PREPARATION OF APICAL MEMBRANE VESICLES OF LENS EPITHELIUM FROM DOGFISH (SQUALUS ACANTHIAS) EYES

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The lens is composed of at least three different populations of cells: the cells of the epithelium, the mature lens fibers and, at the equator of the lens, a "cap" of differentiating cells. On the anterior surface only, a layer of epithelial cells are responsible for the majority of active transport of ions, amino acids and lipids precursors as well as the facilitated diffusion of glucose (Rae, J.L. and Mathias, R.T.: in "The Ocular Lens, Structure, Function and Physiology", H. Maisel, editor, New York, Marcel Dekker, 1985, pp 93-121). These processes are also located in the fiber membranes. Vesicles from dogfish lens fiber membranes have been prepared (S. Pearce et al. Bull MDIBL, 1987-88, 27, 77-79) and have been further described and examined in two other papers in this same volume (Jingjing Ye and J.A. Zadunaisky, Bull MDIBL, 1990, 29).

The primary aim of the experiment reported here was to determine whether apical membrane vesicles could be produced from the epithelial cells of dogfish (Squalus acanthias) lens. The method utilized was a variation of the one described for isolation of apical vesicles of retina pigment epithelium from dogfish (J.A. Zadunaisky et al, 1989, Invest. Ophthal. & Vis. Sci., 30, 2332-2340). The purpose was to test these vesicles for ions transport properties.

Spiny dogfish (Squalus acanthias) eyes were used within an hour after sacrificing the fish. 20 enucleated eyes were dissected and the lens epithelial cell layer and anterior capsule was carefully separated from the whole lens. For a typical membrane isolation about 5.0 g tissue was employed. The tissue was minced with scissors and homogenized in 150 ml buffer in a Waring blender for 60 sec. (30 sec each time with 1 min interval) at full speed in a buffer containing 10 mM mannitol, 2 mM Tris Cl, pH 7.1 adjusted with NaOH. The apical membranes were then enriched by differential precipitation. A 1 M $MgSO_4$ solution was slowly added to the homogenate to achieve a final concentration of 20 mM Mg. The suspension was stored on ice for 15 min with occasional stirring and then centrifuged at 2,100 g for 20 min at 4°C. The pellet, which was rich in basolateral membrane by the Mg^{2+} precipitation, was discarded. The supernatant was centrifuged twice at 21,000 g for 45 min at 4°C and the final pellet containing apical membrane vesicles was resuspended in a vesicles buffer containing 200 mM mannitol, 20 mM HEPES and 1 mM $Mg(NO_3)_2$, pH 7.4 with Tris, homogenized by repeated suction through a 25-gauge needle and stored at -70°C for future use.

Electron micrographs of the vesicles showed very clear circular sealed vesicles with average size of 2000-3000 A. The enzymatic enrichments of its apical markers, Na,K-ATPase (Unakar, N.J. and Tsui, J.Y. 1980, Invest. Ophthlamol. Vis. Sci., 19:630) and acid phosphatase (Gorthy, W.C. 1978, Exp. Eye Res. 27:301) were

25.9 ± 2.1 (4 experiments ± SD) for Na,K-ATPase, and 10.4 ± 1.2 (3 experiments ± SD) for acid phosphatase. The asymmetric properties of the plasma membrane Na^+,K^+-ATPase (Caroni, P. and Carifoli, F. 1983, Eur. J. Biochem. 132, 451-460) were used to estimate the relative proportion of unsealed:sealed vesicles, and the orientation of the sealed vesicles. In the absence or presence of 10 μM valinomycin, a membrane permeabilizing agent, which prevents the depletion of K^+ from inside-out vesicles, the measured Na^+,K^+-ATPase activity reflected the proportions of unsealed and inside-out plus unsealed vesicles respectively. In order to estimate the proportion of right-side-out vesicles, the total Na^+,K^+-ATPase activity, which represents all population of vesicles (right-side out, inside-out and unsealed), was measured in the system with detergent Triton X-100. Orientation studies of the vesicles showed that there are 50.3%±4.5 of right-side out vesicles, 27.2%±2.4 of inside-out and 22.5%±2.8 unsealed vesicles (4 experiment ± SD).

Na influx into the vesicles was studied with SBFI, a fluorescent probe, described in the authors' two other papers in this Bulletin (1990) with an outwardly-directed H^+ gradient. In the presence of an outwardly-directed pH gradient (pH_i=6.1, pH_o=8.1) the amiloride-sensitive Na influx was stimulated by 143.5%±6.3 (4 experiment ± SD) in comparison of the Na uptake in the absence of pH gradient (pH_i=pH_o=8.1). The pH dependent Na influx rate was higher than that in the lens fiber plasma membrane vesicles (122.6%±5.4, Ye and Zadunaisky, this volume). The stimulation was also observed in the vesicles treated with 20 μM valinomycin and 50 mM K gluconate, but was completely blocked by 50 μM amiloride. The results showed that there exists a Na/H antiport. (for detailed method, see two other papers in this same volume). A Ca/Na exchange mechanism was also found in the apical vesicles by using a Ca indicator, Fura-2. We found that Ca influx was stimulated about 270% when a outwardly-directed Na gradient (100 mM Na inside, 0 mM Na outside) was present in the system. The exchange could be blocked by a newly developed inhibitor, bepridil (10 μM) (M.L. Garcia et al. 1988, Biochem. 27, 2410-2415). Both 50 μM epidermal growth factor and 200 μM H_2O_2 had stimulatory effect on the Na/H exchanger (242%±5.6 and 189%±3.9 respectively, 4 experiment ±SD) and Ca/Na exchanger (118%±2.1 and 133%±3.4 respectively, 3 experiment ± SD).

The studies reported here indicated that 1) apical membrane vesicles could be prepared from dogfish lens epithelium; 2) a Na/H exchange mechanism was presented in the system; 3) a Ca/Na exchange mechanism was also found on the membrane.

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