THE EXISTENCE OF A Ca/Na EXCHANGER AND A Na,K,2Cl COTRANSPORTOR IN APICAL MEMBRANE VESICLES OF THE RETINA PIGMENT EPITHELIUM OF DOGFISH (SQUALUS ACANTHIAS) EYE BY USING FLUORESCENT PROBES SBFI, FURA-2 and SPQ

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A Na/H exchange mechanism in the apical membrane vesicles of retinal pigment epithelium (RPE) from dogfish eye has been previously reported (J.A. Zadunaisky et al. 1989, Invest. Ophth. Vis. Sci. 30, 2332-2340). The purpose of the experiments reported here was to detect the existence of other ion transport mechanisms in the vesicles system by using fluorescent probes SBFI, Fura-2 and SPQ.

The newly synthesized fluorescent dye SBFI, which is a specific Na^+ indicator and Fura-2 for Ca^{2+}, along with the Cl^- dye SPQ (all from Mol. Probe, Inc.) were used to measure their ratios of excitation wavelengths at 340 to 380 nm and emission at 505 nm in a dual-wavelength spectrofluorimeter SPEX AR-CM (SPEX Inc., Edison, N.J.).

The RPE apical vesicles were loaded with either 4 μM SBFI or 1 μM Fura-2 (cell permeable form) for 30 min to 1 hour at room temperature. After washing out the unloaded dyes twice with buffer, the pellet was incubated for one hour at room temperature with either sodium-free solution with 100 μM Ca^{2+} (for SBFI loaded vesicles) or calcium-free solution with 100 mM Na^+ in order to make outwardly-directed Ca^{2+} and Na^+ respectively. The vesicles were diluted 100 times in the measuring cuvette containing different buffer conditions. For the calibration of Na^+, 50 μM gramicidin was used to equilibrate in and outside sodium concentrations. For Ca^{2+} calibration, 0.5% of Triton X-100 was used.

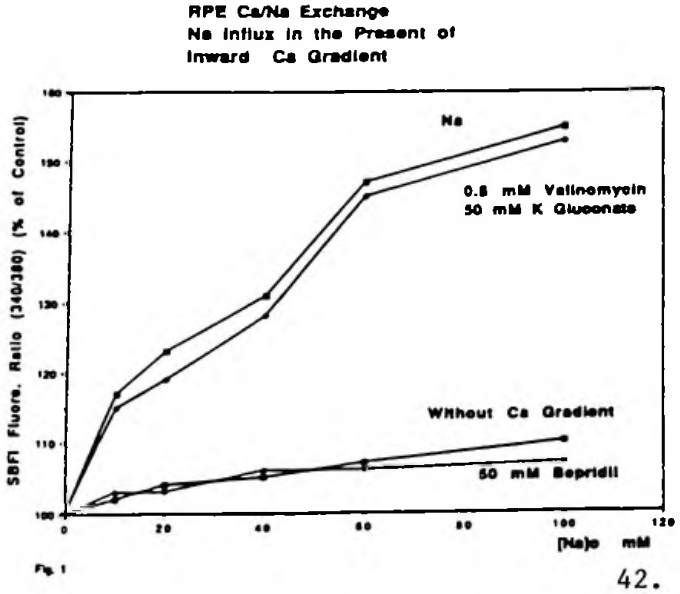

Fig. 1

The results in Figure 1 show that in the presence of a Ca^{2+} outward gradient, the Na^+ influx was significantly stimulated in comparison with that without gradient.When the situation was reversed, i.e. a Na^+ outward gradient was formed, the result remained the same, the influx of Ca^{2+} into the vesicles was also stimulated by the presence of the Na^+ gradient. This means that the Ca/Na exchanger is bi-directional. The stimulations were also observed in membrane vesicles treated with valinomycin or high K^+. The exchange

could be inhibited by bepridil (50 μM), La^{3+} (200 μM) and high concentration of amiloride (2 mM). Epidermal growth factor (RPE) and oxidizing agents such as H_2O_2, diamide as well as extravesicular sucrose (osmolality) had no effect on the exchange.

To investigate Na,K 2Cl cotransport mechanism, vesicles were first incubated with either 4 μM SBFI or 10 mM SPQ (Cl indicator) at room temperature for one hour (For SPQ, 4 hours). The unloaded dyes were washed out and vesicles were incubated again with a Na, K and Cl free buffer for another hour. By measuring the entrance of Cl monitored by intravesicular SPQ in the presence of an inwardly-directed Na gradient and the influx Na by the SBFI fluorescent ratio, the stoichiometry of Na to Cl entering the vesicles was 1:2. As shown in Figure 2, the furosemide-sensitive Cl influx was stimulated in the presence of an inwardly-directed Na gradient (140 mM). If the system was formed by both a Na (70 mM) and a K (70 mM) inwardly-directed gradient, the amount of Cl influx at the extravesicular chloride concentration of 100 mM was enhanced. Valinomycin had no effect to the Na,K 2Cl cotransport mechanism. The inward Na and K gradient dependent Cl influx could be completely inhibited by 50 μM furosemide. EGF had no effect on the cotransport mechanism, but H_2O_2 showed a small inhibitory effect on the chloride uptake into the vesicles.

The evidence provided above leads to the following conclusions. First, there exists a Ca/Na exchange mechanism which could be a secondary regulatory pathway for intracellular Ca^{2+} and Na^{+}. It has been reported (J.A. Zadunaisky et al. 1989, Invest. Ophth. Vis. Sci. 30, 2332-2340) that a Na/H exchange mechanism is presented in the RPE apical membrane. The presence of the Na/H exchange process might have important implications for the control of pH in the subretinal space. Together with the Ca/Na exchange mechanism, the RPE could more precisely regulate its intracellular pH and ion content along with the function of the Na, K-ATPase and the Ca^{2+}-ATPase. The second point is that the presence of Na, K 2Cl cotransport mechanism could play an important role in RPE cell volume regulation.

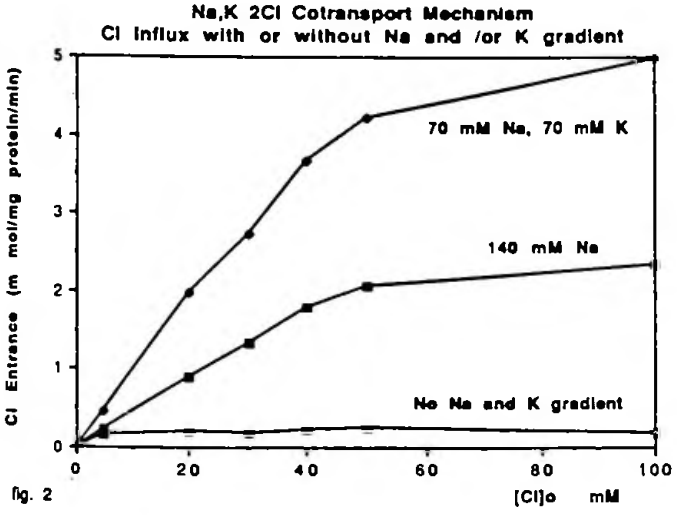

fig. 2

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