Quinine and Quinidine Inhibit K-Cl Cotransport in Low K Sheep Erythrocytes

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M-Pos25
N-ETHYLMALEIMIDE TREATMENT OF LOW K SHEEP RED BLOOD CELLS AT DIFFERENT TEMPERATURES REVEALS ROLE OF THIOLS AND Mg

Exposure of low K sheep red blood cells to low concentrations (<1 mM, 5% hemolysis), N-ethylmaleimide (NEM) (50 mM) or high NEM concentrations (>2 mM) reverses this effect. At least two types of thiol groups differing in their apparent pK, values have been identified. We now report that these two thiols may be further distinguished by NEM treatment at 0°C and 37°C, respectively, followed by analysis of the NEM effect on KCl efflux measured at 37°C in controls and in cells with Mg, reduced by A23187 and EDTA. Results: 1. At 0°C, NEM up to 20 mM solely activated K efflux in CI, with saturation at <2 min and 1 mM-NEM using diithiothreitol to remove NEM. 2. At 37°C, 5 mM NEM reversed KCl efflux stimulation to base values, however, with a tenfold time constant. 3. Both KCl flux activation and reversal were approximately equal in control and in low Mg, cells. Hence Mg, per se does not affect thiol alkylation. 4. Similarly, KCI flux, stimulated in low Mg cells in the absence of NEM, was inhibited by 6 mM NEM. Thus both KCl cotransport states-one activated by reduction of Mg, and the other by thiol alkylation, which affects regulation by Mg, are similar based on their NEM-inhibition through low affinity thiols. Since ATP is required for both NEM activation and swelling-induced Mg inhibition of KCl cotransport, and Mg, fails to inhibit NEM-stimulated KCI flux, MgATP may effect the thiol-catalyzed reversal of KCl cotransport stimulated by NEM, Mg, reduction, and cell swelling, by cosubstrate interventions causing KCI cotransport activation. (Supported by NIH 37,160).

M-Pos26
ALZHEIMER'S DISEASE-INDUCED CHANGES IN HUMAN RED CELL MEMBRANE TRANSPORT PROCESSER (A. Janoshazi, A. Satlin and A. K. Solo-
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An altered rate of SO3- exchange in red cells of patients with Alzheimer's disease (AD) has been reported by Boman et al. (1991). Does this mean that AD red cell band 3 is different from normal? We compared binding kinetics of the fluorescent anion exchange inhibitor, DIDS (4,4'-dibenzamidod-2,2'-stilbene disulfonate) in AD red cells with age-matched controls. In normal red cells, DIDS binding is saturable, a bimolecular association followed by a monomolecular conformation change which locks bound DIDS to band 3 in four preliminary experiments in AD red cells, the observed rate is absent and binding does not saturate in the molar concentration range, consistent with an AD-induced conformation change in red cell band 3. Boman et al. also reported one instance of increased binding of glucose the transport inhibitor, cytochlasin B (Cyt B). In AD red cells, suggesting an AD effect on the glucose transport system (GLUT1). We have previously reported that Cyt B modulates red cell DIDS binding (Janoshazi and Solomon, 1989) indicating that band 3 and GLUT1 are adjacent in the normal cell membrane. Two preliminary experiments show that 2.5 µM Cyt B modulation of DIDS binding kinetics is altered in AD red cells, consistent with an AD-induced conformational change of GLUT1. (Supported by the Council for Tobacco Research and the Alzheimer's Association).

M-Pos28

A candidate aminophospholipid flipase, a lipid-dependent, vanadate-sensitive Mg2+-ATPase, has been purified from human erythrocytes. The detergent solubilized enzyme is not active in the absence of added lipid and shows a high degree of specificity in lipid binding. 1,2-Diacylphosphatidyl serine (PS) stimulates the enzyme maximally, anionic phospholipids afford partial activation and zwitterionic or neutral lipids do not activate the ATPase. Lipid structural elements required for activation were studied using synthetic PS analogs which were modified in the headgroup, phosphate and glycerol backbone. Activation was reduced by removing the carboxyl group (PS, 28% of PS) or the amine moiety (phosphatidylhydroxypropionate, 38% of PS) or by replacing the carboxyl group with the less hydrophilic amine (21% of PS). Presence of the double bond in the headgroup produces 25% less activity than PS, indicating that the enzyme senses headgroup size. Replacement of the phosphate group with an ion-specific, vanadate-sensitive ATPase, indicating that the phosphate group is another recognition element. ATPase activity was dependent on the stereochemistry of the phosphate backbone, but not the serine analog of sn-1,2-PS was equivalent to the natural l-serine derivative in supporting ATPase activity. However, sn-2,3-diaryllip-
ophosphatidylserine and phosphatidylserine with chloroform and hydrogenation caused only 80% and 32% of the activity in the presence of the sn-1,2-isomers. Finally, glycerophosphorylserine and phosphoserine, but not the choline analog or serine alone, significantly inhibited ATPase activation in the presence of PS. These data indicate that the ATPase recognizes several PS structural elements and that each are required for full elaboration of activity. This structural specificity is shared by the aminophospholipid flipase, indicating that these proteins may be identical.

M-Pos29
QUININE AND QUINDINE INHIBIT K-CI COTRANSPORT IN LOW K SHEEP ERYTHROCYTES. N. C. Adragna and P. K. Lauf. WRIGHT STATE UNIVERSITY SCHOOL OF MEDICINE, Ohio, 45401-9027.

Low K (Lk) sheep red blood cells (SRBCs) are used as a model to study K-CI cotransport which plays an important role in cellular dehydration in human erythrocytes. To determine which members of the quinine (Q) and quindine (QD) are effectively used in the treatment of malaria. In the present study we investigated the effect of Q and QD on K-CI cotransport in LK SRBCs. Cells with K-CI cotransport activated by either swelling is hypotonic media, or by thiol alkylation with N-ethylmaleimide, or by cellular Mg removal through A23187 in the presence of external chelators were exposed to various concentrations of Q and QD and the rate constants of K efflux were determined in CI and NO3- K-CI cotransport was defined as the Cl-dependent (CI minus NO3) K efflux. K-CI cotransport stimulated by all three interventions was inhibited by both Q and QD in a dose-dependent manner. Full inhibition of K-CI cotransport occurred at concentrations of Q>2 mM, and of QD of 1 mM. The inhibitory effect of Q was manifested in CI but not in NO3, whereas QD also reduced slightly K fluxes in NO3. Dixon plots of the 1/K-CI flux versus the inhibitor concentration revealed curvilinear behavior indicating complex inhibitory kinetics. In contrast to K efflux, Q and QD inhibited KCl influx less effectively in swollen and NEM-treated cells whereas the effect in low Mg cells was more complex. The inhibitory action of these two drugs was reversible and their mechanism of action likely involves diffusion into the cell and inhibition from the cytoplasmic aspect of the membrane. This is the first report of an inhibition by amimalarial drugs on K-CI cotransport activated by three independent manipulations. (Supported by NIH DK RO1-37160).

M-Pos30
COUPLING OF THE Na FLUX OF THE Na-Pi COTRANSPORTER AND THE Na PUMP. EVIDENCE FOR A MEMBRANE POOL OF Na+. (R. B. Gamliel and T. Dayan) Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322.

The ouabain- and diisofibin-stibine-insensitive Na-Pi cotransporter in human red cells provides 25% of the Na+ influx in a plasma-like medium, but provides 90% of the transport of the nucleotide pyridosamine (Mizukami et al., J. Gen. Physiol. 92:449-474, 1988) that are preferential substrates for the ouabain-sensitive Na-Pi pump. Experiments were performed to test whether the Na-Pi cotransporter with the phosphate also had a special relationship to the pump as compared to cytoplasmic Na+. Human red cells were loaded with Na+ by a 60-s change of medium (10 and 65 mM NaCl, 110 mM KCl) and ouabain-sensitive Na+ efflux was measured in 140 mM NaCl + 1 mM N-methyl- diisofibin-stibine. The external Na+ was able to ionize NaPi-pump, when pump function was assayed by 86Rb influx external NaPO4 had no effect. One explanation is that the influx of neurotransmitter Na+ on the Na-Pi cotransporter was diluting the specific activity of the pump's substrate pool of Na+ and decreasing the tracer efflux but not pump turnover or stoichiometry. One predicted consequence of this is that the NaPi-pump, when the pump is blocked the tracer influx through the Na-Pi cotransporter should increase since 32P is in the pool could no longer be removed out through the pump. We observed that the influx of Na+ into red cells (diaminophenyl-disulfide-treated to block anion exchange on band 3) in the presence of 1 mM PO4-3 was increased by ouabain. The coupling of fluxes whereby the product of one enzyme/transporter is not released but used as substrate for another enzyme/transporter is documented in metabolism and the mitochondrial membrane. This relationship for transporters in the plasma membrane of eukaryotes has not been previously described. Supported by USPHS-NIH-HL 28674.

M-Pos31
SENESCENT ERYTHROCYTE REMOVAL (L. Kantor and H. M. Muzikant) Wayne State University, Detroit, MI 48202. (Spon. by H. M. Muzikant)

An anion transport protein, Band 3 (90-100kd) is involved in the two proposals regarding the senescent rbc's (red blood cells) removal. One by Low et al (1985) suggests that Band 3 molecules become covalently coupled to their n