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Passive Cation Transport in Reversibly Sickling Deer Red Blood Cells

Norma C. Adragna
Wright State University - Main Campus, norma.adragna@wright.edu

Peter K. Lauf
Wright State University - Main Campus, peter.lauf@wright.edu

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GTP·γS in the presence of $[^{32}P]γATP$ demonstrated phosphorylation of several proteins in a pattern similar, but not identical, to that produced by the synthetic diacylglycerol DiC8. Moreover, while the protein kinase C inhibitor staurosporine caused nearly complete (>90%) inhibition of the respiratory burst induced by DiC8, the responses of fMLP and GTP·γS were only partially inhibited. The results suggest the existence of G-protein-mediated activation of the respiratory burst by pathways that are independent of increases in $[Ca^{2+}]$, and protein kinase C, but involve another unidentified kinase.

93. Passive Cation Transport in Reversibly Sickling Deer Red Blood Cells NORMA C. ADRAGNA and P. K. LAUF, Departments of Pharmacology and Toxicology and of Physiology and Biophysics, Wright State University, School of Medicine, Dayton, Ohio

Deer red blood cells (DRBC) reversibly sickle upon exposure to 100% O2 by plasma alkalization, and thus may serve as a model for the irreversible sickling-associated membrane transport changes of human red blood cells (HRBC) containing hemoglobin SS. We report here studies on ouabain-resistant K, Na, and Li fluxes in DRBC of 5-6-mo-old white-tailed deer (Odocoileus virginianus). Ca-activated K flux was studied simultaneously in DRBC and HRBC in choline-Cl media containing A23187 and CaCl2. The ionophore-sensitive K and Na effluxes were (mmol/liter cells·h): 0.21 ± 0.12 (DRBC) and 138 ± 5 (HRBC), and -0.01 ± 0.02 (DRBC) and -0.25 ± 0.01 (HRBC), respectively. Deoxygenation had no effect on K and Na effluxes into choline-Cl, excluding a major role of the Gardos system during deoxygenation in DRBC. Hence, the Ca-induced K channel seems to be absent in DRBC. In addition, DRBC seem to lack significant NEM- and swelling-stimulated K:Cl transport. The activity of the Na/Na exchange pathway was determined as the external Na-stimulated Li efflux from Li-loaded cells. The rate coefficient for Li efflux was 0.987 h⁻¹ and compared with 0.881 h⁻¹ in rabbit RBC. The effect of hypertonicity and amiloride on net Na efflux into either KCl or K-acetate with or without sucrose and with or without amiloride was assessed in both DRBC and rabbit RBC. The results indicate that DRBC, at least at physiological intracellular Na, lack an active Na/H exchange pathway present in rabbit and human RBC. The absence of major "volume-regulatory" pathways in DRBC is also supported by measurements of net ion and water contents under anisosmotic conditions. [Supported by a grant from the Ohio Research Challenge 1987.]

94. Evidence for the Presence of Functional Amino Groups in the NEM-Stimulated K:Cl Transporter of Sheep Red Blood Cells P. K. LAUF, Department of Physiology and Biophysics, Wright State University, School of Medicine, Dayton, Ohio

Ouabain-resistant (OR) K:Cl transport in sheep red blood cells is stimulated irreversibly by alkylation or reversibly by oxidation of thiol groups (Lauf. 1983. J. Membr. Biol. 73:237; 1988. J. Membr. Biol. 101:179). Relative to that of cysteine, the apparent pKα of this "stimulatory" thiol is low, which suggests ionization effects through neighboring amino groups, which, through protonation, may participate in regulation of K:Cl transport (Lauf. 1985. J. Membr. Biol. 88:1). In an effort to directly demonstrate the presence of such amino groups, in particular an imidazole, we exposed both low-K (LK) and high-K (HK) sheep red cells before or after alkylation with N-ethylmaleimide (NEM) to diethylpyrocarbonate (DEPC) and then measured both OR K efflux and Rb influx. We found that, at concentrations between 1 and 7 mM, DEPC alone slightly stimulated OR K(Rb):Cl transport in both LK and HK cells. When LK cells were first treated with NEM and then with DEPC or vice versa, a DEPC-dose–dependent reduction of the "NEM effect" on K:Cl transport was found. Although DEPC may react with amino acids other than histidine, these data indicate that N-carbethoxylation of a thiol-independent amino acid residue prevents or inhibits the NEM-induced stimulation of OR K:Cl transport. This supports our hypothesis that OR K:Cl transport in sheep red blood cells is modulated by intra- and extracellular pH, perhaps by amino group protonation and deprotonation. [Supported by NIH grant DK-37160-04.]