Impromptu

Colloquia in Cellular Signaling

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Institute of Pharmacology, Waehringerstrasse 13a, 1090 Vienna, "Leseraum".
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Subcellular RyR2 Distribution and Function in Ventricular Myocytes and Hippocampal Neurons

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Abstract

Cardiac ryanodine receptors (RyR2) are calcium (Ca2+) release channels most abundantly expressed in the heart and brain. They are clustering in the sarco/endoplasmic reticulum (ER/SR) membrane to form elementary units of Ca2+ release. The distribution of these units plays a critical role in determining the spatio-temporal profile and stability of ER/SR Ca2+ release. Thus, it is believed to be essential for cellular processes, such as excitation-contraction coupling and learning and memory. The distribution of RyR2s has been extensively studied in cells/tissues using anti-RyR2 antibody immunostaining. However, sample preparation required for immunostaining may change cellular structures, besides rendering the cells/tissues non-functional. Hence, the functional relevance of distribution of RyR2 clusters in live cells/tissue remains uncertain. We have recently generated a knock-in mouse model that expresses a green fluorescence protein (GFP)-tagged RyR2. These mice allow us to monitor cellular/subcellular distribution of RyR2 in live cells/tissues by virtue of GFP fluorescence. To improve the detection of GFP-RyR2, we have also developed novel GFP-specific probes based on anti-GFP single domain antibodies (nanobodies). Fluorescence imaging was employed to study Ca2+ release and the distribution of GFP-RyR2 in the interior and periphery of live ventricular myocytes and in intact hearts isolated from GFP-RyR2 expressing mice. We found tightly-ordered arrays of stationary GFP-RyR2 clusters in the interior exclusively along the z-line. In contrast, irregular and dynamic distribution of GFP-RyR2 clusters was observed in the periphery. Imaging of intact GFP-RyR2 brain sections revealed a widespread distribution of RyR2 in various brain regions, most prominently in regions involved in spatial learning and memory, such as the hippocampus. To define the functional role of RyR2 in this region, we performed electrophysiological studies using hippocampal slices prepared from knock-in mice harboring a human RyR2 mutation (R4496C) with increased channel activity. We found that enhanced RyR2 activity reduces long-term potentiation (LTP) in Schaffer collaterals. Thus, RyR2 plays a critical role in LTP. Behavioral studies on RyR2 mutant mice further supported the role of RyR2 in learning and memoryThese results reveal, for the first time, the distribution of RyR2 clusters and its functional correlation in living ventricular myocytes and hippocampal neurons.