Membrane Pumps, Transporters, and Exchangers II

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Regulation of the Cardiac Sodium/Calcium Exchanger by Protein Palmitoylation

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The cardiac sodium/calcium exchanger (NCX1) regulates intracellular Ca in cardiac muscle. Inappropriate NCX1 function contributes to cardiac contraction abnormalities and heart failure: reduced NCX1 activity reduces Ca removal therefore impairing relaxation, whereas overactive NCX1 unloads the intracellular Ca stores and impairs systolic function. Although the structure-function relationship of NCX1 is well characterised, dynamic regulation of NCX1 function by post-translational modifications is controversial. The reversible attachment of the 16-carbon fatty acid palmitate to cysteine residues in proteins (palmitoylation) is an important and common post-translational modification in a variety of tissues. The palmitoyl proteome in cardiac tissue remains largely uncharacterised. Few proteins have been identified as palmitoylated, for example the sodium pump regulator phospholemman. We investigated the palmitoylation of NCX1 from isolated adult rat ventricular myocytes (ARVM), using site-specific resin assisted capture (Acyl Rac). Acyl Rac purified 100% of the constitutively palmitoylated protein caveolin 3 from ARVM lysates, and 70% of NCX1, indicating a biologically meaningful fraction of NCX1 is palmitoylated in ventricular muscle. We transiently expressed NCX1 in HEK-293 cells and measured palmitoylation by Acyl Rac and cell surface localization using membrane impermeable biotinylation reagents. The palmitoyl acyl transferase inhibitor 2-bromopalmitate (2-BP) significantly reduced both NCX1 palmitoylation and cell surface expression to $51 \pm 11\%$ of expression in untreated cells, $p<0.05$. We investigated the effect of palmitoylation on NCX1 function in voltage clamped stably transfected BHK cells. Application of 2-BP decreased inward current density from 1.9-$\pm$0.2 pA/pF to 1.1 ± 0.1 pA/pF, but was without effect on outward current density. In conclusion, palmitoylation of NCX1 influences its transport function and turnover at the plasma membrane and/or processing in the secretory pathway. The site(s) of palmitoylation in NCX1 are currently under investigation.

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The Human Red Blood Cell K^+/Ca^{2+} Exchanger: Effect of Internal pH

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The Human Red Blood Cell (hRBC) is a highly specialized cell in oxygen transport and has an intracellular pH of 7.2, a Vm of -8 mV, and an average life span of 120 days. A rise of $[Ca^{2+}]_i$ has been associated with the senescence process of the hRBC and it has been related with the mechanical stress at the microcirculation level. We have presented evidence for the existence of a mechano-activated K^+ channel (HEMKCA) and a voltage-dependent K^+/Ca^{2+}-Exchanger (1)(2). The activation process of the K^+/Ca^{2+}-Exchanger has sigmoid voltage dependence and a permeability sequence of: $K^+>Rb^+>>Cs^+$ and $Ca^{2+}>Ba^{2+}>>Mg^{2+}$. We have proposed a new hypothesis for the senescence of hRBC (The hypothesis of K^+). Here, when the hRBC passes throughout the capillary suffers mechanical stress, increasing its K^+ permeability, depolarizing its membrane, and consequently activating the K^+/Ca^{2+}-Exchanger, letting the Ca^{2+} enters the hRBC. Using the Patch-Clamp technique, we measured K^+/Ca^{2+}-Exchanger currents and the effect of pH change (from 7.2 to 5.9) in the cytoplasmic side. At an acidic internal pH we registered a complex effect, i/e. the current increased when the exchanger was in the Ca^{2+} entry mode, while in the reverse mode a current decrease was observed. On the other hand (i/e. at pH 6.7), the temporal development of the inactivation was slowed and saturated, from about 430ms to 490ms (14%) at 160mV. These results suggest the existence of at least one histidine residue in the exchanger, exposed to the cell interior which affects its activity, and also, the existence of two different binding sites for intracellular K^+ and Ca^{2+}. We present new evidences for the existence of a K^+/Ca^{2+}-Exchanger in the hRBC and a complex effect of changes of internal pH.

(1)(2005) Biophys.J.88(1):593.
(2)(2006) Biophys.J.89(1):456.

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Functional Characterization of Bacterial NCx by Surface Supported Membrane Technology

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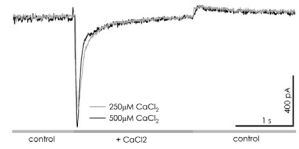
Sodium/calcium exchangers are membrane transporters which play an important role in calcium homeostasis by removing calcium out of the cell using the sodium gradient as driving force. This is essential for cell signaling as well as muscle activity and other functions. Recently NCx from Methanococcus jannaschii (NCx_Mj) has been crystalized and a structure model has been proposed.

Here we show direct functional measurements of this transporter obtained by surface supported membrane technology. The experiments were performed on the SURFE2R device (SURFace Electrogenic Event Reader, Nanion Technologies). Therefore we reconstituted bacterial NCx into lipid vesicles which were then accumulated on a lipid coated gold surface. Due to rapid addition and removal of substrate ions NCx activity could be evoked.

We characterized substrate dependency of sodium and calcium and their interactions. Competing substrates like potassium and magnesium were investigated on their interfering behavior. The results were compared to human NCx in membrane fragments.



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Four Histidines Account for the Inhibitory Effect of Protons on the Cardiac Na^+-Ca^{2+} Exchanger

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Myocardial ischemia and associated cardiogenic shock are leading causes of morbidity and mortality in developed countries. The detrimental effects of ischemia are in large part due to the accompanying cellular acidosis, which markedly diminishes cardiac performance. At the myocyte level, an increase in H^+ causes aberrations in intracellular calcium (Ca^{2+}) handling, thereby affecting contractility. Among the proteins regulated by cytoplasmic H^+ is the cardiac Na^+-Ca^{2+} exchanger (NCX). NCX is a plasma membrane transporter which maintains cellular Ca^{2+} balance by regulating its extrusion on a beat to beat basis. Inhibition of NCX by cytoplasmic H^+ could lead to contractile dysfunction. Remarkably there is little information about the modulatory mechanisms of H^+ on NCX activity. To test the hypothesis that NCX histidines are fundamental to proton inhibition of NCX activity we combined mutagenesis and electrophysiology. Our data reveal that among the 18 histidines found in NCX, four play an important role in its regulation by protons. Replacement of these residues with alanine abolished proton inhibition of NCX. Moreover we extended these findings by demonstrating that the two Ca^{2+} binding domains (CBD1, CBD2) play little role in pH sensitivity: NCX mutants incapable of binding Ca^{2+} were still sensitive to pH. These findings suggest that NCX possesses a true, structurally distinct pH sensor which does not involve the Ca^{2+} binding sites.

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Mitoferrin-2 (MFRN2) Regulates the Electrogenic Mitochondrial Calcium Uniporter and Interacts Physically with MCU

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Background: The mitochondrial calcium uniporter catalyzes electrogenic mitochondrial uptake of both Ca^{2+} and Fe^{2+} (JBC 1975;250:6433). More recently, Mfrn1 and 2 were identified to mediate mitochondrial iron uptake in hemopoietic and non-hemopoietic tissue, respectively (Mol Cell Biol 2009;29:1007), whereas the CCDC109A gene product (MCU) was discovered to be the core protein of the uniporter complex (Nature 2011;476:336&341). Here, our **AIM** was to determine the role of Mfrn2 in uniporter function. **Methods**: Respiration-driven Ru360-sensitive mitochondrial Fe^{2+} and Ca^{2+} uptake was measured in rat liver mitochondria (RLM) and permeabilized UMSCC1 and UMSCC22 squamous carcinoma cells. siRNA knockdown of Mfrn2 was also performed in UMSCC22A cells. Pull down assays were