within 30 s after EGTA treatment. We conclude that NCX activity in ASMC is much more susceptible to inactivation at high concentrations of Na (Na-dependent inactivation) than in transfected CHO cells.

2615-Pos

Scallop Muscle Na - Ca Exchanger can be Activated by Either AMPK and PK-A Through Phosphorylation of Ser621 in CBD2

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Site directed mutagenesis has shown that Ser^{621} in the CBD2 sub-domain of the Ca^{2+}-regulatory domain of scallop muscle Na^+ - Ca^{2+} exchanger (NCX-SCA) is a substrate for cAMP-PK (PK-A) in the native membrane - bound enzyme [1, 2], and under the same conditions Na^+-driven $^{45}Ca^{2+}$ uptake is stimulated [3]. Examination of the amino acid sequence of NCX-SCA (AY567834, GenBank) shows a consensus sequence for AMP-PK in CBD2 in the Ca^{2+}-regulatory domain, with Ser^{621} as the target residue. The consensus sequence for cAMP-PK in CBD2 (K^{618}RG**S**V) is embedded within that for AMP-PK (L^{616}LKRG**S**VEDL). Exposure of native scallop muscle membranes to constitutively active AMP-PK [4] activated Na^+ - Ca^{2+} exchange by approximately the same factor as with cAMP-PK. These results suggest that both intra- and extracellular signals may stimulate the activity of NCX-SCA through phosphorylation of the Ca^{2+}-regulatory domain in the large cytoplasmic loop.

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2616-Pos

Cardiac-Specific Overexpression of NCX1.1-Xip Mutant Causes Dilated Cardiomyopathy in Mice

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The Na^+/Ca^{2+} exchanger (NCX1.1) plays the primary role in Ca^{2+} extrusion from cardiac myocytes during diastole. There have been many reports showing that NCX1.1 expression levels are elevated in heart failure; however, the importance of NCX1.1 in the pathophysiology of cardiac disease is not well understood. To determine the *in vivo* cardiac function of NCX1.1, we generated transgenic mouse lines with cardiac-specific overexpression of wild-type or mutated NCX1.1, with Na^+-dependent inactivation eliminated. Here we show that NCX1.1 mutant transgenic mice develop dilated cardiomyopathy, whereas wild-type NCX1.1 transgenic mice produce hypertrophic cardiomyopathy. Six-month-old mutant transgenic mice showed contractile dysfunction, ventricular arrhythmias, interstitial fibrosis, and mitochondrial defects. Cardiac myocytes isolated from these mice showed reduced Ca^{2+} transients, suggesting greater Ca^{2+} extrusion via overexpressed mutant exchangers. Microarray analysis of their ventricles clearly indicated molecular changes generally associated with the heart disease phenotype. Thus, these mouse models will be useful for understanding the role of NCX1.1 in cardiac diseases.

2617-Pos

Ibogaine Stimulates Ionic Currents Mediated by Serotonin Transporter

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Ibogaine is a non-competitive inhibitor of serotonin transporter (SERT) that is thought to stabilize a conformation of SERT in which the substrate binding site is open to the cytoplasm. Cocaine is a competitive SERT inhibitor thought to stabilize a conformation with the substrate site open to the extracellular medium. As measured by whole-cell patch clamp, the substrate, serotonin (5-hydroxytryptamine, 5-HT), induced inward currents in HEK-293 cells stably expressing SERT. These currents were previously shown to be uncoupled from 5-HT flux. Cocaine, like other previously tested competitive inhibitors, blocked this current and also blocked a small leak current observed in the absence of 5-HT. Ibogaine, in contrast to competitive inhibitors, stimulated an inward current in the absence of 5-HT. This current was, like the 5-HT induced current, blocked by inhibitory concentrations of cocaine. Concentrations of ibogaine that largely inhibited transport stimulated smaller currents than did 5-HT, and those currents were slower to develop than the 5-HT currents. When ibogaine was added after 5-HT, the current decreased to the level observed with ibogaine alone. This decrease occurred with a time course similar to the time course of the appearance of the ibogaine current when ibogaine was added alone. These results suggest that SERT-mediated currents are associated with conformations of SERT that are different from the conformation stabilized by competitive inhibitors such as cocaine. Both ibogaine and 5-HT apparently alter SERT conformation and increase the proportion of transporter conformations that mediate uncoupled ion permeation.

2618-Pos

Overexpression, Purification and Functional Characterization of the Human Serotonin Transporter

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The human serotonin transporter (SERT) regulates the concentration of serotonin, a neurotransmitter, in the synaptic cleft through the reuptake of serotonin. SERT is a member of SLC6 (Solute Carrier) family with 12 transmembrane helices and two N-linked glycosylation sites. We synthesized the SERT gene from 90 oligo DNAs by PCR-based gene assembly. After transient transfection of the codon-optimized synthetic SERT gene to mammalian HEK293 cells and single-step immunoaffinity purification using N-terminal Flag-tag, full-length expression was confirmed by nano-ESI MS/MS analysis. Then, we constructed a mammalian stable cell line which inducibly expresses the human SERT gene. The glycosylation profile showed that the unglycosylated species expressed at an early stage after tetracycline induction eventually decreased and glycosylated species came to be dominant at a later stage (32hr or later). It turned out that the N-linked glycosaccharides at N208 and N217 were deglycosylated with PNGase F without denaturation, which implied that the N-linked glycosaccharides were well exposed. Blue Native PAGE showed that the purified SERT was dominantly in a tetrameric state. It is consistent with a previously published result of the dopamine transporter, other SLC6 family transporter. We further investigated the functionality of SERT both at a cellular level and in a purified form. HEK293 cells overexpressing the SERT on the plasma membrane uptake the neurotransmitter analogues in time dependent manner. Ligand binding assay with [3H] imipramine showed that the purified SERT was fully functional and the functional expression level was determined as mg level per litter culture volume. Those result shows that mammalian overexpression system along with the use of synthetic gene would be useful in elucidating structure and function relationship of the human transporters.

2619-Pos

Inhibition of SERCA1 by a Novel Antimalarial Compound

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Malaria remains one of the most important diseases in developing countries. *Plasmodium falciparum* (*Pf*), the etiological agent of malaria, is developing an increasing resistance to traditional drugs, like chloroquine (CQ). Consequently, the development of new antimalarial agents able to overcome CQ-resistance is an urgent task to be accomplished.

The antifungal agent clotrimazole (CLT) has been shown to have a moderate growth-inhibiting effect on different *Pf* strains (1), and it has been taken as a model for the rational design of innovative classes of antimalarial agents (2). We have previously shown that CLT is able to inhibit the SERCA1 (SR-CaATPase) (3). Here we present some results concerning the effect on SERCA1 of NF1058 (4), a novel compound with potent antimalarial properties that possesses key structural elements of both CLT and CQ. We used an approach that combines biochemical and electrical techniques. Our data show that NF1058 inhibits the steady-state hydrolytic activity of the pump with a medium affinity ($K_{0.5}$ = 41 ± 1 microM), but it does not interfere with Ca-binding to the enzyme. We therefore suggest that the reduction of steady-state hydrolytic activity of SERCA1 by NF1058 may be due to an interference with the Ca-release process. We are planning to explore the possibility that NF1058 can inhibit *Pf*ATP6, a SERCA-type ATPase expressed by the plasmodium.

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