3532-Pos Board B579

The True Ionic Nature of the Na+/Glucose Cotransporter Leak Current

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Expression of the Na^+/glucose cotransporter (SGLT1, SLC5A1) in *Xenopus* oocytes is characterized, by a phlorizin(Pz)-sensitive leak current (observed in the absence of glucose) which represents 5 to10% of the maximal Na^+/glucose cotransport current. This current was considered to represent the glucose-independent steps of the cotranport mechanism and was originally called a Na-leak even if its reversal potential (Vr) was 80 mV more negative than E_{Na}. Using a human SGLT1 mutant (C292A) displaying a large leak current (-290 nA at −155 mV), the leak Vr showed only a modest negative shift when external Na concentration ($[Na^+]_e$) was lowered and was insensitive to changes in external [Cl^-]. When external pH (pH_e) was decreased from 7.5 to 6.5 and 5.5, the leak current (at −155 mV) increased to −600 and −1500 nA and its Vr shifted by +15 and +40 mV, respectively. If protons appear to be the main charge carrier at low pH_e's, other ions need to be involved at pH_e 7.5 since rising pH_e to 8.5 produced no further reduction of the leak current. Starting from a $[Na^+]_e$ of 15 mM (pHe=7.5), adding 75 mM of either Na^+, Li^+, Cs^+ or K^+ generates a similar increase in the leak current amplitude. This is in sharp contrast with the cotransport activity which accepts only Na^+ and, to a lesser extent, Li^+ as driving cations. This demonstrates that a cationic pathway clearly distinct from the cotransport mechanism exists through SGLT1. This is reminiscent of the Pz-sensitive passive water permeability of SGLT1. Interestingly, breaking disulfide bridges in SGLT1 or the C292A mutant with dithiothreitol produces a decrease in both the leak current and the Pz-sensitive water permeability. This suggests that the cationic leak may share a common pathway with water across the cotransporter.

3533-Pos Board B580

The Transport Mechanism of the Human Sodium Myo-Inositol Cotransporter 2 (hSMIT2)

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The myo-inositol molecule (**MI**) is isomerous to glucose and serves as a compatible osmolyte involved in cell volume regulation and as a precursor of important signalling molecules (inositol phosphates, phosphatidylinositols etc.). hSMIT2 (the product of *SLC5A11*) is a secondary active MI transporter expressed in the apical membranes of small intestine and proximal tubule, and the two-electrode voltage-clamp technique was used to characterize hSMIT2's electrogenic properties after expression in *Xenopus* oocytes. As previously observed for the Na/glucose cotransporter (SGLT1, 49% identical with hSMIT2), hSMIT2 has a 2 Na^+: 1 sugar stoichiometry, is inhibited by phlorizin and, in the absence of substrate, exhibits large phlorizin-sensitive pre-steady-state currents with fast (~2 ms), medium (~10 ms) and slow components (~150 ms). The fact that the charge movement recorded during a negative voltage pulse (Q_{on}) can be up to 4 times larger than the charge movement upon return to the normal holding potential (Q_{off}) indicates that at least a component of the transient current does not represent displacement of a charge associated with the transporter across a fraction of the membrane electrical field. If the slow component of the transient current is omitted from the analysis, Q_{on} equals Q_{off} and a 5 state kinetic model can accurately reproduce the electrogenic properties of hSMIT2. The overall resulting model indicates that SMIT2 experiences a slow conformational change of the empty transporter with a voltage-dependent intermediate state followed by a fast Na binding reaction and a slower MI binding step. The slow component of the transient currents is thought to represent a slow relaxation of the hSMIT2 leak current which is relatively large (i.e. 4 times the amplitude of typical SGLT1 leak currents).

3534-Pos Board B581

Roles of Calcium Binding Domains 1 and 2 in Ca^{2+} Regulation of the Na^+-Ca^{2+} exchanger

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The crystal structures of two Ca^{2+} binding domains (CBDs) in the Na^+-Ca^{2+} exchanger (NCX) have been recently resolved. We generated full-length NCXs with mutations in CBD1 and CBD2 to determine their roles in Ca^{2+}-dependent regulation of NCX.

The effects of regulatory Ca^{2+} on the mutant exchangers were measured in giant excised patches. Currents were elicited by applying Na^+ to the cytoplasmic side of the patch in the presence of different regulatory Ca^{2+} concentrations. The pipette contained Ca^{2+} at all times. Under these conditions, NCX current peaks and then decays due to inactivation triggered by high cytoplasmic Na^+. Regulatory Ca^{2+} relieves this inactivation.

Glu 385 coordinates one (Ca3) of the four Ca^{2+} found in CBD1. Peak NCX current of mutant E385A displayed a 4-fold decrease in Ca^{2+} affinity demonstrating the important role of Ca3. NCX-M7 has mutations at 7 of the 10 amino acids that coordinate Ca^{2+} binding to CBD1 which should eliminate Ca^{2+} binding to this region. This mutant showed a further decrease in apparent Ca^{2+} affinity but retained regulation confirming a contribution of CBD2 to Ca^{2+} regulation. Addition of the mutation K585E (located in CBD2) into the NCX-M7 background resulted in an increase in apparent Ca^{2+} affinity. K585E appears to increase Ca^{2+} binding-affinity of CBD2.

Additional mutations within CBD2 were also tested. Previously we have shown that NCX mutants E516L, D578V and E683V lack Ca^{2+} regulation. Introduction of a positive charge either at position 516 or 683 partially rescued NCX Ca^{2+} regulation. The positive charge may mimic Ca^{2+}. These results stress the importance of CBD2 in NCX Ca^{2+} regulation. Additional mutants (including D578R) are under investigation. Our final goal is to gain a better understanding of the interaction between CBD1 and CBD2.

3535-Pos Board B582

Is ATP Required For Activities Of The Na^+/Mg^{2+} Exchange?

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Cytoplasmic Mg^{2+} concentration ($[Mg^{2+}]_i$) was measured with the fluorescent indicator furaptra in rat ventricular myocytes at 25°C. In intact cells loaded with Mg^{2+}, introduction of extracellular Na^+ induced a rapid decrease in $[Mg^{2+}]_i$; the initial rate (initial $\Delta[Mg^{2+}]_i/\Delta t$) was thought to represent the rate of Na^+-dependent Mg^{2+} efflux (putative Na^+/Mg^{2+} exchange). To study whether the Mg^{2+} efflux depends on energy derived from ATP, in addition to transmembrane Na^+ gradient, we estimated the initial $\Delta[Mg^{2+}]_i/\Delta t$ after metabolic inhibition. In the absence of extracellular Na^+ and Ca^{2+}, treatment of the cells with 1 μM FCCP (~10 min) or 5 mM KCN ($\geq$90 min) caused a increase in $[Mg^{2+}]_i$ from ~1 mM to ~2.5 mM (probably due to breakdown of MgATP) and cell shortening by ~50% (probably due to formation of rigor cross-bridges). The initial $\Delta[Mg^{2+}]_i/\Delta t$ was largely reduced, on average by 90% in FCCP-treated cells and 92% in KCN-treated cells. Intracellular Na^+ concentration ($[Na^+]_i$) measured with a Na^+ indicator SBFI was, on average, 5.0-10.5 mM within the time range for initial $\Delta[Mg^{2+}]_i/\Delta t$ measurements, which is lower than that required for 50% inhibition of the Mg^{2+} efflux (~40 mM, Tashiro et al., Biophys J 89:3235-3247, 2005). Normalization of intracellular pH by application of 10 μM nigericin did not reverse the inhibition of the Mg^{2+} efflux. These results suggest that elevation of $[Na^+]_i$ or intracellular acidosis are not primarily responsible for the slowed Mg^{2+} efflux. Instead, in seems likely that a decrease in ATP below the threshold of rigor cross-bridge formation (~0.4 mM estimated indirectly) inhibits the Mg^{2+} efflux, suggesting that absolute requirement of ATP for the Na^+/Mg^{2+} exchange.

3536-Pos Board B583

The K+/Ca2+ Exchanger from Human RBC: Effect of oxidative stress on one or both sides of the membrane

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A internal Ca2+ concentration rise has been associated with the senescence process of the Human Red Blood Cell. Since very little is known about it, using the Patch Clamp Technique we are focused in the study of a current associated with the transport of Ca2+. In this regard, we have already presented direct evidences of the existence of a novel transporter capable to account with the Ca2+ entry during ageing process, the K+/Ca2+ Exchanger (1)(2). At the microcirculation level these membrane are exposed to an oxidative stress by mean of the O2/CO2 interplay, to this respect we have already presented some preliminary results (3). Here, we present a completed study of the differential effect of the oxidative stress in one or both sides of the RBC membrane: No matter how the stress is applied the currents are diminished in a concentration dependence fashion by 28% (inside only) against 40% (both side affected), this effect depends in the fluxes directions. Same dependence is presented by the time course developed, presenting a tmax= 40 min for out to inside Ca2+ flux vs. 16 min for in to outside. On the other hand, when the effect is presented without the activation mechanism influence, the overall effect remains, but there is practically no dependence on the sidedness of the oxidative stress, and in fact, there is no dependence on the direction of the fluxes in any condition, suggesting an effect on the activation mechanism. Interestingly, there is no effect on the kinetic of the deactivation process, no matter how the oxidative stress is applied. Structural implication are discussed.

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