Na\(^+\)/H\(^+\) Antiporter in Lacrimal Acinar Cell Basal-Lateral Membranes

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The first step in the formation of lacrimal gland fluid is believed to depend on transport systems which couple a flux of Cl\(^-\) ions to the passive influx of Na\(^+\) ions across the acinar cell basal-lateral plasma membrane. The transport systems which mediate these fluxes have not yet been characterized, but a review of previous studies (Parod and Putney, Am J Physiol 239:G106, 1980) raises the possibility that Na\(^+\)/H\(^+\) antiporters might represent a major pathway for Na\(^+\) influx. This conclusion is of interest, because antiporter mediated Na\(^+\) fluxes can, potentially, drive net Cl\(^-\) fluxes. We have now examined a sample of basal-lateral membrane vesicles from rat exorbital lacrimal gland to verify the presence of a Na\(^+\)/H\(^+\) antiporter activity. Imposition of an outward H\(^+\) gradient caused a 4.4-fold increase in the \(^{22}\)Na influx rate, while imposition of an outward Na\(^+\) gradient accelerated H\(^+\) uptake as determined by changes in acridine orange absorbance. All transport experiments were done in the presence of valinomycin and symmetrical K\(^+\) concentrations, eliminating the possibility of conductive Na\(^+\) or H\(^+\) fluxes driven by diffusion potentials. The pH gradient dependent Na\(^+\) influx was completely inhibited by 1 mM amiloride, indicating that it was mediated by a Na\(^+\)/H\(^+\) antiporter similar to those described in other tissues. Comparison of the density distributions of Na\(^+\)/H\(^+\) antiporter and standard membrane marker enzyme activities confirmed that the antiporter was primarily localized to the basal-lateral membranes. Invest Ophthalmol Vis Sci 28:1726-1729, 1987

The first step in formation of lacrimal gland fluid occurs in the acini, which secrete a primary fluid characterized by plasma-like concentrations of Na\(^+\) and Cl\(^-\). This process is thought to depend on coupled fluxes of Na\(^+\) and Cl\(^-\) through the secretory cell basolateral plasma membranes, but the precise ionic influx mechanisms have not yet been delineated. Since lacrimal gland fluid formation was partially inhibited by the loop diuretic, furosemide, it appeared likely that the coupled influxes were mediated, at least in part, by NaCl or NaKCl\(_2\) symporters. On the other hand, carbachol-dependent acceleration of \(^{22}\)Na influx and elevation of acinar cytoplasmic Na\(^+\) activity\(^*\) were inhibited by amiloride. These results suggested that Na\(^+\)/H\(^+\) antiporters might represent an important pathway for Na\(^+\) influx. Since it is impossible to discern the localization of transport systems from experiments with isolated cells, we have used an analytical subcellular fractionation approach to verify that Na\(^+\)/H\(^+\) antiporters are present in the lacrimal gland and to establish that they are concentrated in the basal-lateral plasma membranes.

Materials and Methods. Analyzed reagent grade d-sorbitol was from J. T. Baker (Phillipsburg, NJ), \(^{22}\)Na from Amersham (Arlington Heights, IL), and \(^3\)H-mannitol from ICN (Costa Mesa, CA). All other chemicals were reagent grade and were obtained from standard suppliers. Male Sprague-Dawley rats, 240-260 gm, were used in conformity with the ARVO Resolution on the Use of Animals in Research.

For each experiment, 16 exorbital lacrimal glands were cut into 5 mg fragments and placed in a modified Krebs improved Ringer I bicarbonate buffer (KRB) saturated with 95% O\(_2\)-5% CO\(_2\). After a 55 min incubation at 37°C, the fragments were transferred to isolation buffer and subjected to homogenization, differential sedimentation, and equilibrium density gradient centrifugation. KRB, isolation buffer, and density gradient media all contained 0.2

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mM phenylmethylsulfonylfluoride and 9 μg/ml aprotinin as protease inhibitors. The 24 density gradient fractions were assayed for biochemical markers and pooled into density windows. Membranes were harvested by dilution and centrifugation at 250,000 g × 75 min.

For measurements of tracer uptake by standard ultrafiltration techniques, pellets were resuspended in pH 6.0 loading medium, which contained 200 mM sorbitol, 50 mM K-glucanote, and 1 mM Mg-glucanote (pH adjusted to 6.0 with HEPES). Membranes were dispersed by passage through 25 gauge needles, quickly frozen in liquid nitrogen, and stored at −70°C. After rapid thawing, the samples were again dispersed by passage through 25 gauge needles. Five μl aliquots of the membrane suspension were placed on the walls of polypropylene tubes, then rapidly mixed with 20 μl aliquots of reaction media to initiate uptake reactions. Reaction and resuspension medium contained 5 μg/ml valinomycin, and reactions shorter than 15 sec were timed with a metronome. They were terminated by addition of 1 ml aliquots of ice-cold quench solutions which were identical to the reaction media except for deletion of radiotracers and valinomycin. One ml aliquots of the quenched reactions were filtered, rinsed twice with 4 ml aliquots of quench solution, and analyzed in a liquid scintillation counter.

A spectrophotometric method based on the pH-sensitive dye, acridine orange, which is trapped in acidic compartments, was used to measure H+ influx. Vesicles at a protein concentration of 8 mg ml⁻¹ were loaded with a medium containing 150 mM Na-glucanote, 200 mM sorbitol, 50 mM K-glucanote, and 1 mM Mg-glucanote (pH adjusted to 7.0 with HEPES) as described above. Reactions were started by addition of 20 μl aliquots of the vesicle suspension to 1 ml aliquots of reaction media containing 6 μM acridine orange. Loading and reaction media contained 5 μg/ml valinomycin. The differences between acridine orange absorbances at 436 nm and 496 nm were recorded in an Aminco (Urbana, IL) DW-2 spectrophotometer.

Results. Previous studies with freshly obtained lacrimal gland parenchyma indicated that density window II contained the major population of acinar cell basal-lateral membrane vesicles. Therefore, transport activities of membranes from this region of the gradient were characterized in initial experiments.

Figure 1 presents time courses of uptake of ²²Na and ³H-mannitol in the presence (pH₇.₅) and absence (pH₆.₀) of an outwardly directed H⁺ gradient. Vesicles were loaded with pH 6.0 medium as described under Methods. The pH 7.5 medium contained 1 mM Na-glucanote, 200 mM sorbitol, 50 mM K-glucanote, 1 mM Mg-glucanote, and 10 mM Tris. The pH 6.0 medium contained 1 mM Na-glucanote, 200 mM sorbitol, 50 mM K-glucanote, and 1 mM Mg-glucanote. The pH was adjusted with HEPES. Apparent spaces were calculated from the ratio of cpm taken up to cpm ml⁻¹ in the reaction medium. Each point is the mean of three replicates. A similar time-course was observed in a separate window II preparation, and similar rates of ²²Na and ³H-mannitol uptake between 2 and 5 sec were obtained in a total of six separate preparations. Comparison between the time courses in the presence and absence of an H⁺ gradient indicates that the gradient caused a greater than 4-fold increase in the initial rate of ²²Na uptake. That the H⁺ gradient-driven influx of Na⁺ fails to overshoot probably reflects the presence of parallel permeation pathways for both ions.
Fig. 2. Spectrophotometric demonstration of Na⁺ gradient driven uptake of H⁺. Vesicles were loaded with 150 mM Na-glucocinate medium at pH 7.0. They were added to pH = 7.0 media containing 6 nM acridine orange, 200 mM sorbitol, 50 mM K-gluconate, 1 mM Mg-gluconate, and either 150 mM glucamine-gluconate (upper trace) to give an outwardly directed Na⁺ gradient or 150 mM Na-gluconate (lower trace) to give no Na⁺ gradient. (The large negative deflection indicates the time the spectrophotometer sample compartment was opened for vesicle addition.) Similar results were obtained in three replications.

Fig. 3. Effect of amiloride on uptake of 22Na⁺ in the presence (•) or absence (○) of an outwardly directed H⁺ gradient. Each point is the mean of three replicates. Bars, which indicate standard deviations, were not drawn when this range was smaller than the symbol.
The basal-lateral membrane localization contrasts with the well known apical membrane localization of antiporters in proximal tubule and small intestine. This localization is, however, consistent with a possible role in the net secretion of Na⁺ and Cl⁻. That is, Na⁺/H⁺ exchange, driven by an inwardly directed Na⁺ gradient, would tend to alkalize the acinar cytoplasm. Such a pH gradient could drive Cl⁻/OH⁻ or Cl⁻/HCO₃⁻ antiporters. Thus, a reasonable working hypothesis for lacrimal acinar fluid secretion would incorporate an array of Na⁺/H⁺ and Cl⁻/OH⁻ or Cl⁻/HCO₃⁻ antiporters in parallel with NaKCl₂ symporters in the basal-lateral membranes.

Key words: tear film, fluid and electrolyte secretion, plasma membranes, exocrine glands

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