Meeting Report

Principles of Calcium Signaling
Salisbury Cove, Maine, June 28–30, 2007

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ABSTRACT

A three-day International Symposium entitled “Principles of Calcium Signaling” organized by James N. Weiss, Yale E. Goldman, Stéphane Hatem, Lars Cleemann and Nikolai M. Soldatov in honor of the research contributions of Professor Martin Morad was held at the Mount Desert Island Biological Laboratory, Salisbury Cove, Maine. Support for this meeting was provided in part by GlaxoSmithKline, Leica Microsystems, Nikon Corp., St. Jude Medical, Inc., UCLA Cardiac Arrhythmia Center, Dr. Donald S. Orkand, Bob Hills Family and OML, and Mount Desert Island Biological Laboratory. The symposium featured sessions on Cardiac physiology, Ion channels and Calcium signaling.

Calcium ions are important signals that stimulate many processes in live cells including gene expression, excitation-contraction coupling, exocytosis, synaptic plasticity and cell survival. Calcium signaling is initiated by activation of calcium channels that couple membrane depolarization to inward calcium current and a transient increase in intracellular calcium concentration. A large number of proteins are implicated in calcium signal transduction, and elucidation of their coupling to other signaling cascades and microdomains in cell-specific environment has become one of the major directions in biomedical physiology. This symposium focused on three main areas of biomedical investigation into the molecular physiology of calcium signaling. Beginning with the structure-functional links and regulation of calcium channels, the Meeting highlighted new aspects and new players in calcium signaling cross-talk, and demonstrated new links and principles in cardiovascular and neurosecretory function.

The Symposium culminated with the keynote address, The Richard Orkand Memorial Lecture by Erwin Neher (Max Planck Institute for Biophysical Chemistry, Goettingen, Germany) who spoke on a biophysical dissection of glutamate release at the Calyx of Held synapse. Erwin Neher’s keynote talk addressed two important themes of the Symposium: that calcium may have multiple roles in a “simple” physiological event, and that morphological diversity may greatly complicate investigation of such events. At least three distinct roles of Ca²⁺ can be dissected in synaptic glutamate release. (a) Triggering of the release for which at least five Ca²⁺ ions per vesicle are required. (b) Synchronous release, which is facilitated by a mild “conditioning” depolarization. Interestingly, Ca²⁺-sensitivity of glutamate release is not changed by paired pulse facilitation. (c) Vesicle recruitment, which is linearly enhanced by calcium. There are approximately 3,000 glutamate-containing vesicles per synapse, which constitute “fast” and “slow” subsets of vesicles, and also recover with different speeds. Although these subsets of vesicles may have intrinsic differences in Ca²⁺-sensitivity, it is also possible that the differences in the time course of the release occur because not all vesicles dock near Ca²⁺ channels.

In the session on Heart, Clara Franzini-Armstrong (University of Pennsylvania) described the architecture of calcium release units in cardiac muscle revealed by comparative observations with skeletal muscle and by the effect of removing key components. Developing this theme, Franz Hofmann (Technical University of Munich) described analysis of L-type calcium channels by site-specific mutations in mice. Mutation of putative PKA phosphorylation sites predicted from in vitro studies and believed to be associated with PKA-dependent augmentation of the calcium current in cardiomyocytes did not eliminate the PKA effect. Thus its structural determinant remains elusive.

KEY WORDS

calcium signaling, voltage-gated ion channels, intracellular calcium release, heart, atrial cells, ventricular myocytes, arrhythmia, stem cells, synaptic release, transgenic mice

ACKNOWLEDGEMENTS

Support for this meeting was provided in part by GlaxoSmithKline, Leica Microsystems, Nikon Corp., St. Jude Medical, Inc., UCLA Cardiac Arrhythmia Center, Dr. Donald S. Orkand, Bob Hills Family and OML, and Mount Desert Island Biological Laboratory. Social Events were kindly hosted by Julia Merck and Hanse Utsche, Mr. and Mrs. Dick Wolfe, Martha Stewart and Prof. Martin Morad. Help of Dr. John N. Forrest, Jr. and Michael McKernan of MDI Biological Laboratory in organization of the Symposium is greatly appreciated. We are grateful to Tan Duong, who served as a webmaster of the Symposium web site: http://cvrl.mednet.ucla.edu/mm70/
(Vanderbilt University) showed that knock-out of the calsequestrin gene, thought to be critical for calcium storage in the sarcoplasmic reticulum, was not lethal and caused only subtle changes in the structure of calcium release units. However, the knock out resulted in striking acceleration of the functional restitution of intracellular Ca$^{2+}$ release sites after Ca$^{2+}$ discharge in ventricular myocytes, which predisposed the heart to ventricular arrhythmias.

Properties of diseased cardiomyocytes have become an intensive focus of cardiac physiologists. It is becoming evident that these cells undergo significant changes during disease. These include electrophysiological and morphological remodeling in cardiomyopathy and cardiac arrhythmias, dynamic changes in responses of Ca$^{2+}$-induced physiological and morphological remodeling in cardiomyopathy and undergo significant changes during disease. These include electrocardiographic detection of these instabilities, using new signaling processing methods to uncover repolarization alternans, hold promise as a means to identify patients at particularly high risk of sudden cardiac death.

Can cardiac arrhythmia be seen in a cell culture dish? Leslie Tung (Johns Hopkins University) plated rat neonatal ventricular myocytes on which he had stamped precise patterns of proteins known to support growth of cardiac tissue. This stimulated growth patterns of the derived cardiac cells could be implanted into an infarction zone and interconnections resembling normal cardiac tissue architecture. Illustrating the potential of this model, he showed how pacing influenced electrical activity that resembled ventricular tachycardia.

Significant attention has been devoted to the potential of embryonic cardiac cells to repair damaged hearts. In the presentation “Transgenic Embryonic Stem Cells in Cardiovascular Research and Regenerative Medicine” Juergen Hescheler (University of Cologne) showed data on differentiation, lineage selection and freeze storage of early cardiomyocytes from embryonic mouse and human stem cells. The derived cardiac cells could be implanted into an infarction zone of a mouse model where they became well integrated and coupled to the host cells. The role of electrophysiological properties as well as of Ca signaling in integration was discussed. Embryonic stem cell-derived early cardiomyocytes thus represent a promising tool for cell therapy of failing hearts in the field of regenerative medicine.

Philipp Sasse (University of Bonn) showed that pace-making in cardiomyocytes from early embryonic hearts (stages E8.5–E10.5) is generated by cytosolic Ca$^{2+}$ oscillations, which evoke sub-threshold membrane depolarizations through activation of the Na$^{+}$-Ca$^{2+}$ exchanger (NCX). These membrane depolarizations can reach threshold to initiate action potentials. During an action potential, the CICR mechanism has yet to mature, and ~40% of the Ca$^{2+}$ transient is released from the SR. In addition to trans-sacromemmal Ca$^{2+}$ entry through Ca$^{2+}$ channels, the reverse mode of the highly expressed NCX promotes Ca$^{2+}$ entry.

Over the last 10 years it has been recognized that pulmonary vein myocardial sleeves play an important role in atrial fibrillation, leading to pulmonary vein isolation as a therapy. Allan Greenspan (Albert Einstein Medical Center) described arrhythmogenic changes in calcium cycling associated with pulmonary veins that cause these regions to trigger atrial fibrillation.

In the session on Ca signaling, presentations focused on the dissection of molecular components of Ca$^{2+}$ signaling cross-talk, and the defining their functional interactions. Alan Grinnell (UCLA) showed that large conductance Ca$^{2+}$-activated potassium (BK) channels are closely colocalized with Ca$^{2+}$ channels. Thus, BK channels contribute to the termination of action potentials and can be used to accurately measure intracellular Ca$^{2+}$ concentration during Ca$^{2+}$ transients.

Lars Cleemann (Georgetown University) compared mammalian and amphibian NCX1 isoforms with those recently cloned from shark to address the question of the mechanism of cAMP-regulation in non-mammalian NCX. It was proposed that the evolution and differences in cAMP-dependent regulation of cardiac NCXs may depend on the presence of the PKA-site and specific amino acid sequences, such as the variable regions coding for critical binding domains (for ATP, PKA and caveolin) that may also confer flexibility to the NCX molecule, causing a change in access to binding sites.

Yale E. Goldman (University of Pennsylvania) described 3D orientation and stepping behavior of myosins V and VI as they move along actin. His study was based on single molecule fluorescence polarization localization of rhodamine-labeled calmodulin of the myosin light chain domain. Back-and-forth sideways motions of myosin VI explain its variable step size and chaotic path. In contrast, in the case of myosin V there was less abrupt sideways tilting of calmodulin, which explains the relatively straight path of myosin V when carrying large vesicular cargos in the cell.

Characterizing Ca$^{2+}$ signaling events in a noncardiac cell type, James S.K. Sham (Johns Hopkins University) spoke about specifics of global and local Ca signaling in cytoplasmic and nucleoplasmic compartments of pulmonary arterial smooth muscle cells. Based on different response to endothelin of Ca$^{2+}$ transients in these compartments, it was concluded that differential regulation of Ca$^{2+}$ may be essential for the independent regulation of contraction and gene expression in pulmonary arterial smooth muscle cells.

Satomi Adachi-Akahane (Toho University, Tokyo) presented data on regulatory mechanisms of atrial L-type calcium channels. She reported that the inactivation kinetics of the channel strongly depended on the degree of coupling with Ca$^{2+}$ release sites. Sun-Hee Woo (Chungnam University) presented a characterization of microdomain Ca$^{2+}$ signaling and intracellular Ca$^{2+}$ channels in the adult mouse atrial cell line HL-1. It was found that HL-1 cells generate autorhythmic Ca$^{2+}$ spikes (at ~80 Hz) preceded by Ca$^{2+}$ sparks whose occurrences and sizes are higher at the cell periphery and peri-nuclear regions, suggesting a functional role of ryanodine receptors. It appears that IP$_3$ receptors of type 1 and 2 are localized around the nuclei and throughout the cytoplasm, respectively, and that their activation by a direct application of IP$_3$ or by external ATP/phospholipase C activator induce increases in the cytosolic [Ca$^{2+}$], suggesting functional expression of IP$_3$ receptors in these myocytes.

To maintain normal automaticity, sinoatrial nodal cells have a unique ability to generate spontaneous local Ca$^{2+}$ release from sarcoplasmic reticulum (SR) under physiological conditions. To characterize the mechanisms that underlie local Ca$^{2+}$ release, Syeveda G. Sirenko (NIH, NIH) compared SR Ca$^{2+}$ loading and release in saponin-permeabilized single rabbit sinoatrial nodal cells and ventricular myocytes over a wide range of free [Ca$^{2+}$] in a physiological buffer containing 0.5 μM EGTA. It was found that the SR Ca$^{2+}$ content in ventricular myocytes could be elevated to a significantly higher extent than in SANC. A greater local Ca$^{2+}$ release signal mass
in sinoatrial cells than in ventricular myocytes at an equal or lesser SR Ca²⁺ load indicates that spontaneous Ca²⁺ release mechanisms differ in these cells. This may be explained, in part, by the high basal cAMP/PKA phosphorylation of Ca²⁺ cycling proteins in sinoatrial nodal cells.

A growing body of evidence suggests importance of hormones and other bioactive compounds for the regulation of cardiac ion channels. Yoshihisa Kurachi (Osaka University) reviewed the modulatory effect of regulators of G-protein signaling (RGS) on the G protein-activation of inwardly-rectifying muscarinic K⁺ channels in the heart. RGS action is controlled by the interaction of PIP₃ and Ca²⁺/CaM complex with the RGS protein. The GK protein activity thus exhibits an apparent voltage-dependent behavior called “relaxation”. Based on this understanding, the physiological G protein cycle controlling muscarinic K⁺ channel activity could be modeled. Stéphane Hatem (Univ. Pierre et Marie Curie), Niels Danneskiold-Samsøe*, Andreas Top Knudsen*, James N. Weiss (UCLA), James S.K. Sham (Johns Hopkins Univ.), Craig Julius Dietrich, Lars Cleemann (both - Georgetown Univ.), Philipp Sasse (Univ. of Bonn), Shahrazad Movafagh (Georgetown Univ.) and Thomas Lynggaard Sørensen* (* all from Technical University of Denmark & Georgetown Univ.)

Teruyuki Yanagisawa (Tohoku University) gave a presentation on labor and myometrial contraction in relation with oxytocin receptors in mice. Oxytocin receptor expression undergoes dramatic and cell-specific up- and downregulation under the influence of steroids hormones, autacoids and pregnancy. Results suggest that the uterine contractions are mainly controlled by the modification of contractile signal machinery rather than simply the oxytocin receptors quantity.

Yaroslav Shuba (Bogomoletz Institute, Kiev) spoke about links between ion channels and prostate cancer. An overexpression of TRPM8 observed in prostate cancer requires functional androgen receptors. Results show that the DAG-dependent activation of TRPC6 may contribute to augmentation of the calcium current, while TRPC1 may contribute to the activation of IP₃-dependent Ca²⁺ release from ER.

Nutritional benefits of resveratrol, a phytoalexin component of red grape and red wine, are well known. Ming-Jai Su (Taiwan University) showed that its analog astringinin is more potent than resveratrol in cardiac APD prolongation, which is correlated with its potent antiarrhythmic effect against ischemia- and ischemia-reperfusion induced arrhythmia in rats. Comparison of the electrophysiological
effects of resveratrol and astringinin on rat ventricular cells showed that resveratrol-induced APD prolongation was mediated by I_to inhibition at the concentration of 100 μM. However, astringinin, at 10 μM, prolonged APD by slowing the inactivation rate of the sodium current, which can be reversed by lidocaine, in spite of a decrease in the amplitude of the current. Thus, enhanced potency of astringinin may rely on a different mechanism as compared to resveratrol and other stilbens.

Nikolai M. Soldatov (NIA, NIH) discussed the structure-functional remodeling of Ca^{2+} channels in human arterial smooth muscle cells and ventricular myocytes. In atherosclerosis, multiple exon-21 isoforms of the Ca_v1.1C subunit in normal VSMC are replaced by a single exon-22 isoform that is characteristic for proliferating cells and shows significantly different electrophysiological properties. Specific changes in splice variation of the Ca_β and Ca_α1C subunit primary transcripts in ventricular cells on dilated cardiomyopathy reveal an increase in the PKC-insensitive subset of calcium channels, decrease in I_Ca amplitude and slower kinetics of inactivation. Thus, morphological and functional changes in cardiovascular diseases may be directly associated with switches in molecular repertoires of ion channels, and this new face of channelopathy should be carefully examined at the cellular level in live cardiac tissues.

Many of the studies reported at the Symposium were intrinsically related to the long-standing collaborations of former students and colleagues with Martin Morad, Professor of Pharmacology of Georgetown University, who celebrated a memorable birthday this June. Over several decades, he has been a great mentor, colleague and friend to the participants. Sharing appreciation of Professor Morad’s amazing career in science, Clay Armstrong (University of Pennsylvania) put together an entertaining and elegant slide show.