found when myocytes were exposed to bafilomycin A1 (1 μM, presumed to act on intracellular acidic stores). Calcium transients measured using Fluo-5F were also reduced by trans-NED19 (1 μM) and these effects were inhibited by pre-exposure of the myocytes to bafilomycin A1. These observations are consistent with effects of NAADP on myocyte contraction under these conditions, that can be prevented either by interference with the function of acidic stores by bafilomycin A1 or by antagonism of the effects of NAADP by trans-NED19.

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Morphological Alterations in the Cerebellar Granule Cell Layer of Mice Lacking Calretinin

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Calcium binding proteins, such as calretinin, are abundantly expressed in distinctive patterns in the central nervous system, but their physiological functions remain poorly understood. Calretinin is expressed in cerebellar granule cells and calretinin deficient mice suffer from alterations in motor coordination. Using confocal microscopy, we demonstrate that calretinin deficient mice exhibit a significantly decreased density of granule cells at the level of the cerebellar cortex. Moreover, it has been shown that migration of granule cells is tighly associated with intracellular calcium fluctuations. Therefore, we hypothesize that the perturbation of the calcium dynamics in calretinin deficient mice may be the cause of the observed morphological alterations. To test this assumption, we are currently developping two strategies.First, using confocal microscopy and cerebellar microexplant cultures, we are studying calcium transients occuring during granule cell migration in wild type and calretinin knock-out mice. On the other hand, we are developping a dedicated computational model for [Ca2+]i transients that takes into account calcium fluxes through the plasma and ER membrane. This model will shed light on the possible mechanism responsible for the modulation by calretinin of calcium dynamics during granule cell migration.

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Role of Ca^{2+}/H^{+} Exchange and Disturbance of Oxidative Phosphorilation in Glutamate-Induced Delayed Calcium Deregulation in Cultured Neurons

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Prolonged application of toxic doses of glutamate (Glu) to neurons, resulting in their death, induces a biphasic Ca^{2+} response, of which the second phase termed delayed calcium deregulation (DCD) is not fully understood. Using fluorescence microscopy, we monitored cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), mitochondrial membrane potential (V_m) and pH (pH_m), cytosolic pH (pH_c) and NAD(P)H in cultured rat hippocampal and cerebellar granule neurons. Glu (0.1mM+Gly-Mg) caused a rapid drop in pHc and a smaller decrease in pH_m. Oligomycin (Oligo), applied in the presence of Glu prior to DCD, increased V_m and NAD(P)H but did not affect the pH gradient between mitochondria and cytosol. When cyanide was applied in the presence of Glu and Oligo, we observed a rapid increase in $[Ca^{2+}]_i$, drop in V_m, and simultaneous changes in pHm and pHc in opposite directions. Similar changes in $[Ca^{2+}]_i$, V_m, and pH were observed during DCD, except of NAD(P)H became insensitive to Oligo indicating to oxidative phosophorylation uncoupling. PLA2 inhibition increased the period of DCD onset indicating to free fatty acids as possible uncoupling agents. We suppose that DCD is due in part to Ca^{2+} release from mitochondria and can be formally classified as Ca^{2+}/H^{+} exchange. Such exchange acts as a positive feedback loop because H^{+} influx to mitochondria promotes dissociation of Ca^{2+}-phosphate complexes and leads to further Ca^{2+} release into cytosol. Oxidative phosophorylation uncoupling results in ATP deficiency for ion pumps making worse Ca^{2+} extrusion and acting as aggravating factor. Supported by RFBR.

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Requirement of Caspase-3 for Apoptotic Calcium Release

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Calcium is known to be an important second messenger in regulation of various physiological processes including execution of programmed cell death. Calcium release from endoplasmic reticulum (ER) triggers activation of multiple pro-apoptotic factors resulting in apoptosis progression and, ultimately, cell death. Apoptotic calcium release from ER stores is mediated by inositol 1,4,5-trisphosphate receptor (IP3R) channels. Different models have confirmed the importance of IP3R channels in modulation of apoptosis, however many details of IP3R-dependent calcium release still remain enigmatic. Previously, we have demonstrated that calcium release from IP3R channels in response to apoptotic stimuli occurs in two temporally distinct phases. A rapid oscillatory cytosolic calcium release during initiation of apoptotic signaling is followed by delayed and sustained increase in calcium levels associated with persistent opening of IP3R channels. Here we show that early elevations in cytosolic calcium do not require caspase-3 activity. Using MCF-7 cells deficient in caspase-3, we detected robust increase in calcium levels in response to staurosporine treatment indicating that calcium release during initiation of apoptosis occurs independently of caspase-3. To further determine contribution of caspase-3 in regulation of calcium release we generated a MCF-7 cell line stably expressing pro-caspase-3. Additionally, we used TAT-based transducing peptide to deliver active recombinant caspase-3 directly into living MCF-7 cells. Utilizing both methodologies, we found that caspase-3 has a marginal effect on the early events leading to cytosolic calcium elevations and irreversible commitment to apoptotic cell death. These results suggest that caspase-3 mediated truncation of IP3R channels is a consequence, not causative, of apoptotic signaling events.

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Validating a Model of Nitric Oxide-Ca^{2+} Crosstalk in Cardiac Myocytes

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Recently, we have developed a mathematical model of activation of constitutive nitric oxide synthase (NOS)-cGMP signaling pathway and its crosstalk with the excitation-contraction coupling in rat cardiac myocytes. This model describes a dynamic regulation of intracellular nitric oxide ($[NO]_i$) formation by changes in intracellular calcium $[Ca^{2+}]_i$ signals. In particular, a key prediction of the model is that the formation of NO is sensitive to the frequency and magnitude of $[Ca^{2+}]_i$ signaling. To validate these predictions, we tested experimentally correlations between increases in pacing frequency and the level of intracellular $[Ca^{2+}]_i$ and formation of $[NO]_i$ in neonatal rat cardiac myocytes which were loaded with membrane permeable fluorescent indicators DAF-FM and Fluo-4, respectively. As predicted by the model, increasing pacing frequency from 0.5 Hz to 2.0 Hz enhanced diastolic $[Ca^{2+}]_i$ and $[NO]_i$ formation. NO production was markedly blocked by eliminating the extracellular Ca^{2+} with 2 mM EGTA or by increasing Ca^{2+} buffering with BAPTA-AM or by treatment with non-specific NOS inhibitor, N^{G}-monomethyl-L-arginine (L-NMMA), confirming that pacing-frequency-dependent NO synthesis was Ca^{2+} and NOS dependent. Several of our model simulations compared the effects of cytosolic vs. dyadic Ca^{2+} signals in NOS signaling, predicting that cytosolic Ca^{2+} signals may be sufficient to initiate NOS activation. Using the computational model, we found that reduction of extracellular sodium $[Na^{+}]_0$ from 137 mM to 70 mM under condition of inhibited SR would induce a Ca^{2+}signal with magnitude similar to pacing-induced cytosolic Ca^{2+} signals. This occurs due to influx of Ca^{2+} through the Na^{+}/Ca^{2+} exchanger. Experimental testing of this new protocol confirmed a broad, slow Na^{+}-depletion-induced Ca^{2+} signal. The Na^{+} depletion protocol was sufficient to enhance NO synthesis similar to 0.5 Hz pacing. These results confirm the model prediction that cytosolic Ca^{2+} signals regulate NOS activity in cardiac myocytes.

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Cross-Talk Between Acidic Compartments and the Endoplasmic Reticulum Ca^{2+} Store Triggered by Ca^{2+} Release via Two-Pore Segment Channels

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Recent studies have revealed the importance of two-pore segment channels (TPCs) in mediating NAADP-evoked Ca^{2+} release from acidic organelles. This Ca^{2+} signal initiated from the acidic stores can recruit additional Ca^{2+} release from endoplasmic reticulum (ER) via Ca^{2+}-induced Ca^{2+} release (CICR). Both humans and mice express two TPCs, TPC1 and TPC2 whereas many other vertebrates express also TPC3. We have shown that each TPC subtype may be targeted to different acidic organelles, with TPC1 predominantly localized to a subpopulation of endosomes while TPC2 almost exclusively expressed in lysosomes. Consistent with these expression patterns and the fact that endosomes are smaller and less clustered than lysosomes, TPC1, when overexpressed in HEK293 cells, mediated Ca^{2+} release in response to 10 nM NAADP that was spatially restricted in nature and did not trigger global Ca^{2+} transient. However, application of 10 nM NAADP in cells overexpressing TPC2 evoked a biphasic Ca^{2+} response with a pacemaking phase followed by a large secondary and global Ca^{2+} transient. The responses to NAADP in both TPC1 and TPC2 expressing cells were abolished upon depletion of acidic Ca^{2+} stores with bafilomycin. By contrast, Ca^{2+} transients evoked in TPC2-, but not TPC1-, expressing cells were attenuated by prior depletion of ER Ca^{2+} stores with thapsigargin. Therefore, only TPC2 appears to couple to the ER by CICR. Interestingly, the expression of chicken and rabbit TPC3 in HEK293 cells yielded distinct subcellular localizations and functional data are consistent with differential organelle targeting. Our data suggest that cross-talk between acidic stores and ER is governed by the capacity of local Ca^{2+} signals from acidic Ca^{2+} stores to summate, and in a manner that may determine whether the threshold for CICR from ER is breached.