Kinetics of Na/Li Exchange in High-K (HK) and Low-K (LK) Sheep Red Cells (SRC)

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MEMBRANE ASSOCIATED AND AGE RELATED CHANGES IN HUMAN RED BLOOD CELL MEMBRANES MEASURED BY QUASI-ELASTIC LIGHT SCATTERING. Roy B. Tishler, The Thomas C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218

This report identifies further results of quasi-elastic light scattering (QELS) studies of the membrane/cytoskeleton (m/c) of human red blood cells (RBCs). The effects of specific alterations of the m/c on the QELS signal and changes associated with cellular age have been identified. Measurements of the intensity autocorrelation function, $g(2)$, were made using a QELS microscope spectrometer. The average correlation time, $\tau_{\text{corr}}$, of $g(2)$, determined by fitting the data with a single exponential, was used for comparing data from different cell populations. The QELS signal from RBCs has been previously shown to arise from spontaneous motion of the m/c (Tishler & Carlson, Biophys. J., 1986, 49:145A) Consequently, modifications of the RBC which alter the physical properties of the membrane or cytoskeleton would be expected to change the QELS signal. Increasing the cholesterol/phospholipid ratio of the RBC membrane led to an increase in $\tau_{\text{corr}}$. Cross-linking the cytoskeletal proteins by increasing the intracellular Ca++ concentration also led to an increase in $\tau_{\text{corr}}$. The effect of increasing NaCl concentration was previously interpreted in terms of osmotic changes. Further studies using salts which have cations that are permeable or non-permeable to the RBC membrane clearly identified an osmotic basis for the NaCl changes. Density centrifugation of RBCs was used to separate cells on the basis of age. As RBCs age, they become more dense and their membranes become less flexible. This change in the membrane would be expected to alter the QELS signal. The mean value of $\tau_{\text{corr}}$ for older, denser RBCs was greater than that for the younger, less dense cells. Supported by USPH/NIH grant AM12803 to F. D. Carlson (Dept. of Biophysics, JHU) and 5T32GM07309 (R.B.T.)

KINETICS OF NA/LI EXCHANGE IN HIGH K (HK) AND LOW K (LK) SHEEP RED CELLS (SRC). K.H.Ryu, N.C. Adragna*, D. Bradley, and P.K. Lauf. Depts. Physiology & Biophysics, and*Pharmacology and Toxicology, Wright State University School of Medicine, Dayton, Ohio, 45401-0927.

More than 90% of the ouabain-resistant Na flux in HK and LK SRC is mediated by the Na/Na homo-exchange (Tofteson & Hoffman, J. Gen.Physiol. 44:169,1960) the physiologic contribution of which to the steady state ion composition of both cell types is unclear. Although there are a few subsequent studies, no detailed analysis of the kinetic parameters of Na/Na exchange in sheep is available. In defining these properties in HK and LK SRC we adjusted cellular Na and Li levels by the nystatin method using choline as replacement ion. In HK SRC the maximum Li influx exchange rate ($V_m$) was 2.5 ±0.5 mmoles/L.cell water x hr (+ SE,n=5) with an apparent $K_m^L$ of 0.63 ± 0.04 mM external Li concentration, ($L_i$). In HK SRC the $V_m$ and $K_m^L$ values were 1.5 ± 0.1 (n=4) and 0.70±0.06, respectively. Corresponding values for Li efflux were: $V_m = 1.5 ± 0.3$ (n=5) and $K_m^L = 0.27 ± 0.05$ in HK SRC, and 0.97 and 0.26 in HK (n=2). $V_m$ and $K_m^L$ values for Na influx in HK SRC were 2.4 ± 0.5 and 14.0 ± 3.6 (n=3), respectively. When Na influx and Li efflux were measured simultaneously, saturation curves of Li efflux versus varying extracellular Na concentrations, ($Na_o$), were obtained with a trans-stimulatory effect of ($Na_o$) on Li efflux. The $V_m$ and $K_m^L$ values for Li efflux vs $Na_o$ were 1.4±0.3 mmoles/L.cell water x hr, and 12.0±1.4 mM ($Na_o$), respectively. The transeffect of ($Na_o$) on Li efflux was studied at different cellular Li concentrations. After correction for major Li leaks in choline or Mg media the ratio of the apparent $V_m$ to $K_m^L$ values for Li seemed to be independent of ($Na_o$). Such behavior has been reported for human red cells to be consistent with a consecutive or"ping-pong" mechanism of Na/Na(Li) exchange (Hannaert & Garay, J.Gen.Physiol. 87:353,1986). NIH grant 5 ROI-AM 37160 (to PRL).