayer electrophysiology system. Lacrimal gland polycystin-2 channels exhibited very similar properties to those reported by us previously for other tissues[9] and organisms.[22] Specifically, the slope conductance of lacrimal gland polycystin-2 channels was similar to those measured in kidney epithelial cells and *C. elegans* (114 and 104 pS, respectively[9,22]). Furthermore, voltage-dependent open probability and activity, inhibition by 3 mM and 10 mM cytoplasmic Mg^{2+}, suggesting a dose-dependent effect, and the

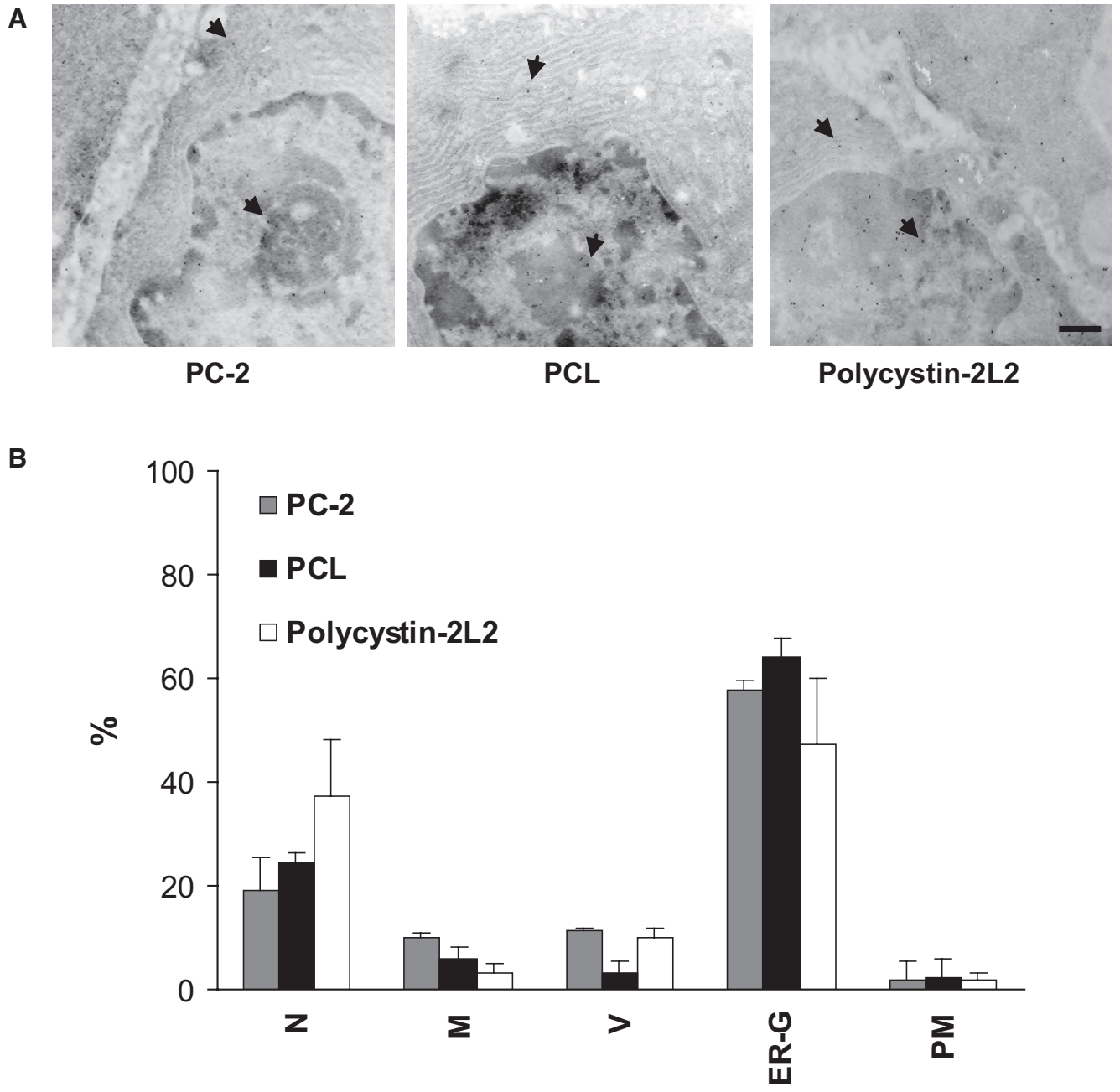

FIGURE 3. Polycystins localize to the membranes of the ER/Golgi and the nucleus. (**A**) Representative images of the subcellular localization of polycystin-2, PCL, and polycystin-2L2. *Arrows* indicate 15 nm immunogold label in the different subcellular compartments. Scale bar, 100 nm. (**B**) Quantification of immunogold label expressed as percentage of total particles measured and corrected for control (omission of primary antibody). Polycystin expression is strongest in the membranes of the ER/Golgi and nucleus, in accordance with their function as intracellular Ca^{2+} release channels. Data are shown as mean ± SEM ($n = 3$). ER-G, ER-Golgi apparatus; M, mitochondria; N, nucleus; PM, plasma membrane; V, vesicles.

bimodal dependence on cytoplasmic Ca^{2+} concentrations were similar to those reported by us for other tissues[9] and organisms.[22]

The results presented here complement existing data correlating activation and function of intracellular calcium channels with tear fluid secretion from the lacrimal gland, as we show that polycystin-2, given its localization and biophysical properties, forms functional intracellular Ca^{2+} release channels in mouse lacrimal gland.

It has previously been shown that colocalization of polycystin-2 and IP_3Rs in microdomains is essential for local Ca^{2+}-induced Ca^{2+} release mechanisms,[22–24] and that disruption of this interaction likely accounts for pathophysiological aberrations in autosomal dominant polycystic kidney disease.[22,24] Similarly, it has been shown that polycystin-2 regulates RyR-mediated Ca^{2+} signaling in cardiac myocytes.[25] Based on these findings, it is likely that polycystin-2 channels are modulators of intracellular Ca^{2+} signaling in the lacrimal gland. However, little is known to date about the contributions of polycystin-2 to the complex Ca^{2+} signaling in exocrine glands.[26] Besides the present report of polycystin-2 expression in the lacrimal gland, polycystin-2 expression has only been identified in adult pituitary gland.[27]

Elevated levels of oxidative stress contribute to the pathophysiology of dry eye disease.[28] Oxidative stress often results in dysregulated Ca^{2+} homeostasis and intracellular

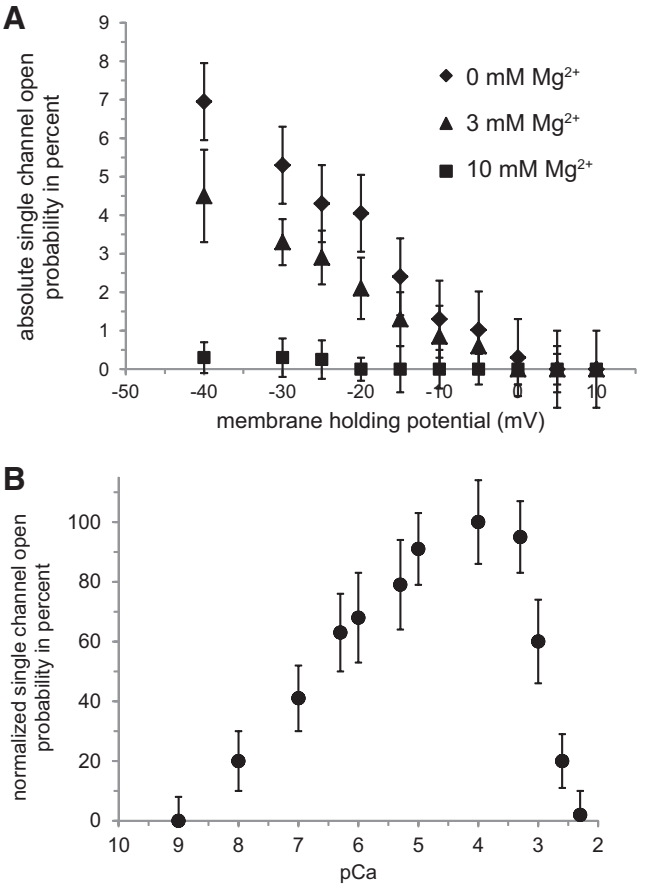

Figure 4. Single channel electrophysiology of polycystin-2 isolated from mouse lacrimal gland. (**A**) Single-channel channel activity of polycystin-2 in ER microsomes isolated from mouse lacrimal gland was determined at pCa 7 as a function of the holding potential of the lipid bilayer membrane at varying concentrations of cytosolic Mg^{2+} and is shown as the absolute channel open probability. (**B**) The activity dependence of lacrimal gland polycystin-2 on the cytosolic Ca^{2+} concentration was measured and normalized to maximal activity at pCa 4. Increased activity was due to elevated mean open time and frequency of single-channel openings with lower activity at low and high physiological cytosolic Ca^{2+} concentrations. Data are shown as mean ± SEM ($n = 3$).

Ca^{2+} channels are targets for oxidative stress-induced modulation.[29,30] For instance, polycystin-2 is downregulated in response to radical oxygen species in term human syncytiotrophoblasts.[31] In contrast, we have previously shown that IP_3Rs are upregulated in response to oxidative stress.[32] Besides oxidative stress, hormonal regulation contributes to the etiology of dry eye disease, with females being at higher risk for developing the disease.[3,4,17] Hormone replacement therapy even further increases the risk for developing dry eye disease.[33] Interestingly, polycystin-2 mRNA levels in the lacrimal gland are directly correlated with hormone levels. Overall mRNA levels have been shown to be significantly higher in females than in males.[34] Furthermore, polycystin-2 mRNA levels could be increased by estrogen treatment,[35] and reduced by testosterone exposure.[36] Based on these findings, it could speculated that increased levels of polycystin-2 under conditions of oxidative stress, together with changes in other intracellular Ca^{2+} channels,[32] result in Ca^{2+} dyshomeostasis in the lacrimal gland and ensuing deficits in lacrimal fluid secretion that contribute to etiology of dry eye disease.

Future studies are warranted to demonstrate the specific involvement of polycystin-2 channels in Ca^{2+} handling in the lacrimal gland under physiological conditions, as well as the possible involvement of polycystin-2 in the pathophysiology of diseases affecting the lacrimal gland and their modulation by oxidative stress. Results from these studies will determine the potential benefit and feasibility of targeting polycystin-2 channels as novel pharmaceutical intervention approaches in dry eye disease and Sjögren's syndrome.

In conclusion, we here report that the intracellular Ca^{2+} channel polycystin-2 is expressed at significant levels in mouse adult lacrimal gland and exhibits properties similar to those reported in other tissues and organisms. Our results highlight the need to further decipher mechanisms and pathways of Ca^{2+} signaling in the lacrimal gland and support the strategy of using Ca^{2+} signaling as a therapeutic target for the treatment of ocular diseases affecting tear production.

Acknowledgments

Excellent technical assistance for electron microscopy was provided by A.M. Brun-Zinkernagel. The authors thank Margaret, Richard, and Sara Koulen for generous support and encouragement.

References

1. Bromberg BB. Autonomic control of lacrimal protein secretion. *Invest Ophthalmol Vis Sci.* 1981;20:110–116.
2. Sundermeier T, Matthews G, Brink PR, Walcott B. Calcium dependence of exocytosis in lacrimal gland acinar cells. *Am J Physiol Cell Physiol.* 2002;282:C360–C365.
3. Gayton JL. Etiology, prevalence, and treatment of dry eye disease. *Clin Ophthalmol.* 2009;3:405–412.
4. Yu J, Asche CV, Fairchild CJ. The economic burden of dry eye disease in the United States: a decision tree analysis. *Cornea.* 2010;30:379–387.
5. Koulen P, Thrower EC. Pharmacological modulation of intracellular Ca(2+) channels at the single-channel level. *Mol Neurobiol.* 2001;24:65–86.
6. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol.* 2003;4:517–529.
7. Yang J, McBride S, Mak DO, et al. Identification of a family of calcium sensors as protein ligands of inositol trisphosphate receptor Ca(2+) release channels. *Proc Natl Acad Sci U S A.* 2002;99:7711–7716.
8. Chen XZ, Vassilev PM, Basora N, et al. Polycystin-L is a calcium-regulated cation channel permeable to calcium ions. *Nature.* 1999;401:383–386.
9. Koulen P, Cai Y, Geng L, et al. Polycystin-2 is an intracellular calcium release channel. *Nat Cell Biol.* 2002;4:191–197.
10. Volk T, Schwoerer AP, Thiessen S, Schultz JH, Ehmke H. A polycystin-2-like large conductance cation channel in rat left ventricular myocytes. *Cardiovasc Res.* 2003;58:76–88.
11. Gromada J, Jorgensen TD, Dissing S. The release of intracellular Ca2+ in lacrimal acinar cells by alpha-, beta-adrenergic and muscarinic cholinergic stimulation: the roles of inositol triphosphate and cyclic ADP-ribose. *Pflugers Arch.* 1995;429:751–761.
12. Gromada J, Jorgensen TD, Dissing S. Cyclic ADP-ribose and inositol 1,4,5-triphosphate mobilizes Ca2+ from distinct intracellular pools in permeabilized lacrimal acinar cells. *FEBS Lett.* 1995;360:303–306.
13. Gromada J, Jorgensen TD, Dissing S. Role of protein kinase C in the regulation of inositol phosphate production and Ca2+ mobilization evoked by ATP and acetylcholine in rat lacrimal acini. *Pflugers Arch.* 1995;429:578–586.
14. Medina-Ortiz WE, Gregg EV, Brun-Zinkernagel AM, Koulen P. Identification and functional distribution of intracellular Ca channels in mouse lacrimal gland acinar cells. *Open Ophthalmol J.* 2007;1:8–16.

15. Stokely ME, Hwang SY, Hwang JY, et al. Polycystin-1 can interact with homer 1/Vesl-1 in postnatal hippocampal neurons. *J Neurosci Res.* 2006;84:1727–1737.
16. Fabiato A. Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. *Methods Enzymol.* 1988;157:378–417.
17. Tavares Fde P, Fernandes RS, Bernardes TF, Bonfioli AA, Soares EJ. Dry eye disease. *Semin Ophthalmol.* 2010;25:84–93.
18. Zoukhri D, Hodges RR, Rawe IM, Dartt DA. Ca2+ signaling by cholinergic and alpha1-adrenergic agonists is up-regulated in lacrimal and submandibular glands in a murine model of Sjogren's syndrome. *Clin Immunol Immunopathol.* 1998;89:134–140.
19. Liu Y, Li Q, Tan M, et al. Modulation of the human polycystin-L channel by voltage and divalent cations. *FEBS Lett.* 2002;525:71–76.
20. Guo L, Schreiber TH, Weremowicz S, Morton CC, Lee C, Zhou J. Identification and characterization of a novel polycystin family member, polycystin-L2, in mouse and human: sequence, expression, alternative splicing, and chromosomal localization. *Genomics.* 2000;64:241–251.
21. Nomura H, Turco AE, Pei Y, et al. Identification of PKDL, a novel polycystic kidney disease 2-like gene whose murine homologue is deleted in mice with kidney and retinal defects. *J Biol Chem.* 1998;273:25967–25973.
22. Koulen P, Duncan RS, Liu J, et al. Polycystin-2 accelerates Ca2+ release from intracellular stores in Caenorhabditis elegans. *Cell Calcium.* 2005;37:593–601.
23. Li Y, Wright JM, Qian F, Germino GG, Guggino WB. Polycystin 2 interacts with type I inositol 1,4,5-trisphosphate receptor to modulate intracellular Ca2+ signaling. *J Biol Chem.* 2005;280:41298–41306.
24. Sammels E, Devogelaere B, Mekahli D, et al. Polycystin-2 activation by inositol 1,4,5-trisphosphate-induced Ca2+ release requires its direct association with the inositol 1,4,5-trisphosphate receptor in a signaling microdomain. *J Biol Chem.* 2010;285:18794–18805.
25. Anyatonwu GI, Estrada M, Tian X, Somlo S, Ehrlich BE. Regulation of ryanodine receptor-dependent calcium signaling by polycystin-2. *Proc Natl Acad Sci U S A.* 2007;104:6454–6459.
26. Petersen OH, Tepikin AV. Polarized calcium signaling in exocrine gland cells. *Annu Rev Physiol.* 2008;70:273–299.
27. Markowitz GS, Cai Y, Li L, et al. Polycystin-2 expression is developmentally regulated. *Am J Physiol.* 1999;277:F17–F25.
28. Wakamatsu TH, Dogru M, Tsubota K. Tearful relations: oxidative stress, inflammation and eye diseases. *Arq Bras Oftalmol.* 2008;71:72–79.
29. Zundorf G, Reiser G. Calcium dysregulation and homeostasis of neural calcium in the molecular mechanisms of neurodegenerative diseases provide multiple targets for neuroprotection. *Antioxid Redox Signal.* 2011;14:1275–1288.
30. Duncan RS, Goad DL, Grillo MA, Kaja S, Payne AJ, Koulen P. Control of intracellular calcium signaling as a neuroprotective strategy. *Molecules.* 2010;15:1168–1195.
31. Montalbetti N, Cantero MR, Dalghi MG, Cantiello HF. Reactive oxygen species inhibit polycystin-2 (TRPP2) cation channel activity in term human syncytiotrophoblast. *Placenta.* 2008;29:510–518.
32. Kaja S, Duncan RS, Longoria S, et al. Novel mechanism of increased Ca(2+) release following oxidative stress in neuronal cells involves type 2 inositol-1,4,5-trisphosphate receptors. *Neuroscience.* 2011;175:281–291.
33. Schaumberg DA, Buring JE, Sullivan DA, Dana MR. Hormone replacement therapy and dry eye syndrome. *JAMA.* 2001;286:2114–2119.
34. Richards SM, Jensen RV, Liu M, et al. Influence of sex on gene expression in the mouse lacrimal gland. *Exp Eye Res.* 2006;82:13–23.
35. Suzuki T, Schirra F, Richards SM, et al. Estrogen's and progesterone's impact on gene expression in the mouse lacrimal gland. *Invest Ophthalmol Vis Sci.* 2006;47:158–168.
36. Sullivan DA, Jensen RV, Suzuki T, Richards SM. Do sex steroids exert sex-specific and/or opposite effects on gene expression in lacrimal and meibomian glands? *Mol Vis.* 2009;15:1553-1572.