Na/H EXCHANGE AND STIMULATORY EFFECT OF H_2O_2 ON LENS FIBER PLASMA MEMBRANE VESICLES OF THE SHARK (SQUALUS ACANTHIAS)

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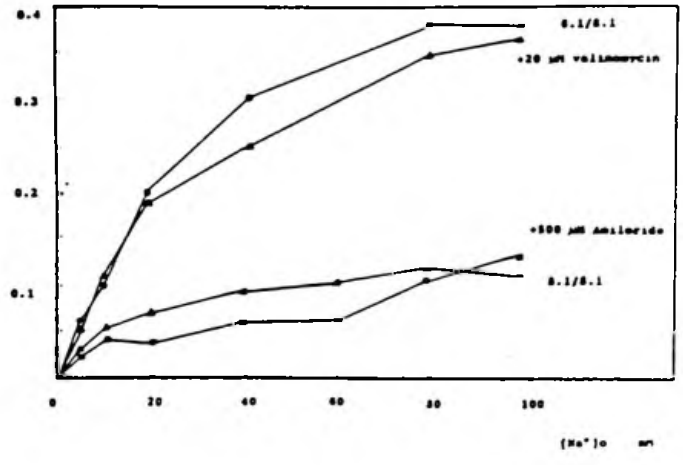
Lens fiber plasma membrane vesicles from Spiny Dogfish (Squalus acanthias) were prepared according to the method described before by this laboratory (Pearce, S. et al. 1987-88 MDIBL Bulletin, 27, 77-79) with further measurement of its quality by electron microscopic examination and enzymatic markers. The EM pictures showed that it contained at least 50% of single-membrane sealed vesicles with average size of 2000 A°. The enrichments of membrane enzymatic markers, such as Na,K-ATPase, Acid Phosphatase and Adenyl Cyclase were 7.7±0.3, 12.3±0.5 and 3.2±0.1 (4 experiment ± SD) respectively. The vesicles orientation experiment showed that 52%±3 of vesicles were in the so called right-side-out state, 30%±2 were in unsealed state and 18%±3 were in inside-out state (4 experiment ± SD).

The sodium uptake study was carried on in a dual-wavelength spectro-fluorometer (SPEX, Edison, N.J.) by using the newly synthesized fluorescence dyes SBFI, which is a specific intracellular Na^+ probe, and SNARF-1, a pH indicator (Molecular Probe Inc. Eugene, OR).

Vesicles were incubated at room temperature with 4 μM SBFI or with 4 μM SNARF-1 (both in cell permeable forms) for 60 min. Then, the unloaded dyes were washed out by twice suspending the spun vesicles in vesicle buffer (0.2M mannitol, 1 mM $Mg(NO_3)_2$ and 20 mM HEPES, pH 7.1). After final spinning down the washed vesicles, the pellet was resuspended in a pH 6.1 buffer containing 100 mM mannitol, 1 mM $CaSO_4$ and 50 mM MES. For the condition of no pH gradient, a pH 8.1 buffer of 100 mM mannitol, 1 mM $CaSO_4$ and 50 mM HEPES was used (J.A. Zadunaisky, et al. 1989, Invest. Ophthal. Vis. Sci. 30, 2332-2340). The whole system was incubated again at room temperature for another 60 min. before the fluorescence assay. The vesicles were diluted 100 times in the cuvette in a pH 8.1 buffer. The ratios of 340nm to 380nm (for Snarf-1, 380 to 340 nm) were collected at an emission wavelength of 505 nm. The calibration curve for SBFI was obtained by adding 50 μM gramicidin into the measuring system in order to equilibrate inside and outside sodium concentrations. For SNARF-1 calibration, 1 mM nigericin was used to keep intra and extravesicular pH the same.



The amiloride-sensitive sodium uptake into the vesicles was significantly stimulated to 70% above controls when a outwardly-directed pH gradient (pH_i=6.1 and pH_o=8.1) was formed across the vesicle membrane,

utilizing the no pH gradient Na^+ uptake condition as control. The addition of valinomycin and high K^+ concentration had no effect on pH dependent sodium uptake. The pH gradient-dependent influx of sodium could be completely blocked by a specific Na/H exchange inhibitor, amiloride (5×10^{-4} M). The intracellular pH in the first minute after adding 100 mM Na^+ into the extravesicular compartment was increased by 0.3+0.02 pH units (3 experiment + SD) as shown in Fig. 1. It means that the pH_i is shifting to the alkaline state. Epidermal growth factor at 50 μM stimulates the Na/H exchange by two fold.

The system was further examined as a model of cataract production by oxidizing agents. After incubating the vesicles with 200 uM H_2O_2 for 10 min. the pH gradient dependent sodium influx was stimulated to 175% of control. Glutathione in its reduced form but not oxidized form could prevent such stimulation. Other oxidizing agent such as diamide also acts in the same way as H_2O_2 in preliminary experiments.

The results of this study show that in the lens fiber membrane of the dogfish there exists a Na/H exchange mechanism which might play an important role in intracellular pH and Na content regulations in the lens. Also, the Na/H exchange might be important in the process of cataract formation. The stimulating effect of oxidizing agents might induce the accumulation of Na inside the cell, the alkalization of the intracellular compartment, and cataract formation. The increase of intracellular sodium can also in turn lead to increased intracellular concentrations of calcium by the Ca/Na exchange mechanism located in its plasma membrane (preliminary data not shown). The increase of intracellular calcium is believed to be a significant feature of cataracts.

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