Platform: Calcium Signaling Proteins

2151-Plat

Structural Basis for the Mg^{2+}-Dependent Modulation of Calmodulin Activity

F. Timur Senguen, Zenon Grabarek.
Boston Biomedical Research Institute, Watertown, MA, USA.

The physiological function of calmodulin (CaM) depends on its ability to respond specifically to micromolar Ca^{2+} signals in the presence of ~1000-fold excess of the chemically similar Mg^{2+}. The intracellular concentration of Mg^{2+} is tightly controlled in a narrow range of 0.5-1.0 mM, which is sufficient to fully or partially saturate the Ca^{2+}-binding sites of many EF-hand proteins, including CaM, at the resting Ca^{2+} levels. To explain the mechanism of metal ion binding specificity we have compared the effects of Mg^{2+} (ionic radius r=0.72 Å), Mn^{2+} (r=0.67 Å) and Ca^{2+} (r=1.06 Å) on the structure and stability of the N-terminal domain of calmodulin (N-CaM). The far UV-CD melting profiles show that all three metal ions stabilize the structure of N-CaM, as indicated by the concentration dependent increase in unfolding temperature from 52 °C (apo) to >100 °C, 95 °C and 75 °C in the presence of Ca^{2+}, Mn^{2+} and Mg^{2+}, respectively. We have determined the X-ray structures of N-CaM complexed with the three metal ions. Only the Ca^{2+}-N-CaM complex features the open-domain active conformation, whereas Mg^{2+}-N-CaM and Mn^{2+}-N-CaM have a closed-domain, apo-like conformation. The relative positions of all bound metal ions with respect to the EF-hand-β-scaffold are similar, however the bidentate ligand, the Glu sidechain in the 12th position of the loop, interacts with Ca^{2+} only and does not bind directly to either Mn^{2+} or Mg^{2+}. These results strongly support our proposal (Grabarek, BBA, 1813, 913, 2011) that the stereochemical constraints imposed by the two-EF-hand domain structure preclude Mg^{2+} from engaging the EF-hand ligands in the same way as Ca^{2+}. Mg^{2+} stabilizes the apo-like conformation of an EF-hand, thus contributing to the switching off CaM activity at the resting Ca^{2+} concentrations.

2152-Plat

Lobe-Specific Functions of Calcium.Calmodulin in Alpha-Calcium. Calmodulin-Dependent Protein Kinase II Activation

Katalin Torok[1], Abdirahman M. Jama[1], Jonathan P. Gabriel[1], Stephen Martin[2].
[1]SGUL, London, United Kingdom, [2]NIMR, London, United Kingdom.

N-methyl-D-aspartic acid receptor-dependent long-term potentiation (LTP), a model of memory formation requires calcium.calmodulin-dependent protein kinase II (αCaMKII) activity and Thr286-autophosphorylation via both global and local calcium signalling but the mechanisms of signal transduction are not understood. We tested the hypothesis that the calcium-binding activator-protein calmodulin (CaM) is the primary decoder of calmodulin signals thereby determining the output, e.g. LTP. Thus we investigated the function of CaM mutants, deficient in calcium binding at sites 1, 2 of the N terminal lobe or sites 3, 4 of the C terminal CaM lobe, in the activation of αCaMKII. Occupancy of CaM calcium binding sites 1, 3 and 4 is necessary and sufficient for full activation. Moreover, the N and C terminal CaM lobes have distinct functions: calcium binding to N lobe calcium binding site 1 increases the turnover rate of the enzyme 5-fold, while the C lobe plays a dual role: it is required for full activity, but in addition, via calcium binding site 3, it stabilizes ATP binding to αCaMKII 4-fold. Thr286-autophosphorylation too is dependent on calcium binding sites on both the N and C lobes of CaM. As the CaM C lobe sites are populated by low amplitude/low frequency (global) calcium signals, but occupancy of N lobe site 1 and thus activation of αCaMKII requires high amplitude/high frequency (local) calcium signals, lobe-specific sensing of calcium signalling patterns by CaM is proposed to explain the requirement for both global and local calcium signalling in the induction of LTP via αCaMKII.

2153-Plat

Microtubule Dependent Mechano-Transduction Drives Oxidative Stress and Calcium Dysregulation in Dystrophic Skeletal Muscle

Ramzi J. Khairallah[1], Francesca Sbrana[2], Guoli Shi[1], Mariateresa Tedesco[3], Benjamin L. Prosser[1], Roberto Raiteri[3], W. Jonathan Lederer[1], Christopher W. Ward[1].
[1]University of Maryland, Baltimore, MD, USA, [2]Institute of Biophysics, Genova, Italy, [3]University of Genova, Genova, Italy.

Duchenne muscular dystrophy (DMD) is a common X-linked progressive muscle wasting disease characterized by an increased susceptibility to stretch-induced damage. While an abnormal regulation of reactive oxygen species (ROS) and calcium (Ca^{2+}) signaling cascades are established contributors, little mechanistic insight has been revealed for how mechanical stress induces the dysregulation of these signaling pathways. With novel tools to mechanically manipulate enzymatically isolated skeletal muscle fibers, we show for the first time that a small physiologic stretch of a single *mdx* myofiber produces an almost instantaneous increase in ROS. The inhibition of NADPH oxidase 2 (NO**X**2) abrogated the stretch-induced **ROS** consistent with the recently reported **X-ROS** signaling in the heart (Prosser et al *Science*, 2011); however X-ROS was not detectable in wild-type fibers. Our group and others have demonstrated that the microtubule cytoskeleton is an important mechano-transduction element in muscle. Our investigation shows increased microtubule density in *mdx* muscle that displays X-ROS signaling. In this regard, microtubule depolymerization with colchicine was sufficient to inhibit X-ROS signaling in *mdx* myocytes implicating microtubules as critical proximate mechano-transducers in this pathway. Accordingly, proteins necessary for X-ROS (tubulin subunits and the NOX2 subunits $gp91^{phox}$ and rac1) are increased in dystrophic muscle. An important consequence of X-ROS signaling in *mdx* is increased sarcolemmal Ca^{2+} influx which is abrogated by the stretch-activated channel blocker GsMTx4 or microtubule destabilization. Lastly, we find increased surface membrane stiffness in *mdx* (measured by atomic force microscopy) suggesting that the abundance of microtubules contributes to important functional changes in skeletal muscle properties including stiffness which impacts X-ROS signaling. Our discoveries suggest that X-ROS may underscore the pathogenic stretch-dependent signaling in DMD and thus may provide novel therapeutic targets. Funding: RJK- T32AR007592, CWW- RC2 NR011968, WJL- R01 HL106059, R01 HL36974

2154-Plat

NAADP-Induced Calcium Release in Pulmonary Arterial and Aortic Smooth Muscle Cells is Mediated by Cross-Activation of NAADP-Receptor and Ryanodine Receptor-Gated Calcium Stores

Yong-Liang Jiang, Amanda H.Y. Lin, Suengwon Lee, Yang Xia,
James S.K. Sham.
Johns Hopkins University, Baltimore, MD, USA.

Ca^{2+} signaling in vascular smooth muscle cells involves dynamic interactions between multiple Ca^{2+} influx and release pathways. Ca^{2+} release pathways include the IP_3 receptor-, ryanodine receptor-, and nicotinic acid adenine dinucleotide phosphate (NAADP) receptor-gated Ca^{2+} stores. Recent studies demonstrated that the two-pore channels (TPC1 and TPC2) are NAADP receptors located in the endo-/lysosomes. Here we quantified the expression of TPC channels and characterized the NAADP-mediated Ca^{2+} signals in vascular tissues. Western blot analysis and real-time RT-PCR detected TPC1 and TPC2 protein and mRNA respectively in aorta, pulmonary, mesenteric, renal, femoral, tail, and cerebral artery of rat, with TPC1 mRNA level five to ten-fold higher than that of TPC2. Application of the membrane permeable NAADP-AM to pulmonary arterial smooth muscle cells (PASMCs) elicited concentration-dependent increase in global $[Ca^{2+}]_i$. The Ca^{2+} response was independent of extracellular Ca^{2+} influx, and was unaffected by the IP_3 receptor blocker xestospongin C. In contrast, the NAADP-induced Ca^{2+} response was partially inhibited by ryanodine or thapsigargin, and was abolished by the NAADP antagonist NED-19 or the acidic vacuolar H^+-ATPase bafilomycin A. Moreover, NAADP caused dramatic increase in local Ca^{2+} release events, which was inhibited by ryanodine or NED-19. The temporal and spatial properties of the NAADP-induced local release events were comparable to the spontaneous Ca^{2+} sparks. Similar observations were also made in aortic smooth muscle cells. Our results, hence, show that the NAADP channels, TPC1 and TPC2, are functionally expressed in vascular smooth muscle; and Ca^{2+} signals from the NAADP channel-gated lysosomal stores cross-activate ryanodine receptors to amplify Ca^{2+} release in pulmonary arterial and aortic smooth muscle cells.

2155-Plat

Increased Calcium Influx and Decreased Buffering Capacity of Intracellular Stores Underlie Neuropathology Induced by Over-Expression of α-Synuclein

Lidia Reznichenko[1], Qun Cheng[1], Kristal Nizar[1], Sergey L. Gratiy[1], Payam A. Saisan[1], Edward M. Rockenstein[1], Tanya González[1], Cristina Patrick[1], Brian Spencer[1], Paula Desplats[1], Anders M. Dale[1], Anna Devor[1,2], Eliezer Masliah[1].
[1]UCSD, La Jolla, CA, USA, [2]Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA.

Abnormal accumulation of α-synuclein is centrally involved in the pathogenesis of many disorders with Parkinsonism and dementia. Although the mechanism through which α-synuclein triggers neuronal vulnerability is not entirely clear, *in vitro* studies suggest that neuronal accumulation of α-synuclein might lead to increase in intracellular calcium levels. In this study, we investigated *in vivo* calcium dynamics in an α-synuclein transgenic mouse model of Parkinson's Disease and dementia with Lewy bodies using two photon