New Experimental Method to Study Acid/Base Transporters and Their Regulation in Lacrimal Gland Ductal Epithelia

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The function of the lacrimal gland is to produce the preocular component of the tear film covering the surfaces of the cornea and the conjunctiva. This secreted fluid, which contains proteins, ions, and water, is essential for maintaining healthy, normal function of the ocular surface. When tear secretion decreases in amount or changes in composition, dry eye syndrome (keratoconjunctivitis sicca) can develop and, in the worst case, can induce corneal ulceration and vascularization leading to serious visual impairment.1,2

As do all exocrine glands, such as the pancreas and the submandibular gland, the lacrimal gland has three major cell types—acinar, ductal, and myoepithelial (surrounding acinar and ductal cells).3,4 Preocular tear film is mostly secreted by acini and ductal cells. Because of the convenient accessibility of the external end of the lacrimal gland duct, the precorneal tear has been characterized in detail using tear-collecting techniques in which the main duct is cannulated and the secreted fluid is collected.5

Methods have been published by which proteins and fluids secreted by the acini can be studied.6 In principle, the gland is removed and minced into small pieces. These pieces undergo enzymatic digestion, resulting in small groups of acini or single acinar cells.7 These techniques are mostly used for animal studies. However, some investigators have also isolated acini from human lacrimal gland biopsy specimens or cadavers.8,9

Despite the large number of studies on the whole lacrimal gland and acini, less is known about the lacrimal gland ductal cell (LGDC).10,11 Ubels et al.11 have recently described a laser capture microdissection technique for cDNA microarray analysis and immunohistochemistry using frozen lacrimal gland, but no methods have been developed to characterize the LGDC secretion in viable ductal cells. Nevertheless, the secretory mechanisms of ductal epithelia may play a physiological role in the maintenance of the standard environment of the cornea and the conjunctiva. More important, the failure of ion and water secretion, as may occur in dry eye syndrome or in cystic fibrosis,12,13 has serious consequences for the integrity of the cornea and can lead to potentially sight-threatening disease that diminishes the patient’s quality of life. Therefore, it is imperative to separate the functions of acini and ductal cells in the secretion of preocular tear. Better understanding of LGDC secretion at the cellular and molecular levels under normal and pathologic conditions may help in the development of drugs that stimulate tear secretion in patients with dry eye.

Our aims in this study were to develop a method to isolate lacrimal ducts, to make it possible to obtain more information on the regulation of lacrimal gland epithelial tissue, and to characterize LGDC acid/base ion transporters (mediating fluid secretion).

MATERIALS AND METHODS

Ethics

All experiments were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.
and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. In addition, the experimental protocol was approved by the local ethical board of the University of Szeged, Hungary.

**Solutions and Chemicals**

The standard HEPES-buffered solution contained 130 mM NaCl, 5 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM [SCAP]-glucose, and 10 mM Na-HEPES. The Na$^+$-free HEPES-buffered solution contained 140 mM NMDG-Cl, 5 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM [SCAP]-glucose, and 10 mM Na-HEPES-acid. The ammonium pulse HEPES-buffered solution contained 110 mM NaCl, 20 mM NH$_4$Cl, 5 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM t-glucose, and 10 mM Na-HEPES. The Cl$^-$-free HEPES solution contained 140 mM N-a-gluconate, 2.5 mM K$_2$-gulonate, 1 mM Mg$_2$-gulonate, 10 mM [SCAP]-glucose, 10 mM Na-HEPES, and 0.01 mM nigericin. HEPES-buffered solutions were gassed with 100% O$_2$, and their pH was set to 7.4 with NaOH or HCl at 37°C. The standard HCO$_3^-$-buffered solution contained 115 mM NaCl, 25 mM NaHCO$_3$, 5 mM KCl, mM 1 CaCl$_2$, mM 1 MgCl$_2$, and 10 mM t-glucose. The ammonium pulse HCO$_3^-$-buffered solution contained 95 mM NaCl, 20 mM NH$_4$Cl, 25 mM NaHCO$_3$, 5 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, and 10 mM [SCAP]-glucose. The Na$^+$-free HCO$_3^-$-buffered solution contained 115 mM NMDG-Cl, 5 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM [SCAP]-glucose, 10 mM choline-HCO$_3^-$, and 0.01 mM atropine, and pH was set to 8.0 with HCl. The Cl$^-$-free HCO$_3^-$-buffered solution contained 115 mM Na-glucocinate, 25 mM NaHCO$_3$, 2.5 mM K$_2$-gulonate, 1 mM Mg$_2$-gulonate, and 10 mM t-glucose. HCO$_3^-$-buffered solutions were gassed with 95% O$_2$/5% CO$_2$ to set pH to 7.4 at 37°C. Cell and tissue adhesives was obtained from Becton Dickinson Labware (Cell Tak; Bedford, MA). 2.7-bis-(2-carboxyethyl)-5-(and-6-)carboxyfluorescein, acetoxymethyl ester (BCECF-AM), FURA 2AM, and 4,4'-disothiocyanato-3,2'-disulfonate (H$_2$DIDS) were obtained from Molecular Probes (Eugene, OR). BCECF was dissolved in dimethyl sulfoxide (DMSO), and FURA 2AM was dissolved in DMSO containing 20% Pluronic acid. Nigericin was dissolved in absolute ethanol, and amiloride was dissolved in DMSO. All other chemicals were obtained from Sigma-Aldrich (Budapest, Hungary).

**Solutions and Chemicals for Isolation**

Chromatographically pure collagenase was obtained from Worthington (Lakewood, NJ). Ingredients for culture medium (Dubecco modified Eagle medium [DMEM], McCoy 5A medium modified [McCoy 5A], fetal calf serum, glutamine, and bovine serum albumin) were from Sigma-Aldrich. The isolation solution contained DMEM supplemented with 100 U/mL collagenase and 1 mg/mL bovine serum albumin. The storage solution contained DMEM and 5% (wt/vol) bovine serum albumin. The culture solution contained McCoy 5A tissue culture medium, 10% (vol/vol) fetal calf serum, and 2 mM glutamine.

**Preparation of Micropipettes**

Micropipettes were prepared for transferring ducts after isolation. Glass tubes were obtained from Drummond Scientific Company (Broome, PA) and were pulled by a vertical pipette puller (Technical Product International Inc., St. Louis, MO) from glass tubing (inside diameter, 0.075 inch; outside diameter, 0.090 inch). Inside diameters of tips were between 50 and 150 μm.

**Animals**

Adult male New Zealand White rabbits weighing 2 to 2.5 kg were sedated with 50 mg/kg pentobarbital and humanely killed by cervical dislocation. Superotemporal and inferotemporal portions of the conjunctival fornices were dissected after wide temporal canthotomy. The eyeball was then dislocated inferonasally, and the temporal part of the orbital connective tissues were excised using stereomicroscopy. The preparation procedure revealed the main lobes of the lacrimal gland under the roof of the orbit, which were removed by gentle pressure with forceps and final separation with scissors. Both intraorbital lacrimal glands were carefully dissected.

**Isolation Process**

Intraorbital lacrimal glands were dissected as described above and were transferred to a sterile, small, flat-bottom glass Erlenmeyer flask containing cold (4°C) storage solution, as described, to minimize damage to the cells. Then the glands were placed on a 4°C sterile glass plate. First, the glands were trimmed of fat and then 1 mL isolation solution was injected into the interstitium of the glands using 26-gauge × 0.5-inch (0.45 × 12 mm) medical stainless steel needles (Braun Melsungen AG, Melsungen, Germany). Microinjected glands were cut into small pieces using a razor blade and were transferred, using Pasteur pipettes, to a glass flask containing 2 mL isolation solution. The flask was briefly gassed with 100% O$_2$ and was incubated in a shaking water bath (80 cyc/min) at 37°C for 25 minutes. After incubation, the isolation solution was removed, and 5 mL fresh cold storage solution was added to the flask. Digested tissue was washed two more times with storage solution to minimize the amount of collagenase in the solution. Finally, the tissue was transferred to a disposable 10 mL polycarbonate tube and was kept at 4°C until microdissection.

Tissue sample suspension was transferred, using a Pasteur pipette, to a glass microscope slide and viewed under a stereo microscope (Jencons-PLS; Nikon, Grinstead, UK) equipped with a cold-light source. Intralobular and interlobular ducts were microdissected under 50× magnification with 26-gauge × 0.5 inch (0.45 × 12 mm) medical stainless steel needles. Isolated ducts were aspirated into a micropipette (described in Methods) and transferred to a Petri dish containing storage solution. After 20 to 30 minutes, the tissue sample was discarded and replaced by a fresh cold piece of tissue. Fifteen to 25 ducts were isolated from each animal.

**Culturing**

After microdissection, intact lacrimal gland ducts were transferred to a polycarbonate hydrophilic membrane (10-μm pore size; Whatman International Ltd., Kent, UK) placed on top of the culture solution in a Petri dish. Ducts were cultured overnight in a 37°C incubator gassed with 5% CO$_2$/95% air.

**Transmission Electron Microscopy**

For electron microscopic studies, ducts were fixed in 2.5% glutaraldehyde immediately after isolation. Samples were then postfixed in 1% osmium tetroxide, dehydrated in a series of graded ethanol, and subsequently embedded in epoxy resin. Ultrathin sections were contrasted with uranyl acetate and lead citrate. Tissue sections were analyzed under a transmission electron microscope (CM10; Philips, Eindhoven, The Netherlands; Fig. 1).

**Intracellular pH Measurement**

Cultured lacrimal ducts were attached, using an adhesive (Cell Tak; Becton Dickinson Labware), to a coverslip (24 mm) forming the base of a perfusion chamber mounted on a microscope (Olympus, Budapest, Hungary). Ducts were bathed in standard HEPES solution at 37°C and loaded with the pH-sensitive fluorescent dye BCECF-AM (2 μM) for 20 to 30 minutes. Thereafter, the ducts were continuously perfused with solutions at a rate of 4 to 5 mL/min. Intracellular pH (pHi$_{5+}$) was measured using an imaging system (Cell; Olympus). Four to five small areas (regions of interest [ROIs]) of 5 to 10 cells each in an intact duct were excited with light at wavelengths of 490 nm and 440 nm, and the 490/440 fluorescence emission ratio was measured at 535 nm (Fig. 2). One pH$_{5+}$ measurement was recorded per second. In situ calibration of the fluorescence signal was performed using the high K$^+$-nigericin technique.$^{14,15}$ During calibration, ducts were bathed in high K$^+$-
HEPES solution containing 10 μM nigericin, and extracellular pH was stepped between 5.95 and 8.46. Initial rates of pHi recovery (over the first 30 seconds) were calculated by linear regression analysis.

Measurement of Intracellular Ca\(^{2+}\) Concentration

Cultured lacrimal ducts were attached to a coverslip and mounted on an microscope (Olympus), as described above. The ducts were bathed in standard HEPES solution at 37°C and loaded with the Ca\(^{2+}\)-sensitive fluorescent dye FURA 2AM (4–5 μM) for 60 minutes. After loading, the ducts were continuously perfused with solutions at a rate of 4 to 5 mL/min. Changes in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) were measured using an imaging system (Cell; Olympus). Four to 5 small ROIs of 5 to 10 cells in each intact duct were excited with light at wavelengths of 340 nm and 380 nm, and the 380/340 fluorescence emission ratio was measured at 510 nm (see Fig. 5). One [Ca\(^{2+}\)]\(_i\) measurement was obtained per second.

Statistical Analysis

Results are expressed as mean ± SEM (n = 3–6 ducts/10–30 ROIs). Statistical analyses were performed using ANOVA. P ≤ 0.05 was accepted as significant.

RESULTS

Morphology of Isolated Ducts

Ultrastructural examination revealed that small ducts were characterized by numerous microvilli in the apical regions, tight junctions, secretory granules, mitochondria, and basolateral infoldings (interdigitations) of the cell membrane and basement membrane in the basal region (Fig. 1). Cells were relatively rich in vesicles and secretory granules (Fig. 1). Figure 2A shows an isolated interlobular duct attached to a coverslip, as described in Methods. Lumen (L), epithelial cells (ECs), and connective tissue (CT) are visible. This figure confirms that acini-free epithelial cells can be chosen for data recordings (ROI).

pH Regulation in Lacrimal Gland Ductal Epithelia

In the first series of experiments, we wanted to determine the resting pHi of LGDC. Ducts were exposed to standard HEPES solution (pH 7.4), followed by an 8-minute exposure to a high-K\(^+\)-HEPES solution (pH 7.28) and then to an 8-minute

FIGURE 1. Electron micrographs of intact lacrimal ducts that had been maintained in culture for 24 hours. (A) Horizontal sections of isolated ducts. LM, luminal membrane; BM, basolateral membrane; EC, epithelial cell. (B) Luminal side of the lacrimal duct. MC, mitochondria; MV, microvilli; SG, secretory granule. (C) Basolateral side of the lacrimal duct. BL, basolateral interdigitation.

FIGURE 2. Resting pHi of lacrimal ductal epithelial cells. Cultured lacrimal ducts were attached to a coverslip. (A) Four to five small areas (ROIs) of 5 to 10 cells each in an intact duct were excited with light at wavelengths of 490 nm and 440 nm, and the 490/440 fluorescence emission ratio was measured at 535 nm (see Fig. 5). One [Ca\(^{2+}\)]\(_i\) measurement was obtained per second.

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exposure to a high-K\(^+\)-HEPES solution (pH 7.4). We used the classical linear model\(^{14,15}\) to determine the resting pHi. The resting pHi level of five ducts (22 ROIs) was found to be 7.40 ± 0.01. The resting pHi of LGDCs was virtually the same, confirming that the experimental conditions could be kept constant for pHi experiments (Fig. 2B).

Na\(^+\)/H\(^+\) Exchanger (NHE). In this series of experiments, we tested whether isolated lacrimal glands were suitable for functional experiments. Na\(^+\)/H\(^+\) transport proteins that mediate electroneutral exchange of Na\(^+\) and H\(^+\) ions were examined. Removal of Na\(^+\) from the standard HEPES solution caused rapid and marked intracellular acidosis (0.20 ± 0.01 pH U/min; n = 3 ducts/15 ROIs; Fig. 3A). Adding Na\(^+\) back to the solution resulted in complete pHi recovery. Because the solution did not contain HCO\(_3^−\), this finding confirms the presence of a Na\(^+\)-dependent H\(^+\) efflux mechanism on the basolateral side of each LGDC. Removal of Na\(^+\) from the HCO\(_3^−\)/CO\(_2\)-containing solution also caused mark acidification (0.22 ± 0.04 pH U/min; n = 3 ducts/15 ROIs; Fig. 3B).

Na\(^+\)/HCO\(_3^−\) Cotransporter (NBC). We also tested whether LGDCs express a functionally active Na\(^+\)-dependent HCO\(_3^−\) transporter on the basolateral membrane (Fig. 3B). The administration of basolateral HCO\(_3^−\)/CO\(_2\) rapidly and greatly decreased pHi. This marked change in pHi could be explained by the quick diffusion of CO\(_2\) into the cytoplasm. A small pHi recovery (0.04 ± 0.02 pH U/min; n = 6 ducts/30 ROIs) was found after acidification, suggesting the marginal role of HCO\(_3^−\) efflux into the lacrimal duct cells.

Cl\(^−\)/HCO\(_3^−\) Exchange Activity. To test the activity of the Cl\(^−\)/HCO\(_3^−\) exchange mechanisms, we used the Cl\(^−\) removal technique in the presence and absence of HCO\(_3^−\) ions. In the absence of HCO\(_3^−\), Cl\(^−\) removal caused a small reversible

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933443/)
alkalization in LGDC (Fig. 3C; 0.020 ± 0.002 pH U/min), suggesting the small availability of HCO₃⁻ ions in the cytoplasm. However, in standard HCO₃⁻ solution, significantly higher alkalization was observed (0.16 ± 0.02 pH U/min, respectively). In addition, the anion exchange inhibitor H₂DIDS (250 μM) significantly inhibited pH/t (Figs. 3E, 3F; 0.067 ± 0.015 pH U/min). These results confirmed functionally active Cl⁻/HCO₃⁻ exchange mechanisms on the basolateral membranes of LGDCs.

pHᵢ Recovery from Alkali and Acid Load

An alternative method for characterizing these transporters is the ammonium-pulse technique.¹⁶ Administration of 20 mM NH₄Cl initially increases pHᵢ because of the rapid entry of NH₃ into the cell. Recovery from alkali load may reflect the activity of the Cl⁻/HCO₃⁻ exchanger (AE).¹⁶ Removal of NH₄Cl causes the typical acidic undershoot of pHᵢ (Fig. 4A). Transporters (if present in LGDCs) most likely involved in the recovery process from acidosis are the basolateral Na⁺/HCO₃⁻ cotransporter, the Na⁺/H⁺ exchanger, and the H⁺ pump.

Recovery (ΔpH/Δt) from alkali load was significantly higher in the presence of HCO₃⁻ (0.049 ± 0.004 pH U/min and 0.08 ± 0.001 pH U/min, respectively), suggesting an active Cl⁻/HCO₃⁻ exchanger.

Recovery from acid load was 0.12 ± 0.01 pH U/min in standard HCO₃⁻ solution (containing Na⁺ and HCO₃⁻/CO₂). The absence of HCO₃⁻ did not significantly change the rate of recovery (0.11 ± 0.015 pH U/min). However, the removal of Na⁺ from the HEPES solution significantly decreased recovery from acid load to 0.012 ± 0.002 pH U/min by switching off the NHE. The small remaining recovery from acid load may represent an active proton pump in LGDCs. Finally, we tested the NHE inhibitor amiloride (0.2 mM). Amiloride administration greatly inhibited the NHE (0.04 ± 0.01 pH U/min) located on the basolateral membranes of LGDCs. Furthermore, the removal of amiloride immediately turned on the NHE, suggesting the reversible effect of amiloride.

Ca²⁺ Signaling during Parasympathomimetic Stimulation

Parasympathetic neurotransmitters acetylcholine (ACh) and vasointestinal peptide are potent stimuli of lacrimal gland secretion¹⁷ and have been shown to act through the intracel-
Carbachol dose dependently elevated intracellular Ca\textsuperscript{2+} concentration in LGDCs. Each experiment was performed on the same duct using a 10-minute washout period between the pulses. Representative curves are shown. Maximal [Ca\textsuperscript{2+}]\textsubscript{i} elevation was observed 2 ± 0.5 seconds after stimulation. Data were taken from the ROI marked in the image. Similar results were obtained when the experiments were performed on different ducts (n = 3). (B) Typical patterns of [Ca\textsuperscript{2+}]\textsubscript{i} changes in an intact duct perfused with different concentrations of carbachol. Increase in [Ca\textsuperscript{2+}]\textsubscript{i} is denoted by a change from a cold color (blue) to a warmer color (yellow to red; see color scale at the top). Pictures 1 and 2 were taken at the times indicated by the circles in (A). Bar, 30 \textmu m.

**Effects of Carbachol on NHE and AE**

Administration of 1 mM carbachol significantly elevated the pH\textsubscript{i} in standard HEPES solution (containing Na\textsuperscript{+} and Cl\textsuperscript{-} but not HCO\textsubscript{3}\textsuperscript{-}; Fig. 6A). However, this elevation was not observed in an Na\textsuperscript{+}-free HEPES solution (Fig. 6B). Because HCO\textsubscript{3}\textsuperscript{-} was absent, the alkalization in the Na\textsuperscript{+}-containing solution must have been the result of a stimulated Na\textsuperscript{+}-dependent H\textsuperscript{+} efflux mechanism through the NHE (Fig. 6A). When the LGDCs were treated with 1 mM carbachol in standard HCO\textsubscript{3}\textsuperscript{-} solution, a small pH\textsubscript{i} elevation was observed (Fig. 6C). However, this brief alkalization (most likely caused by the stimulation of an NHE) was followed by acidification. Importantly, this acidification was absent in a Cl\textsuperscript{-}-free HCO\textsubscript{3}\textsuperscript{-} solution, suggesting that this decrease in pH\textsubscript{i} was caused by a Cl\textsuperscript{-}-dependent HCO\textsubscript{3}\textsuperscript{-} efflux mechanism through a Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchanger (Fig. 6D). These data indicate that carbachol stimulates Na\textsuperscript{+} and Cl\textsuperscript{-} influx into the cell through the basolateral membrane of the LGDC. Parasympatholytic atropine (0.2 mM) completely blocked the stimulatory effect of 1 mM carbachol (Fig. 6E).
To confirm this hypothesis, we analyzed the recoveries from acid and alkali load using the ammonium pulse technique. Figure 7 shows a representative trace of the experiments. We found that 1 mM carbachol significantly stimulated NHE (recovery from acid load in an HCO₃⁻/H₂¹⁰₀₂⁻ free solution; Figs. 7A, 7B). No differences were observed in the recovery from alkali load in a HCO₃⁻/H₂¹⁰₀₂⁻ free (HEPES) solution. However, when the experiments were performed in standard HCO₃⁻/H₂¹⁰₀₂⁻ solution, the AE (recovery from alkali load; Fig. 7C) was stimulated by 1 mM carbachol. As we found earlier, atropine (0.2 mM) totally blocked the stimulatory effect of carbachol on the NHE and AE (data not shown).

DISCUSSION

Lacrimal gland secretion consists of two fractions derived from acinar and ductal cells. The regulation of ion and water secretion has been well investigated in intact glands,¹⁷ but no available method has been described to study the role of LGDCs in the process of lacrimal fluid secretion. The precocular tear secreted by the lacrimal gland contains Na⁺, Cl⁻, and K⁻ in high concentrations. This lacrimal gland fluid contains 42 ± 4 mM K⁺, 107 ± 4 mM Na⁺, 126 ± 5 mM Cl⁻ in rabbit,¹⁸ 46 ± 3 mM K⁺, 135 ± 5 mM Na⁺, 123 ± 1 mM Cl⁻ in rat,¹⁹ and 38 ± 5 mM K⁺, 144 ± 5 mM Na⁺, 149 ± 16 mM Cl⁻ in mouse.⁷ Ductal epithelia, at least in part, must be involved in this hypertonic fluid secretion.

In other secretory glands, such as the pancreas, duct isolation techniques have been described,²⁰ and the secretion by acini and ductal cells can therefore be studied separately. In the present study, we developed an isolation technique suitable for investigating the ion transporters of LGDCs and the regulation of fluid secretion. The microdissection technique was similar to what we used in the pancreas.¹⁵,¹⁶ However, because the connective tissue sticks to the lacrimal ducts more strongly than in the pancreas, isolation takes more time and fewer ducts can be isolated.
cell.21 Therefore, to show the viability of isolated and cultured epithelial cells that is able to transport acid/base equivalents through the basolateral membranes between the acinar cells and LGDCs. Many epithelial cells express proton pumps25 and NBC,26 which, in addition to other physiological roles, can protect epithelial cells from acidosis. We demonstrated that NBC ion transporters, if present, have only a marginal role in the pHi regulation of LGDCs. After CO2-induced acidosis, only a small amount of HCO3− entry was detected (Fig. 3B). Furthermore, no difference was found in the regeneration after acid load caused by an ammonium pulse between the presence and absence of HCO3−. Removal of Na+ decreased this recovery by 93% in HEPES solution, suggesting a functionally very active Na+-dependent H+ efflux mechanism.

We also detected a functionally active Cl−-dependent HCO3− efflux mechanism in LGDCs. When HCO3− was absent from the solution, Cl− removal only caused a small pHi change, suggesting reduced HCO3− concentration inside the cell. However, when HCO3− was present in the solution, Cl− removal caused a marked pHi elevation. We found that the classic and defining inhibitor of SLC4 family AE1-AE4,27,28 H2DIDS,29 strongly inhibited the Cl−-dependent HCO3− efflux mechanism. AE1 has been identified in rat lacrimal ducts.11 Thus far, however, no other AEs have been confirmed in lacrimal ductal epithelium.

We also tested whether the isolated and cultured ducts are suitable for studying the regulation of LGDC secretion. The main function of the lacrimal gland is to secrete water, electrolytes, and proteins onto the eye surface. The relative contribution of acinar cells and LGDCs to this secretion is yet to be determined. Regulation of lacrimal gland secretion can be mediated by neurotransmitters (e.g., ACh) and growth factors (e.g., cyclodulin and cyclodulin growth factor family).30 Activation of muscarinic receptors by ACh released from parasympathetic nerves stimulates lacrimal gland secretion. The glandular subtype of M3 muscarinic receptors have been identified in the lacrimal gland.31 It is more than likely that the ductal epithelium are involved in the hypersecretory effect of parasympathetic stimulation. We tested the effect of carbachol on the intracellular Ca2+ signaling using the Ca2+-sensitive fluorescence dye FURA 2AM. Our results showed that carbachol dose dependently increased [Ca2+].

Finally, we investigated the effects of parasympathetic stimulation on the acid/base transporters of LGDCs and found that carbachol strongly stimulates NHE activity, hence driving Na+ into the cell. This stimulation is followed by the activation of the AE on the basolateral membrane, which drives Cl− into the LGDC. Na+ and Cl− influxes require H+ and HCO3− inside the cell; they become available after the dehydration of carbonic acid (H2CO3) by carbonic anhydrase.32,33 indicating that there must be other differences in ion transport mechanisms on the basolateral membranes between the acinar cells and LGDCs. Na+/K+ ATPase expression is three to five times higher on duct cells than on acinar cells.34 Therefore, elevated intracellular Na+ concentration after parasympathetic activation may stimulate the basolateral Na+/K+ ATPase, which increases the intracellular K+ concentration in LGDC. Our data suggest that Na+/K+ ATPase may be a crucial basolateral transporter in the mechanisms of K+ secretion in LGDCs. After the intracellular accumulation of K+ and Cl−, these ions can be secreted through a coupled mechanism (K+/Cl− cotransporter)11 or through a separate K+-selective cation channel (IK, BKCa, or both) and a Cl−-selective anion channel (cystic fibrosis transmembrane conductance regulator (CFTR), chloride channel (ClC), or both; Fig. 8).

The regulation of pHi in epithelial cells is important because most of the biological processes (e.g., water and ion secretion) are pH sensitive.21 Epithelial cells have a polarized membrane that is able to transport acid/base equivalents through the cell.21 Therefore, to show the viability of isolated and cultured interlobular lacrimal ducts, we characterized the most common acid/base transporters.

Our results showed the functional presence of an Na+-dependent but HCO3−-independent H+ efflux mechanism (most probably through NHEs) on LGDCs. Amiloride partially inhibited this Na+/H+ exchange mechanism. However, we must note that this K+-sparing diuretic can also inhibit electrogenic Na+ channels25 and the Na+/Ca2+ exchanger.25 Given that NHE1 and NHE2 are the most sensitive to amiloride inhibition and NHE3 and NHE4 are amiloride resistant,24 our results indicate that approximately 60% of the functionally active NHEs are NHE1 and NHE2 isoforms.

FIGURE 7. Effects of carbachol on recovery from acid and alkali load. Duct cells were exposed to a 3-minute, 20-mM NH4Cl pulse, followed by its sudden withdrawal. Initial rates of pHi recovery from the acid and alkali load (over the first 30 seconds) were calculated in each experiment. Carbachol (1 mM) was administered from 7 minutes before the NH4Cl pulse. All experiments were performed at 37°C. Each experiment was performed on a different duct. (B) Summary of the calculated initial rates of recovery (ΔpHi/Δt) from acid load (A). Experiments were performed in standard HEPES solution (without HCO3−). (C) Summary of the calculated initial rates of recovery (ΔpHi/Δt) from alkali load in standard HCO3−/CO2 solution.
Taken together, we described a lacrimal gland duct isolation technique in which the intact ducts remain viable and in which the role of duct cells in precocious tear film secretion can be characterized. We also added new insights into the regulation of lacrimal gland ductal secretion. Our data and our new isolation method open up the possibility of understanding the physiological and pathophysiological (such as dry eye syndrome) characterization of epithelial cells of rabbit lacrimal glands in tissue sections and cell cultures. Tissue Cell. 1996;28:301–312.

References
