Secretagogue-Induced Redistributions of Na,K-ATPase in Rat Lacrimal Acini

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Lacrimal acinar cells secrete macromolecular products in an approximately isotonic, sodium chloride (NaCl)-rich fluid. The mechanisms of macromolecular product secretion depend in part on a recycling traffic of membrane constituents between the Golgi complex and the apical plasma membrane. In contrast, the acinar cell's mechanisms for secreting Na⁺ and Cl⁻ depend largely on the fluxes of these ions through transporters expressed in the apical and basal-lateral membranes. In addition to accelerating the recycling of secretory vesicle membrane constituents, the cholinergic agonist carbachol also triggers a net redistribution of sodium potassium adenosine triphosphatase (Na,K-ATPase) ion pumps between Golgi-associated pools and the basal-lateral plasma membranes (Yiu SC, et al: J Membrane Biol 102:185, 1988). In the present study, acinar preparations from rat lacrimal glands were stimulated with either carbachol, epinephrine, or isoproterenol. All three agonists stimulated release of the secretory protein lactoperoxidase, but only carbachol significantly accelerated Na⁺ unidirectional influx. Subcellular fractionation analyses of resting and stimulated preparations indicated that carbachol caused a significant translocation of Na,K-ATPase activity from a Golgi-associated compartment to the basal-lateral plasma membranes. Neither adrenergic agonist significantly increased the basal-lateral membrane Na,K-ATPase activity, but each triggered a distinct pattern of redistributions of Na,K-ATPase and the Golgi membrane marker, galactosyltransferase. The carbachol-induced augmentation of basal-lateral membrane Na,K-ATPase activity represents a mechanism by which the cell might compensate for increased Na⁺ influx. However, it remains to be determined whether the signal for Na,K-ATPase recruitment is an elevation of cytosolic Na⁺ activity or whether the redistribution is mediated more directly by the intracellular messenger systems activated after cholinergic stimulation.
chemical potential gradient, whereas Na\(^{+}/\)H\(^{+}\) exchange generates the HCO\(_{3}^{-}\) anions that drive Cl\(^{-}\) influx through the Cl\(^{-}/\)HCO\(_{3}^{-}\) antiporters.\(^2\)

Muscarinic cholinergic stimulation triggers a number of events related to the diverse lacrimal secretory functions. As has been reviewed elsewhere,\(^3\) these include activation of apical Cl\(^{-}\) channels,\(^4\)-\(^7\) activation of basal-lateral Na\(^{+}/\)H antiporters,\(^2\),\(^8\),\(^9\) release of macromolecular secretory products, and internalization and recycling of secretory vesicle membrane constituents.\(^13\) Subcellular fractionation analyses of resting and carbachol-stimulated lacrimal gland fragment preparations indicate that cholinergic stimulation also is accompanied by net translocations of Na,K-ATPase pumps\(^14\) and cholinergic receptors\(^15\) from intracellular, presumably Golgi-associated, pools to the basal-lateral plasma membranes.

The carbachol-induced recruitment of Na,K-ATPase pumps to the basal-lateral membrane should contribute to the cell's ability to compensate for accelerated Na\(^{+}\) influxes. The relationship between this phenomenon and the recycling traffic of retrieved secretory vesicle membrane constituents has not been explored. The fact that various secretagogues have differential effects on the lacrimal acinar secretory functions offers one avenue for approaching this question. Epinephrine, presumably acting through both \(\alpha\)-adrenergic\(^16\)-\(^18\) and \(\beta\)-adrenergic\(^18\)-\(^22\) receptors, stimulates the release of lacrimal macromolecular secretory products, and it has been reported to accelerate the flux of Na\(^{+}\) into isolated lacrimal acinar cells.\(^8\) Other reports indicate that isoproterenol, presumably acting through \(\beta\)-adrenergic receptors, stimulates protein release and accelerates the endocytic component of basal-lateral membrane recycling traffic\(^23\) but has little effect on Na\(^{+}\) influx.\(^8\) Thus, in this study we have compared the abilities of carbachol, epinephrine, and isoproterenol to stimulate protein release and Na\(^{+}\) influx with their abilities to stimulate Na,K-ATPase redistributions as measured by newly developed procedures for subcellular fractionation analysis of acinar preparations from rat exorbital lacrimal glands.\(^24\) In our study, the acinar preparations released comparable amounts of lactoperoxidase in response to carbachol, isoproterenol, and epinephrine. Carbachol accelerated Na\(^{+}\) influx 2.7-fold over resting values, but neither isoproterenol nor epinephrine had a significant effect on Na\(^{+}\) influx. Only carbachol caused a net translocation of Na,K-ATPase from Golgi-associated pools to the basal-lateral membranes. Isoproterenol and epinephrine each induced a unique pattern of Na,K-ATPase redistribution, but these redistributions appeared to be related to changes in the properties of Na,K-ATPase-containing Golgi membranes.

### Materials and Methods

**Materials**

Rats were used in accord with the ARVO resolution on the use of animals in research. Male Sprague-Dawley rats (240–260 g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Analyzed reagent grade sorbitol was obtained from J.T. Baker (Philipsburg, NJ). Purified collagenase was acquired from Gibco (Grand Island, NY), hyaluronidase from Worthington (Freehold, NJ), and DNase from Boehringer Mannheim Biochemicals (Indianapolis, IN). \(^2\)Na\(^{+}\) as NaCl was obtained from Amersham (Arlington Heights, IL). All other chemicals were reagent grade and were obtained from standard suppliers. Tris-HEPES buffered saline (THBS) contained 120 mM NaCl, 6.2 mM KCl, 1.6 mM MgCl\(_2\), 1.0 mM CaCl\(_2\), 10 mM glucose, 10 mM Tris, and 10 mM HEPES; pH was adjusted to 7.4 with H\(_3\)PO\(_4\). Isolation buffer contained 5% sorbitol, 5 mM histidine-imidazole (pH 7.5), 0.5 mM Na\(^{2}\)EDTA, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), and 0.9 \(\mu\)g/ml Aprotinin. Sorbitol concentrations of density gradient media are given as weight/volume; other components of density gradient media were as in isolation buffer.

**Isolation of Lacrimal Acini**

Six rats were killed by cervical dislocation for each experiment. Acini were isolated with the enzymatic digestion method of Oliver\(^25\) as modified by Yiu et al.\(^28\)

**Secretagogue-Induced Peroxidase Release**

After isolation, acini were resuspended in a modified Kreb's phosphate buffer (KPB) in which the NaCl and CaCl\(_2\) concentrations were reduced to 110 mM and 0.5 mM. KPB also contained 5 mM \(\beta\)-hydroxybutyric acid, 0.2 mM PMSF, and 0.9 \(\mu\)g/ml Aprotinin. The suspension was divided into 1-ml aliquots, which were allowed to equilibrate for 30 min under an atmosphere of 100% O\(_2\). At the end of the equilibration period, acini were sedimented in a clinical centrifuge and resuspended in KPB or KPB containing either carbachol, isoproterenol, or epinephrine bitartrate at a final concentration of 10 \(\mu\)M; these agonist concentrations are typical of those used in previous studies and are near the values that Parod et al.\(^8\) found to optimally stimulate Na\(^{+}\) influx. After an additional 30-min incubation, acini were sedimented. Supernatant medium was withdrawn and analyzed for peroxidase activity according to the method of Herzog et al.\(^16\) Acini were resuspended, homogenized, and assayed for protein. The relative peroxidase release was
calculated as the ratio of the amounts of activity released in the presence and absence of agonist.

Secretagogue-Accelerated Sodium Influx

After isolation, acini were equilibrated for 30 min in THBS plus 0.5% bovine serum albumin at 37°C under an atmosphere of 100% O2. They were washed twice by centrifugation, resuspended in 14 ml THBS plus 0.5% bovine serum albumin, and incubated in a gently shaking siliconized Erlenmeyer flask at 37°C. For each transport reaction, a 1.0-ml sample was removed, and acini were sedimented in a clinical centrifuge. The supernatant was removed, and the acini were resuspended in 0.4 ml THBS or THBS containing 10 μM carbachol, epinephrine, or isoproterenol.

Saito and co-workers9 have shown that within 2 min of stimulation with acetylcholine, mouse lacrimal acinar cells reach a steady state, maintained for at least 30 min, that is characterized by a hyperpolarized membrane potential, a decreased cytosolic Cl− activity, and an elevated cytosolic Na+ activity. Therefore, 22Na+ was added to the uptake reaction mixture in an additional 100-μl aliquot of THBS 2.0 min after the acini had been resuspended. At 15 and 45 sec after addition of the tracer, 0.2-ml aliquots were removed from the reaction mixtures, and influx was quenched by rapid dilution into 1.0-ml aliquots of ice-cold THBS. Pilot experiments2 confirmed that 22Na+ uptake increased linearly through this interval, thus providing an acceptable measure of the Na+ unidirectional influx.

Acini were collected on 0.45-mm pore size nitrocellulose filters (Schleicher and Schuell, Keene, NH), which were washed with two 4-ml aliquots of ice-cold THBS. Filters were placed in 20-ml glass scintillation vials, and acini were dissolved with 1.0 ml 0.2% sodium dodecyl sulfate. Eight milliliters of Filtron X chamber gradient maker were used to construct gradients in Ultraclear tubes for an SW 28 rotor (Beckman Instruments). The first stage was formed from 11.5 ml 35% sorbitol and 13.2 ml 55% sorbitol, and the acini were dissolved in 10-μl samples of the dissolved acini had been removed for protein determinations, and radioactivity was counted in a Beckman LS 8000 liquid scintillation counter (Beckman Instruments, Fullerton, CA). Relative Na+ influx was calculated as the ratio of the influxes in the presence and absence of agonist.

Subcellular Fractionation Analysis

After isolation, acini were allowed to equilibrate for 60 min at 37°C in Ham’s F-12 medium supplemented with insulin, transferrin, selenium, and bovine serum albumin as described previously.24 At the end of the equilibration period, acini were transferred to 50-ml polypropylene flasks containing KP-50. After an additional 30-min incubation, acini were harvested by centrifugation at 50 g for 4 min and resuspended in 3-ml aliquots of ice-cold isolation buffer.

Acini were disrupted with a Tissumizer (Tekmar Instruments, Cincinnati, OH) equipped with an SDT 080 EN shaft and generator. During the initial homogenization, the Tissumizer was run for 20 min at a Thyristor setting of 45. The homogenate was analyzed by a sequence of differential and isopycnic centrifugation steps based on procedures that originally were designed for large-scale analyses of lacrimal gland fragments11,14,15 and isolated lacrimal acini.24 The initial homogenate was centrifuged at 2,000 g for 10 min. The resulting pellet was resuspended in 2 ml isolation buffer, homogenized for 10 min, and again centrifuged at 2,000 g. The two initial low-speed supernatants, collectively designated S0, were pooled. The final low-speed pellet, designated P0, was resuspended in isolation buffer and set aside for marker determinations. The sorbitol concentration of S0 was increased to approximately 55% by the addition of a concentrated sorbitol stock solution. The sample was mixed thoroughly and allowed to stand at 4°C for 60 min.

Two-stage, hyperbolic density gradients were prepared one day in advance and stored at 4°C. As described elsewhere,26,27 a Buchler Auto-Densi Flow (Fort Lee, NJ), peristaltic pump, and standard two-chamber gradient maker were used to construct gradients in Ultraclear tubes for an SW 28 rotor (Beckman Instruments). The first stage was formed from 11.5 ml 35% sorbitol and 13.2 ml 55% sorbitol, and the second from 8.1 ml 55% sorbitol and 10.9 ml 70% sorbitol; a cushion of 80% sorbitol was inserted beneath the second stage. S0 was introduced between the first and second stages, followed by an additional 1.7 ml of 55% sorbitol. The volume of the tube was completed with an overlay of approximately 3 ml of 5% sorbitol. Gradients were centrifuged for 5 hr at 122,000 g.

After centrifugation, the Auto-Densi Flow was used to collect 12 fractions from each gradient. Each density gradient fraction was diluted to 6 ml with isolation buffer, and membranes were harvested by centrifugation for 75 min at 250,000 g in Type 65 (Beckman Instruments) and T 865.1 (Sorvall, Wilmington, DE) rotors. The resulting series of pellets, collectively designated 2P0, were resuspended in 1.0-ml aliquots of isolation buffer. The series of high-speed supernatants, collectively designated 2S0, were pooled, and samples were set aside for marker determinations.
Analytical Methods

Na,K-ATPase was determined with the K⁺-dependent p-nitrophenylphosphatase (K-p-NPPase) reaction described by Murer et al.²⁸ and modified by Mircheff;²⁷ pilot experiments have confirmed that the density distribution of K-p-NPPase is similar to the density distribution of ouabain-sensitive (Na⁺ + K⁺)-dependent ATPase activity.²⁹ Other marker determinations were performed with the methods used in previous fractionation analyses of lacrimal gland fragments.¹¹,²⁷,²⁹ Marker distribution data are presented as the cumulated percentage yields from each of the sequence of separation steps, so that cumulative enrichment factors may be calculated from the ratios of percentage of recovered marker activity to percentage of recovered protein. Because resting and secretagogue-stimulated acinar samples were processed in parallel from common starting preparations, stimulation-induced changes were tested for significance with Student's t-test for paired analyses.

Results

Secretagogue-Induced Peroxidase Release and Sodium Influx

As illustrated in Figure 1, carbachol, epinephrine, and isoproterenol were similarly effective in stimulating peroxidase release. However, as indicated in Figure 2, carbachol induced a 2.7-fold acceleration of Na⁺ unidirectional influx, while neither epinephrine nor isoproterenol significantly changed the Na⁺ transport rate.

Subcellular Fractionation Analyses of Resting Acinar Preparations

Nine separate acinar preparations were analyzed. Each was divided into a resting and a stimulated sample, and the effects of each secretagogue were tested in three separate preparations. The distributions of markers among the fractions generated by the differential centrifugation steps of the analyses of resting preparations (ie, P₀, SP₀, and SS₀), are summarized in Table 1. These distributions were qualitatively similar to those observed in large-scale fractionation analyses of isolated acini.²⁴ However, apparently because the present analyses employed fewer cycles of homogenization and centrifugation of the low-speed pellet, relatively larger amounts of the marker activities were recovered in P₀, and correspondingly smaller amounts were recovered in SP₀ and SS₀. The overall recoveries of marker activities from S₀ were somewhat smaller than encountered in previous studies.¹¹,²⁴,³⁰ The apparent losses of activity may have been the consequence of underestimates of the activity, associated with slowly sedimenting vesicles remaining in SS₀.¹⁴ These losses did not differ between control and agonist-treated preparations.

The distributions of membrane-associated markers after density gradient analyses of the resting samples are presented in Figure 3. Although the resolution of these analyses was somewhat inferior to that of large-
Table 1. Marker distributions after differential centrifugation steps of analyses of resting acinar preparations

<table>
<thead>
<tr>
<th>Marker</th>
<th>Initial activity</th>
<th>Percent recovered activity</th>
<th>Recovery from $S_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P₀</td>
<td>ΣS₀</td>
<td>ΣP₀</td>
</tr>
<tr>
<td>Na,K-ATPase</td>
<td>65 ± 3</td>
<td>13.4 ± 2.0</td>
<td>11.2 ± 0.8</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>317 ± 16</td>
<td>12.8 ± 2.0</td>
<td>14.2 ± 2.6</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>1748 ± 110</td>
<td>9.2 ± 1.5</td>
<td>33.6 ± 4.7</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>99 ± 7</td>
<td>40.7 ± 4.0</td>
<td>8.2 ± 4.6</td>
</tr>
<tr>
<td>Galactosyltransferase</td>
<td>927 ± 288</td>
<td>4.3 ± 1.2</td>
<td>9.1 ± 2.9</td>
</tr>
<tr>
<td>Protein</td>
<td>15.8 ± 8.4</td>
<td>8.7 ± 0.9</td>
<td>59.4 ± 5.0</td>
</tr>
</tbody>
</table>

Values presented are means ± SEM. Initial protein is in mg, initial galactosyltransferase is in arbitrary units, and initial activities of other markers are in n mole/mg protein hr. Galactosyltransferase was measured in seven preparations. All other markers were determined in nine preparations.

scale analyses performed with a zonal rotor (Yiu et al24), several of the major features noted in the earlier analyses could be discerned. Fractions 2–5 contained a peak in the alkaline phosphatase distribution. The Na,K-ATPase distribution had distinct modes in fractions 5, 7, and 9. The galactosyltransferase distribution, which had a mode in fraction 7, extended from fraction 5 to fraction 12. The mitochondrial marker, succinate dehydrogenase, had a clearly defined mode in fraction 10. Although no marker for endoplasmic reticulum membranes was measured in this study, the results of large-scale fractionation analyses of lacrimal gland fragment preparations indicate that a major population of endoplasmic reticulum-derived membranes must have accounted for a substantial component of the peak of protein that spanned fractions 5 through 12.11,29,31

Secretagogue-Induced Changes in Marker Distributions

The secretagogues had no consistent effects on the manner in which membrane markers were distributed among the fractions defined by differential centrifugation, but each caused a unique pattern of changes in the density distributions of Na,K-ATPase and galactosyltransferase. The distributions of markers between P₀, ΣP₀, and ΣS₀ were similar for resting and carbachol-stimulated preparations. Epinephrine decreased the yield of alkaline phosphatase in P₀ to 6.3% ± 1.5% (P < 0.01), but the compensating increases in the other fractions were too small to be statistically significant. Isoproterenol decreased the yield of protein in P₀ to 1.6% ± 0.4% (P < 0.001) but did not significantly alter the protein yield in any other differential centrifugation fraction. Because P₀ contained mixtures of intact nuclei, undisrupted secretory vesicles, large membrane fragments, and aggregates of membrane fragments and extracellular matrix material, the basis of the epinephrine- and isoproterenol-induced changes in P₀ is not clear.

As summarized in Figure 4, carbachol consistently increased the yield of Na,K-ATPase in density gradient fractions 3 and 4 by 3.4% ± 0.4% (P < 0.01) of the total recovered activity. The addition of Na,K-ATPase to fractions 3 and 4 amounted to a 1.3-fold increase over the resting value. This increase appeared to be accompanied by a decrease in the Na,K-ATPase yield of fraction 10 of 3.8% ± 1.5% (P
< 0.08) of the total recovered activity. A possible effect of carbachol on the galactosyltransferase distribution was tested in one acinar preparation. In that experiment, the position of the peak appeared to shift toward lower densities, with the yields in fractions 5 and 6 increasing and the yields in fractions 7–10 decreasing; carbachol did not change the yields of galactosyltransferase in fractions 3 and 4. Apart from a small decrease in the yield of alkaline phosphatase in fraction 12, the only other statistically significant changes induced by carbachol were increased protein yields in fractions 2 and 3.

Like carbachol, epinephrine increased the yield of protein in fraction 2; it also increased the protein yield in fraction 5. In contrast to carbachol’s action in significantly increasing the Na,K-ATPase yields in fractions 3 and 4, epinephrine increased the Na,K-ATPase yields in fractions 5 and 6. Epinephrine also increased the yields of acid phosphatase in fractions 2 and 6.

Isoproterenol failed to increase the yield of Na,K-ATPase from fraction 10 to fractions 3 and 4 (Figure 4) is similar to the redistribution noted in lacrimal gland fragment preparations,14 and the redistributions in both preparations appear to reflect translocations of Na,K-ATPase pump units from a domain of the Golgi complex to the basal-lateral plasma membrane. In acinar cell preparations, internalization and release of the fluid phase marker Lucifer yellow also is accelerated by cholinergic stimulation,32 so the net translocation of Na,K-ATPase must be occurring in the context of an overall acceleration of plasma membrane recycling.

Both epinephrine and isoproterenol stimulated secretory protein release to nearly the same extent as carbachol (Fig. 1), but neither adrenergic agonist caused a significant acceleration of Na+ unidirectional influx (Fig. 2), and neither caused a significant recruitment of Na,K-ATPase to the basal-lateral membranes. The effects of the adrenergic agonists differ somewhat from those reported previously by other laboratories. The ability of isoproterenol to elicit protein and peroxidase release has varied considerably between different laboratories, with some groups finding significant effects20-22 and others de-
Carbachol  Epinephrine  Isoproterenol

Fig. 4. Secretagogue-induced changes in marker density distributions. Values presented are mean differences ± SEM from paired controls. Carbachol effects on galactosyltransferase distribution were determined in one acinar preparation. Effects of each secretagogue on other marker distributions were determined in three separate acinar preparations.
tecting responses only in the presence of phosphodiesterase inhibitors.\textsuperscript{33} The inability of isoproterenol to significantly accelerate Na\textsuperscript{+} influx in our acinar preparation is in accord with the results of Parod et al.\textsuperscript{8} However, in contrast to our results, Parod and co-workers found that the effect of epinephrine was intermediate between the effects of isoproterenol and carbachol.\textsuperscript{21} It is difficult to account for the discrepancies between results from different laboratories, and a variety of factors may be involved; one factor that may influence the ability of isoproterenol to elicit secretion is the extent to which phosphodiesterase activity is retained during tissue preparation.

Although the adrenergic agonists failed to cause net translocation of Na,K-ATPase from intracellular pools to the basal-lateral membranes, they triggered significant changes in the Na,K-ATPase density distribution pattern. These changes appeared to reflect alterations occurring within the Golgi complex. Epinephrine shifted Na,K-ATPase and galactosyltransferase toward lower densities without dissociating one activity from the other. This shift could be interpreted simply as the result of decreases in the modal densities of Golgi membranes that contained Na,K-ATPase and galactosyltransferase. Isoproterenol triggered discrete increases of the galactosyltransferase yields in fractions 8 and 10 and discrete decreases of the Na,K-ATPase yields in fractions 7 and 9. These changes suggest that \(\beta\)-adrenergic stimulation might change the biochemical organization of several domains within the Golgi complex.

Because epinephrine and isoproterenol both stimulated secretory protein release from our acinar preparation (Fig. 1), it seems reasonable to suggest that the physical and biochemical changes these agonists induced in domains of the Golgi complex were in some way related to the process of protein secretion. Most secretory vesicles are fragmented to a population of microsomal membranes upon cell disruption,\textsuperscript{11} and this population's location in the density gradient analysis depicted in Figure 3 is not known. Indirect fluorescence localization of Na,K-ATPase immunoreactivity indicates that little, if any, of the Na,K-ATPase is associated with the secretory vesicles,\textsuperscript{12} so it is unlikely that the changes depicted in Figure 4 can have been direct results of the fusion of secretory vesicles with the apical plasma membranes. However, secretory vesicles are assembled in, and internalized secretory vesicle membrane constituents are recycled through, the Golgi complex.\textsuperscript{13} Therefore, the changes induced by epinephrine and isoproterenol probably reflect the patterns of membrane recycling traffic that these agonists trigger. It is striking that the changes instigated by an epinephrine, a mixed \(\alpha\)- and \(\beta\)-adrenergic agonist,\textsuperscript{34-36} should have differed so markedly from the changes instigated by isoproterenol, a \(\beta\)-agonist. One possible explanation for this difference may be that the \(\alpha\)-receptor responses predominate over the \(\beta\)-receptor responses.

As noted, the net recruitment of Na,K-ATPase to the basal-lateral membranes observed in response to carbachol should represent a significant contribution to the cell's ability to compensate for the acceleration of Na\textsuperscript{+} influx that is also induced by carbachol (Fig. 2). Such a recruitment of Na,K-ATPase pumps would not be required for cellular Na\textsuperscript{+} homeostasis in the cell's responses to the adrenergic agonists, which do not accelerate Na\textsuperscript{+} influx. The question arises as to the mechanism(s) by which the lacrimal acinar cell coordinates the recruitment of Na,K-ATPase pumps with increases in Na\textsuperscript{+} influx. The augmentation of basal-lateral membrane Na,K-ATPase \(J_{\text{max}}\) produced by the insertion of additional pumps is not strictly commensurate with the increase in Na\textsuperscript{+} influx because cytosolic Na\textsuperscript{+} activities remain elevated during sustained cholinergic stimulation.\textsuperscript{9} Thus, the elevation of cytosolic Na\textsuperscript{+} could represent a signal for mobilization of Na,K-ATPase from Golgi-associated pools. However, the spectrum of intracellular messengers released after cholinergic stimulation is distinct from that released by either \(\beta\)- or mixed \(\alpha\)- and \(\beta\)-adrenergic stimulation,\textsuperscript{34-36} so the redistribution of Na,K-ATPase could be a direct response to the unique set of messengers, one that also has the effect of activating Na/H antiporters in the basal-lateral membranes.

**Key words:** lacrimal gland, tear film, fluid secretion, plasma membranes, Golgi complex, stimulus-secretion coupling, carbachol, epinephrine, isoproterenol

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