Membrane Transporters & Exchangers I

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Cisteine Residues Involved in Transport Activity and Nitric Oxide Sensitivity of the Cationic Amino Acid Transporter CAT-2A
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The cationic amino acid transporter CAT-2A is a low-affinity, high-capacity carrier expressed in cardiomyocytes, liver and other tissues. CAT-2A is sensitive to N-ethylmaleimide (NEM) and nitric oxide (NO) inhibition. Both reactions involve cysteine (Cys) modification. Our previous studies showed that mutating Cys347 to alanine (C347A) in mouse CAT2A (mCAT2A) results in a ~60% decrease in transport activity, and insensitivity to NO inhibition without affecting membrane expression levels of this transporter. The decreased activity might be due to a disruption of the Cys local environment. Alternatively, this Cys could be directly implicated in transport activity. We decided to test these possibilities by replacing Cys347 with a ''more conservative'' serine (C347S). Serine has similar size/polarity as Cys, but lacks the -SH group. The C347S mutant shows transport activity comparable to that of wild type; however, it retains NO insensitivity. To learn more about the involvement of Cys347 in transport, we measured the L-Lys concentration dependence of uptake in the presence of radiotracer amounts of [^{14}C]L-Lys in both wild-type and C347A mCAT-2A variant. Hyperbolic fitting yielded not significantly different K_m values (6.3 ± 2.8 vs. 8.2 ± 0.4 mM) and a substantial decrease in V_{max} (2.6 ± 0.6 vs. 4.7 ± 0.1 nmol/mg prot./min) for C347A compared to wild-type control. These data suggest an impaired transporter turnover rate when Cys347 is replaced with Ala. In conclusion, Cys347 -SH side chain is required for NO modulation of mCAT-2A and the size/polarity of residue 347 is critical for the normal functioning of this transporter.

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Topology of Human Proton-Coupled Folate Transporter Expressed in E. Coli
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The proton-coupled folate transporter (PCFT) is an important membrane protein involved in the absorption of folates in humans. Folic acid is considered a vitamin that plays important roles in crucial one carbon transfer reactions. Folates are especially important for processes where DNA synthesis is involved, as for example fetal development and cancer. Humans cannot synthesize folates and therefore, absorption from the diet is the only source. Point mutations in PCFT manifest in form of Hereditary Folate Malabsorption Syndrome (HFM). PCFT is also involved in cancer treatment by providing the uptake pathway for antifolate anticancer agents. So far very limited information is available about PCFT's structure and function. To obtain large quantities of human PCFT for structural studies, we have generated a PCFT construct that expresses well in membranes of E. coli. To ascertain correct folding of PCFT after over-expression in E. coli, we carried out a detailed topological study using the substituted Cysteine accessibility method (SCAM), comparing the accessibility of engineered single-Cys in PCFT expressed in E. coli vs. PCFT expressed in Xenopus laevis oocytes. Correctly folded PCFT produced in E. coli will be a potent tool for further structural and mechanistic studies of folate translocation by PCFT that will aid in the development and optimization of therapies against HFM and cancer.

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Biophysical Properties of a Human Plasma-Membrane Creatine Transporter
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Creatine (Cr) and phosphocreatine play central roles in energy storage and transport/shuttle of high-energy phosphate in cardiac and skeletal muscle. Cr is taken up from the circulation by a Na,Cl-dependent cotransporter. The mechanism of this secondary-active transport process as well as the effect of the cell membrane potential (V_M) on Cr transport are poorly understood. To study the biophysical features of this process, the human isoform of the plasma-membrane creatine transporter (SLC6A8) was subcloned and expressed in *Xenopus* oocytes. Cr-activated inward currents were measured by two-electrode voltage clamp as a function of 2-200 μM $[Cr]_o$ in buffer containing 150 mM NaCl at 22°C. The $[Cr]_o$ that half-maximally activated current ($K_{0.5}$) was largely V_M-independent (range −180 to 0 mV) and had an average value of 12 ± 1 μM (S.E., $n = 9$). Maximal current levels (I_{max}), normalized to the value at the holding potential (−60 mV), were modestly V_M-dependent, increasing exponentially with a fractional electrical distance $\lambda = 0.17 \pm 0.00$ as V_M was made more negative. These findings suggest that Cr binding reactions are intrinsically V_M-independent (Cr has no net charge), and that V_M-dependent steps may relate to Na^+ and/or Cl^-, which are not rate-limiting at 150 mM NaCl. This point is currently being studied by determining the $[Na^+]$ dependence of the $K_{0.5}$ for $[Cr]_o$ activation of current. Interestingly, an additional inward current component appears at −20 mV, displaying a bell-shaped current-V_M relationship that peaks at +10/+20 mV, and increases with $[Cr]_o$. However, this current is independent of Cr transporter expression since oocytes not injected with mRNA for SLC6A8 display similar currents (although not bell-shaped) when are exposed to 500 μM Cr_o in the presence of 1 mM intracellularly-injected Cr. This second component is also being characterized.

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Modulation of Proximal Na^+ Reabsorption is Related to the Fructose-Induced Hypertension
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Methods/Results: Wistar rats were fed for 2 or 8 weeks with control diet (CTRL) or isocaloric 60% fructose diet (HF). Systolic blood pressure (SBP) was measured weekly by tail cuff plethysmography. There was no difference in the SBP after 2 weeks; however, there was an increase in SBP after 8 weeks ($P < 0.0001$). 24h urine collected in metabolic cages showed a reduction in urine flow (2 weeks - $P = 0.0007$ and 8 weeks - $P = 0.001$). Glomerular filtration rate (GFR) was not altered after 2 weeks, but was decreased after 8 weeks ($P = 0.001$). Na^+ fractional excretion (FE) was decreased after 2 weeks ($P = 0.0131$), but was increased after 8 weeks ($P = 0.038$). After 2 or 8 weeks, rats were subjected to in situ microperfusion experiments and had their proximal tubules (TP) perfused with an alkaline solution to investigate Na^+ reabsorption by means of bicarbonate flux (JHCO3-, in nmol/cm2 x s). JHCO3- was increased after 2 weeks ($P < 0.0001$) but was reduced after 8 weeks ($P < 0.0001$). Perfusion using S3226, a specific NHE3 inhibitor, showed that fructose acts stimulating NHE3 activity after 2 weeks ($P < 0.0001$) and inhibiting it after 8 weeks ($P < 0.0001$). Conclusions: Reduced Na^+ FE and urine flow, associated with increased NHE3 activity after 2 weeks of HF suggest that initially fructose leads to a state of Na^+ overload, which may contribute to the development of hypertension observed after 8 weeks of HF intake. Kidney damage was increased after 8 weeks, since GFR was decreased, explaining the persistence of reduced urine flow, despite reduced NHE3 activity. These data suggest that the mechanism of pressure-natriuresis was activated after 8 weeks, in order to compensate for volume expansion.

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Fluoride Toxicity in Extreme Acid Stress: What's a Bacterium to do?
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Microorganisms face an environmental threat from F^- anions (present at 100μM in soil), which inhibit enolase and pyrophosphatase. Many microorganisms possess fluoride exporters to reduce cellular F^- concentrations. Fluoride toxicity is more troublesome at acidic pH levels, because membrane-permeant HF (pK_a ~3.4) from the acidic extracellular fluid diffuses into the cytoplasm where it dissociates at the more neutral intracellular pH, thus trapping and accumulating F^- at levlels much higher than the extracellular concentration. Enteric bacteria, such as *Escherichia coli*, face a particularly extreme acidic stress (pH < 2.5) encountered during their transit through the host stomach. How, then, does *E.coli* survive the combination of elevated F^- concentration and extreme acidity? To address this, we exposed WT *E.coli*, *E.coli* with its native F^- channel knocked out (ΔcrcB), and ΔcrcB cells expressing F^- exporters from *Piruella staleyi* (pst) acid-shock media containing 1μM-10mM NaF for 2 hours. Cells were then transferred to neutral LB media and their survival rates and recovery times were monitored. For all strains, absolute survival rates decreased only as F^- concentration during acid-shock increased above physiological levels (>100μM). But, different strains showed major differences in the lengths of their recovery periods before growth after acid-shock with F^-, even at low F^- concentrations. The ΔcrcB cells, despite having the same absolute survival rate as WT cells, took 4 hours longer to begin dividing than WT at 10μM F^-, whereas the lag time for ΔcrcB cells expressing the F^- exporter Pst matched or was less than that of WT at F^- concentration as high as 1mM.

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Prolongation of Beating Rate Caused by Reduction of NCLX, a Mitochondrial Na^+-Ca^{2+} Exchanger, in Hl-1 Cardiomyocytes
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