(2)(2006) Biophys. J. 89(1):456
(3)(2007) Biophys. J. 90(1):1101
CDCH PI 03-00-6135-2005

3537-Pos Board B584

β-adrenergic Regulation of a Novel Isoform of NCX: Sequence & Expression of Shark Heart NCX in Human Kidney Cells

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Overexpression of the Na^+/Ca^{2+} exchanger (NCX) in mammals is associated with arrhythmias in cardiac hypertrophy and failure. The function and regulation of NCX genes varies significantly among vertebrates likely reflecting differences in molecular structure. We have previously reported that β-adrenergic suppression of the Ca^{2+}-efflux and -influx modes of amphibian cardiac NCX1.1 is associated with specific molecular motifs. In contrast, cardiac NCX of shark *(Squalus acanthias)* shows 'bimodal' adrenergic regulation with preferential suppression of the Ca^{2+}-influx mode (Woo and Morad, PNAS 98:2023, 2001); and its sequence (DQ 068478) reveals two novel proline/alanine-rich AA-insertions. Here we examined the effects of deleting the longer of these inserts.

Shark and mutant shark cardiac NCX were expressed in mammalian cells (HEK 293 and FlpIn 293), and their activity was measured as Ni^{2+}-sensitive Ca^{2+}-fluxes (Fluo-4) and membrane currents (I_{NaCa}) by changing $[Na^+]_o$ and/or membrane potential (V_m). Bimodal regulation, defined as differential regulation of Ca^{2+}-efflux and influx pathways with a strong suppression of its Ca^{2+}-influx mode and no change, or enhancement, of the Ca^{2+}-efflux mode, persisted in the shark NCX regardless of Ca^{2+} buffering, closely resembling the β-adrenergic regulation of native shark cardiomyocytes. In contrast, β-adrenergic stimulation of the shark mutant NCX produced an equal suppression of the inward and outward currents as well as the Ca^{2+} fluxes (as found with frog NCX), thereby abolishing the bimodal nature of the regulation. Control experiments were carried out with untransfected and dog cardiac NCX expressing cells.

We conclude that shark NCX is physiologically functional in mammalian cells, retaining the essentials of its bimodal β-adrenergic regulation. In addition, the deleted shark-specific insert was found to affect the modality of cAMP-dependent regulation, possibly because it provides essential intramolecular flexibility and/or binding sites.

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Calmidazolium Inhibits Na+/ca2+ Antiporter In Mitochondria

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A major function of Ca2+ in mitochondria is the regulation of intramitochondrial enzyme activity. A rise of Ca2+ in mitochondria can stimulate the oxidative metabolism and ATP production through activating Ca2 ± sensitive matrix dehydrogenases and controlling the synthesis of ATP. The main mechanism of the Ca2+ efflux from mitochondria in the cells of heart, brain, skeletal muscles, pancreas, and the majority of tumor cells is the Na+/Ca2+ exchange (NCXmito). Application of the NCX inhibitors (tetraphenylphosphonium cation, CGP37157) increases the concentration of Ca2+ ([Ca2+]m) and NADH level in mitochondria, and stimulates the oxidative metabolism. Little is known about the regulation of NCXmito. In this work it is shown, that calmodulin (CaM) participates in the regulation of the antiporter activity. To study the NCXmito activity, we used the Ca2 ± signal generating purinoreceptors of Ehrlich ascites tumor cells (EATC) and an inhibitor of CaM, R24571. R24571- and ATP-induced changes in the [Ca2+]i, [Ca2+]m, NADH fluorescence, and the membrane potential of mitochondria were recorded. ATP produced a transitory increase in [Ca2+]i, [Ca2+]m and NADH fluorescence. A preliminary addition of Na+/Ca2+ exchange inhibitors slightly changed the cytosolic signal, but suppressed the efflux of Ca2+ from mitochondria. Addition of ionomycin (0.5μM) in the time of the plateau in the mitochondrial Ca2 ± signal caused a decrease in Rhod-2 and NADH fluorescence up to the initial level, which indicated the Ca2 ± efflux from mitochondria. R24571 (3-5μM) caused a transitory Ca2 ± signal in the cytosol and an irreversible increase of NADH and Rhod-2 fluorescence. Ionomycin caused NADH and Rhod-2 fluorescence to recover to the initial level. Thus, R24571 inhibits the NCXmito in EATC, which suggests that its activity is regulated by CaM.

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High-Yield Expression and Purification of a Plasma Membrane Citrate Transport Protein

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Citrate is prominently and uniquely involved in metabolic crossroads that govern the energy balance of human and other organisms, yet little is known about the molecular mechanisms determining its entry into cells. The unique ability of plasma membrane citrate transporters (PMCTs) to import extracellular citrate into the cytoplasm confers upon them an essential role in determining an organism's energy state. Thus, PMCTs located in liver and brain figure prominently in: i) fatty acid, lipid, and cholesterol biosyntheses; ii) control of glucose metabolism via the citrate-mediated allosteric inhibition of PFK-1; and iii) neuronal cell synthesis of neurotransmitters. Therefore, inhibition of human PMCT function may prove to be a novel strategy for altering energy balance in a manner that mimics caloric restriction. With this background in mind, we recently cloned PMCTs from *C. elegans, Drosophila,* mouse, rat, and human into the *Pichia pastoris* expression system and developed a biofermentation protocol for high-yield expression of the human and the *C. elegans* PMCTs. We have been able to successfully purify detergent-solubilized *C. elegans* PMCT via sequential chromatography on Ni-NTA, Talon, and Superdex, resulting in material that is > 90% pure. Mass spectrometry confirms that full-length *C. elegans* PMCT was the purified entity. Preliminary studies indicate that we have been able to successfully reconstitute detergent-solubilized *C. elegans* PMCT function in liposomal vesicles. Sulfhydryl reagents such as NEM and pCMB effectively inhibit the reconstituted transporter. These studies provide the foundation for a detailed structure/function analysis of this metabolically essential plasma membrane transport protein. Supported by NIH grant GM-054642 to R.S.K.

3540-Pos Board B587

What are the structural determinants of Cr transport regulation?

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Phosphocreatine (PCr) and Creatine Cr play key roles in energy metabolism by replenishing ATP levels via creatine kinases and ADP. The sole source of Cr to skeletal and cardiac myocytes is transport by the Cr transporter (CrT), a membrane protein belonging to the neurotransmitter transporter family (SLC6) that also includes the serotonin, dopamine and norepinephrine transporters. Previous work suggests that Cr transport is modulated by AMPK and PKC. For example, Cr transport in HL-1 cardiomyocytes expressing the human CrT is decreased when the cells are treated with β-PMA, or incubated in culture media enriched for Cr. Cr transport increases if these cells are incubated in culture media depleted of Cr or supplemented with AICAR, an AMPK activator. To identify phosphorylation sites that regulate CrT function, we created single and multiple site mutants lacking putative intracellular phosphorylation sites that scored highly (greater than 0.7) when the transporter protein sequence was evaluated by the NetPhos 2.0 algorithm (CBS, Technical University of Denmark). These sites tended to cluster at the beginning of the N-terminus and the end of the C-terminus of the protein. Ablation of these potential phosphorylation sites had no effect on transport capacity, or the ability to respond to β-PMA, AICAR or substrate availability. We also generated mutants with incremental deletions of the N-or C-termini of the CrT protein. Deletions that remove the entire N or C-terminus resulted in the expression of an inactive transporter protein, while smaller truncations yielded functional Cr transporters that still responded to PKC and AMPK activation and Cr availability like the phosphorylation mutants. Therefore, either low-probability phosphorylation site(s) modulate CrT activity and/or a yet to be identified signalling molecule or interacting protein(s) are responsible for modulation of Cr transport by AMPK, PKC and substrate availability.

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What Localization Of UCP4 Can Tell Us About Its Function?

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UCP4 is a member of the mitochondrial uncoupling protein subfamily und is suggested to be mainly involved regulation of reactive oxidative species and/or calcium concentration. Here we investigate for the first time the subcellular, cellular and tissue distribution of UCP4 at physiological conditions using antibodies against UCP4. Affinity purificated polyclonal anti-body against UCP4 was first designed and evaluated using recombinant protein UCP4-GFP. We present evidences that UCP4 is expressed in central nervous system, as previously shown at mRNA level. The described distribution of UCP4 mRNA in kidney was not confirmed an the protein level. Protein are expressed in both fetal and adult murine brain tissue. UCP4 dis-tribution pattern in neuronal cell bodies does not support the idea of thermogenesis in syn-apses. The hypothesis that proapoptotic stress may induce transient UCP4 expression as a component of an apoptotic pathway during neuronal differentiation is discussed.