product only appears when the glass is dry. The results are consistent with water being an effective competitor for the ferric heme site in the presence of nitrate.

Platform BA: Calcium Signaling Pathways

2875-Plat

Isoform-specific Regulation Of The Ca-sensitive Transcription Factor NFAT In The Cardiovascular System

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NFAT transcription factors (Nuclear Factor of Activated T-cells) mediate Ca-sensitive gene transcription and are involved in cardiovascular remodelling. Nuclear localization of NFAT is dynamically regulated by intracellular Ca signals yielding to dephosphorylation and nuclear translocation of NFAT, and activity of intracellular kinases that induce nuclear export. The aim of this study was to analyze the regulation of NFAT in vascular endothelial cells and adult cardiomyocytes. Subcellular distribution of NFAT-GFP fusion proteins (isoforms NFATc1 and NFATc3) was analyzed with confocal microscopy and intracellular Ca ($[Ca]_i$) was measured simultaneously using rhod-2. In calf pulmonary arterial endothelial (CPAE) cells, application of ATP (5 μM) induced nuclear localization of both isoforms (quantified as an increase in $NFAT_{NUC}/NFAT_{CYT}$ ratio). Subsequent attenuation of $[Ca]_i$ to facilitate nuclear export resulted in substantial export of NFATc3 to the cytoplasm, which was sensitive to Leptomycin B (40 nM). Previously translocated NFATc1 was only partially affected by nuclear export, indicating isoform-specific regulation of NFAT in endothelial cells.

In cardiac myocytes regulation of NFAT was isoform-, and tissue-specific: NFATc1 displayed nuclear localization in quiescent myocytes, which was dependent on $[Ca]_i$ and further enhanced by blocking nuclear export (Leptomycin B) or by inhibition of intracellular kinases (20 mM LiCl, 1 μM alsterpaullone or 1 μM SP600125). In contrast, NFATc3 was distributed in the cytoplasm of quiescent cells. Incubation with Leptomycin B, but not inhibition of nuclear kinases induced nuclear localization of NFATc3 in ventricular cells. Incubation with the G_q protein-coupled receptor agonists endothelin-1 (100 nM) and Ang II (2 μM) induced nuclear localization of NFATc3 only in atrial, but not ventricular cells. We conclude that (i) regulation of nuclear NFAT in the cardiovascular system is isoform- and tissue specific and (ii) dynamically regulated by activity of nuclear export pathways.

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Alterations In Binding Properties Of Myocardial Nuclear Membrane Receptors Induce Nuclear Calcium Overload In Rat Ischemia-reperfusion

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Cell nuclei possess an independent calcium regulatory system consisting of nuclear Ca^{2+}-ATPases (NCA), IP_3 receptors (IP_3R), IP_4 receptors (IP_4R), ryanodine receptors(RyR), and nuclear pore complexes(NPC). We studied the changes in $Ca^{2+}{}_n$ and its regulatory system in rat model of myocardial ischemia-reperfusion injury(IRI) induced by 30 min coronary occlusion followed by 180 min reperfusion. The $Ca^{2+}{}_n$ content in isolated nuclei was measured with atomic absorption spectrophotometer. NPC permeability was assessed through the amount of calmodulin conjugated Alexa FluoTM 488 as fluorescent probes. NCA activity was evaluated by phosphate group released from ATP in enzymatic reaction. The maximum binding capacity(B_{max}) and dissociation constant(K_d) of IP_3R, IP_4R and RyR were determined by radioligand binding of $[^3H]IP_4$, $[^3H]IP_4$ and $[^3H]$-ryanodine to isolated cardiomyocytic nuclei. All results are shown in the table. Our findings suggest that in vivo rat myocardial IRI is characterized by $Ca^{2+}{}_n$ overload, upregulations of nuclear IP_3R and IP_4R, downregulations of NCA activity and RyR, and an increase in permeability of NPC. The upregulation of nuclear IP_3R and IP_4R may be responsible for the nuclear calcium overload.

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ATP-evoked Ca2 ± waves Stimulate Gene Expression In Human Airway Fibroblasts

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We sought to investigate the effects of a variety of autacoids on Ca^{2+}-handling in human airway fibroblasts. Primary cultured fibroblasts were loaded with the Ca^{2+}-indicator dye fluo-4 and studied using confocal fluorimetric microscopy. ATP (10^{-5} M) evoked recurring Ca^{2+}-waves . This fluorimetric change was greater and longer lasting within the nucleus of the cell than in the non-nuclear portion of the cytosol, and was only sometimes accompanied by a contraction. These responses were completely occluded by cyclopiazonic acid (10^{-5} M; depletes the internal Ca^{2+}-store) or the phospholipase C inhibitor U73122 (10^{-6} M). Pretreatment of the cells with ryanodine (10^{-5} M), on the other hand, had no effect on the ATP-evoked responses. With respect to the receptor through which this response was exerted, we found it to be mimicked by UTP or ADP but not by adenosine or α,β-methylene-ATP, and to be blocked by the purinergic receptor blocker PPADS; interestingly, PPADS itself appeared to sometimes evoke a rise in $[Ca^{2+}]_i$ on its own. ATP also evoked a membrane conductance change with characteristics of a non-selective cation current, markedly enhanced synthesis of the cytokine TGFβ and the matrix proteins fibronectin and collagen I; these changes in protein synthesis were blocked by PPADs and were partially reduced by ryanodine. We conclude that, in human pulmonary fibroblasts, ATP acts upon P2Y receptors to liberate internal Ca^{2+} through ryanodine-insensitive channels, leading to a Ca^{2+}-wave which courses throughout the cell and triggers protein synthesis.

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Store-operated Ca^{2+} Entry Is Suppressed During Mitosis Due To Phosphorylation Of The Endoplasmic Reticulum Ca^{2+} Sensor Stim1

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When ER Ca^{2+} stores are depleted due to physiological Ca^{2+} release or pharmacological perturbation, Ca^{2+} influx via plasma membrane Ca^{2+} channels is activated by a process known as store-operated Ca^{2+} entry (SOCE). The current associated with SOCE is Ca^{2+} release-activated Ca^{2+} current (I_{crac}). SOCE involves Orai Ca^{2+} influx channels and STIM ER Ca^{2+} sensors. When ER Ca^{2+} stores are full, STIM1 is localized throughout the ER membrane; however, ER Ca^{2+} store depletion induces rearrangement of STIM1 into punctate structures near the plasma membrane where it activates Orai channels. Interestingly, mitosis is the only known physiological situation in which Ca^{2+} store depletion is dissociated from SOCE or I_{crac} activation. Identification of the molecular components of the SOCE signaling pathway has facilitated analysis of the mechanism underlying mitotic SOCE suppression. We found that in mitotic HeLa cells, an enhanced yellow fluorescent protein-tagged STIM1 (eYFP-STIM1) did not rearrange into puncta in response to Ca^{2+} store depletion and accordingly, SOCE was not activated. We hypothesized that mitosis-specific phosphorylation of STIM1 may underlie the block of STIM1 rearrangement and SOCE suppression. To this end, the phospho-specific MPM-2 antibody recognized eYFP-STIM1 immunoprecipitated from mitotic but not interphase cells. MPM-2 recognizes phosphorylated serine or threonine followed by proline, and human STIM1 contains 10 instances of S/T-P, all located in the cytoplasmic, C-terminus. STIM1 truncation mutants indicate that at least 2 sites within the C-terminus account for the mitosis-specific phosphorylation. Individual phosphorylation site mutants are being created to identify specific phosphorylated residues and to determine the functional consequences of phosphorylation during mitosis. Suppression of SOCE during mitosis may be an important signaling event, because mitotic processes such as chromosome separation and cytokinesis are exquisitely sensitive to small changes in cytoplasmic Ca^{2+}.

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Heteromeric channel assembly of Orai1 and Orai3 exhibits altered Ca2+ selectivity

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Coexpression of STIM1, targeted to the endoplasmic reticulum and each of the three Orai (also termed CRACM) channels located in the plasma-membrane leads to store-operated, highly Ca2+ selective currents. While Orai1 has been reported to form the native Ca2+ release activated Ca2+ (CRAC) channels in human T-cells, the molecular architecture of less Ca2+ selective store-operated currents remains unknown. Here we show employing confocal fluorescence resonance energy transfer (FRET) that all three Orai proteins are able to form homo- and hetero oligomers. Overexpressed homomeric Orai1 or Orai3 together with STIM1, resulted in store-operated inward rectifying, highly Ca2+ selective currents, as resolved by whole-cell patch-clamp recordings. Coexpression of Orai1 together with Orai3 and STIM1 yielded similar store-depletion activated Ca2+ currents, yet with a leftward shifted reversal potential, pointing to less selective currents. In line, a tandem construct where Orai1was linked to Orai3 exhibited a similarly reduced Ca2+ selectivity that allowed for robust Cs+ permeation. Moreover, Orai3 pore mutants coexpressed with wild-type Orai3 affected Ca2+ and Cs+ selectivity/permeability. These