Proton (H) Modulation of K-Cl Cotransport Through Both Internal and External Sites in DIDS-pH-Clamped Low Magnesium LK Sheep Erythrocytes

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Membrane transport: Glutamate transporter-mediated uptake is associated with a Cl⁻ conductance increase, but unlike the gradients for Na⁺, K⁺, and H⁺, the Cl⁻ gradient is not measurably coupled to that of glutamate. This conductance is not activated by external binding of the non-competitive antagonist kainate. The temperature coefficient (Q10, 20/7(29)) for D-aspartate transport mediated by the human subtype EAAT1 was 3.2 ± 0.2, while that of the transporter current was 1.0 ± 0.1 (n=5), indicating that the macroscopic anion conductance is independent of the transport rate. The NO₃⁻ and SCN⁻ permeabilities of the anion channel (relative to Cl⁻) were 11 and 58, respectively. No anomalous mole fraction effect was observed with mixtures of anions. The anion conductance was also activated during reverse transport induced by raising extracellular K⁺ (EC₅₀ = 35.3 ± 5.7 mM; n=5). A macroscopic anion current with a similar permeability profile (P(Na⁺)/P(Cl⁻)=61) was observed in excised inside-out patches from oxycephalic transporters when excitatory amino acids and Na⁺ were applied to the intracellular membrane face with K⁺ in the pipette. This current was not observed when K⁺ was replaced by choline (n=6). A mutant EAAT3 transporter (E374D) was constructed which mediated K⁺-independent obligate amino acid exchange; this mutant exhibited a similar anion conductance. The results suggest that an anion conducting state is associated with a glutamate- and Na⁺-occupied state of the transporter rather than a K⁺-transporting limb of the cycle.

ION PERMEATION IN GLUTAMATE TRANSPORTERS

Glutamate transporter-mediated uptake is associated with a Cl⁻ conductance increase, but unlike the gradients for Na⁺, K⁺, and H⁺, the Cl⁻ gradient is not measurably coupled to that of glutamate. This conductance is not activated by external binding of the non-competitive antagonist kainate. The temperature coefficient (Q10, 20/7(29)) for D-aspartate transport mediated by the human subtype EAAT1 was 3.2 ± 0.2, while that of the transporter current was 1.0 ± 0.1 (n=5), indicating that the macroscopic anion conductance is independent of the transport rate. The NO₃⁻ and SCN⁻ permeabilities of the anion channel (relative to Cl⁻) were 11 and 58, respectively. No anomalous mole fraction effect was observed with mixtures of anions. The anion conductance was also activated during reverse transport induced by raising extracellular K⁺ (EC₅₀ = 35.3 ± 5.7 mM; n=5). A macroscopic anion current with a similar permeability profile (P(Na⁺)/P(Cl⁻)=61) was observed in excised inside-out patches from oxycephalic transporters when excitatory amino acids and Na⁺ were applied to the intracellular membrane face with K⁺ in the pipette. This current was not observed when K⁺ was replaced by choline (n=6). A mutant EAAT3 transporter (E374D) was constructed which mediated K⁺-independent obligate amino acid exchange; this mutant exhibited a similar anion conductance. The results suggest that an anion conducting state is associated with a glutamate- and Na⁺-occupied state of the transporter rather than a K⁺-transporting limb of the cycle.

FUNCTIONAL ANALYSIS OF hu MDR 1 PROTEIN EXPRESSED IN YEAST

A truncated hu MDR 1 cDNA (missing the first 17 amino acid codons of the open reading frame) fused to the Ste 5 promoter of S. cerevisiae (generously provided by Dr. K. Kuchler and J. Thorne [PNAS. 89, 2302-2306]) was cloned into the yeast expression vector pVX2. The resulting construct (pMDR1) places both the Ste 6 and alcohol dehydrogenase (ADH) promoters upstream of the hu MDR 1 cDNA. Genes of pMDR1 stabil transfected to form high levels of hu MDR 1 protein expression. The strains are resistant to valinomycin, indicating the expressed protein is functional. The expressed protein migrates at about 140 kDa on SDS gels, due to inefficient glycosylation, and we estimate hu MDR 1 protein is about 0.8-0.9% of the total membrane protein in our highest expressing strains. Isolated plasma membranes exhibit about a 2-3 fold higher rate of ATP hydrolysis in this buffer (pH 7.5, 5 mM Mg²⁺) relative to control, and the increased ATPase activity can be further modulated by 10-100 µM levels of a variety of hydrophobic compounds. A summary of rates of growth in media of various toxicity and ion composition, cell volume, intracellular pH, plasma membrane potential, and ion transport for these strains will be presented. Similar data for hu MDR 1 expressing yeast strains created in erg1 and K⁺ uptake mutants will also be presented. This work was performed in the Sackler Laboratory of Membrane Biophysics and was supported by grants from the NIH (GM54516, GM53549) and a Cancer Center Support Grant (NCI-P30-CA-08745). P.D.R. is a Sackler Scholar at MKCC. pVX2 was generously provided by the NRC, Canada.

ALTERED pH REGULATION IN JT3 / CFTR CLONES AND THEIR CHEMOTHERAPEUTIC DRUG - SELECTED DERIVATIVES

Recently (Wei et al., Biophys. J. 69: 883-895, 1995) we found that JT3 cells overexpressing the CFTR exhibit traits of multidrug resistant (MDR) cells. In this work, JT3 / CFTR clones were selected with either doxorubicin or vincristine to generate series of stable cell lines which exhibit increased levels of multidrug resistance. Thus, C3D6 (grown in the presence of 600 and nM vincristine) was selected from C3D1 (grown in 100 µM vincristine), which was selected from the original JT3 / CFTR clone C3 (M.J. Suzuki et al. JBC 286, 20553-20568 (1981)) that was selected from the parent cell line K95. NaCl, Na; Na-Hepes, pH 7.2 and variable concentrations of MgCl₂ (0, 0.2, 0.5, 1.0, 2.0, 5.0, 10). The basal (Mg⁺²) was 0.74 ± 0.06 mM (cf. Alvarez-Leefmans et al. J. Physiol. 354: 303, 1984). Replacement of external Na⁺ with n-methyl-D-glucamine, produced an increase in [Mg⁺²], from 0.8 ± 0.3 mM up to 1.7 ± 0.4 mM at an initial rate of 0.2 ± 0.1 mM/min. Mole by mole replacement of 20 mM external Na⁺ with N-hCl, produced a transient intracellular alkalization, followed by an acidification upon removal of N-hCl. During the alkalization, basal (Mg⁺²) decreased from 0.9 mM to 0.1 mM. Upon removal of N-hCl, (Mg⁺²) recovered even in the absence of external Mg⁺², indicating that the changes in (Mg⁺²), were due to intracellular redistribution. The data suggests that pH₅ modulates intracellular Mg⁺², probably by affecting cystolic Mg⁺² binding or transport. (Supported by NINDS USA Grant NS29227 to FIAL).