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Review

Intracellular calcium channels: Inositol-1,4,5-trisphosphate receptors

Olena A. Fedorenko [a,b], Elena Popugaeva [c], Masahiro Enomoto [d], Peter B. Stathopulos [d], Mitsuhiko Ikura [d], Ilya Bezprozvanny [c,e,*]

[a] Department of Brain Physiology, Bogomoletz Institute of Physiology, 01024 Kiev, Ukraine
[b] State Key Laboratory of Molecular and Cellular Biology, 01024 Kiev, Ukraine
[c] Laboratory of Molecular Neurodegeneration, Department of Medical Physics, St. Petersburg State Polytechnical University, 195251 St. Petersburg, Russia
[d] Princess Margaret Cancer Centre, Department of Medical Biophysics, University of Toronto, M5G1L7 Toronto, Ontario, Canada
[e] Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

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ABSTRACT

The inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$) are the major intracellular Ca^{2+}-release channels in cells. Activity of $InsP_3Rs$ is essential for elementary and global Ca^{2+} events in the cell. There are three $InsP_3Rs$ isoforms that are present in mammalian cells. In this review we will focus primarily on $InsP_3R$ type 1. The $InsP_3R1$ is a predominant isoform in neurons and it is the most extensively studied isoform. Combination of biophysical and structural methods revealed key mechanisms of $InsP_3R$ function and modulation. Cell biological and biochemical studies lead to identification of a large number of $InsP_3R$-binding proteins. $InsP_3Rs$ are involved in the regulation of numerous physiological processes, including learning and memory, proliferation, differentiation, development and cell death. Malfunction of $InsP_3R1$ play a role in a number of neurodegenerative disorders and other disease states. $InsP_3Rs$ represent a potentially valuable drug target for treatment of these disorders and for modulating activity of neurons and other cells. Future studies will provide better understanding of physiological functions of $InsP_3Rs$ in health and disease.

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1. Introduction

The inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$) are the major intracellular Ca^{2+}-release channels in cells. The investigation of mechanisms of inositol-1,4,5-trisphosphate ($InsP_3$)-induced Ca^{2+}-release started in 1980s and $InsP_3R$ was first solubilized and purified from the rat cerebellum in 1988 by Snyder's group (Supattapone et al., 1988). Reconstitution of the purified receptor into lipid vesicles showed that $InsP_3$ and other inositol phosphates stimulate calcium flux (Ferris et al., 1989). Then cDNA of $InsP_3R$ was cloned (Furuichi et al., 1989; Mignery et al., 1990) that helps to initiate structure-function studies (Mignery and Sudhof, 1990; Miyawaki et al., 1991). Reconstitution of $InsP_3R$ into planar bilayer membranes revealed its single channel permeability, its modulation by Ca^{2+} and by ATP (Bezprozvanny and Ehrlich, 1993, 1994; Bezprozvanny et al., 1991). Since these initial publications the functional properties of native and recombinant $InsP_3R$ have been extensively characterized by Ca^{2+} flux measurements, planar lipid bilayer or nuclear envelope patch-clamp recordings (Bezprozvanny, 2005; Foskett et al., 2007;

* Corresponding author at: Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.
E-mail address: ilya.bezprozvanny@utsouthwestern.edu (I. Bezprozvanny).

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Mikoshiba, 2007; Wagner and Yule, 2012). Although some of these studies initially resulted in conflicting data, an agreement has been reached by the field regarding key $InsP_3R$ functional properties.

The variety of $InsP_3$-activated Ca^{2+} channels, including three mammalian $InsP_3R$ isoforms $InsP_3R$ type 1 ($InsP_3R1$), $InsP_3R$ type 2 ($InsP_3R2$), $InsP_3R$ type 3 ($InsP_3R3$), different splicing variants of $InsP_3R1$, *Drosophila melanogaster* $InsP_3R$ and *Caenorhabditis elegans* $InsP_3R$ have been discovered and characterized (reviewed in (Bezprozvanny, 2005; Foskett et al., 2007; Mikoshiba, 2007)). The three mammalian $InsP_3R$ isoforms are 60–70% identical in sequence (Furuichi et al., 1994) and share a common domain structure (Mignery and Sudhof, 1990; Miyawaki et al., 1991) that consists of an amino-terminal $InsP_3$-binding domain, a carboxyl-terminal Ca^{2+} channel domain, and a middle coupling domain containing most of the putative regulatory sites and is the most divergent (Fig. 1). $InsP_3R1$ is predominant in the central nervous system, but most other tissues express at least two and often all three $InsP_3R$ isoforms at different ratios (Taylor et al., 1999).

The $InsP_3R$ are subjected to multiple levels of regulation (Bezprozvanny, 2005; Foskett et al., 2007; Mikoshiba, 2007; Wagner and Yule, 2012). $InsP_3Rs$ are the targets of a number of allosteric regulators, including protein kinases, adenine nucleotides, pH and divalent cations, all of which may play a part in $InsP_3$-induced Ca^{2+} signaling. Significant effect of phosphorylation on $InsP_3R$ is also well documented (Bezprozvanny, 2005; Foskett et al., 2007; Mikoshiba, 2007). Many protein binding with $InsP_3R$ have been described, and physiological relevance of these interactions is under intense investigation.

At this moment one can find thousands of papers from different research groups dedicated to various aspects of $InsP_3R$ structure, regulation or functional role, but there are still many questions remain to be answered. In this review we focus on $InsP_3R$ type 1, which are predominant isoform expressed in mammalian neurons. Here we will briefly review the structure and basic properties of these channels, their role in the cell functions and in several neurodegenerative disorders, such as Hungtington's disease, spinocerebellar ataxias and Alzheimer's disease.

2. $InsP_3Rs$ in cell functions

A rise in intracellular calcium in neurons in response to $InsP_3Rs$ activation is implicated in the control of a numerous cellular functions, including neurotransmission and synaptic plasticity, proliferation, differentiation, development, gene expression, and cell death (Berridge et al., 1998). Evidence at both cellular and behavioral levels implicates $InsP_3Rs$ in memory formation, in particular during long-term memory formation (Baker et al., 2013). It was demonstrated that $InsP_3R1$ is extremely important in embryonic development. $InsP_3R1$ knock-out mice have severe ataxia and tonic or tonic-clonic seizures and die by the weaning period (Matsumoto et al., 1996). Besides, $InsP_3R1$ is a critical regulator of synaptic circuit maintenance in the mature cerebellum; this mechanism may underlie motor coordination and learning in adults (Sugawara et al., 2013). Thus, $InsP_3R1$ are essential for proper brain development and function.

$InsP_3R1$ are highly concentrated in the Purkinje cells of the cerebellum, with lower levels being found in other regions of the brain (Sharp et al., 1993a; Sharp et al., 1993b; Taylor et al., 1999) and in a variety of peripheral tissues (Taylor et al., 1999). Immunohistochemical studies in Purkinje cells, *Xenopus* oocytes and pancreatic epithelial cells have revealed that at a subcellular level $InsP_3Rs$ are localized in the rough and smooth endoplasmic reticulum (ER), Golgi complex and nuclear envelope, but not mitochondria or plasma membranes (Lam and Galione, 2013; Ross et al., 1989; Solovyova and Verkhratsky, 2003). Though, it has been indicated that the plasma membrane in some cell types may also contain $InsP_3R$ (Barrera et al., 2004; Dellis et al., 2006; Tanimura et al., 2000), but the functional role of these receptors has not been clarified.

$InsP_3$ is not the only regulator of $InsP_3Rs$ function; Ca^{2+} plays a critical role in shaping the $InsP_3R$-evoked Ca^{2+} signals. Low Ca^{2+} concentrations (<300 nM) activate the channel and increase its open probability, whereas high Ca^{2+} concentrations inhibit channel opening (Bezprozvanny et al., 1991; Finch et al., 1991; Iino, 1990). These positive and negative feedback cycles are well suited for generating Ca^{2+} oscillations or waves. It appears that $InsP_3Rs$ are activated by simultaneous binding of the two agonists, $InsP_3$ and Ca^{2+}, to the cytoplasmic domain of the molecule that leads to the conformational change in the receptor complex and an increase in the frequency of its Ca^{2+} channel opening, resulting in Ca^{2+} release from the intracellular stores. Both $InsP_3$ and Ca^{2+} are the two main intracellular messengers with their own regulatory pathways (Berridge, 2009, 2012; Decrock et al., 2013). So $InsP_3R$ acts as a skilled "analyst" which coordinates two complex streams of signals and forms an integrated response.

Because of complex Ca^{2+}-mediated feedback on $InsP_3R$ activity, Ca^{2+} signals evoked by the receptor activation are complex, restricted in space and time, and this spatiotemporal organization determines physiological effect of the signal (Berridge, 1997; Bootman et al., 2001; Konieczny et al., 2012). Intracellular $InsP_3$-activated Ca^{2+} signals are organized at three levels, each of them provides different signaling functions and serves as a building block for Ca^{2+} signals at the next level (Berridge, 1996, 1997; Bootman et al., 2001). At the first, so called "fundamental", level signals result from openings of a single $InsP_3R$ channel. At the resting conditions the cytosolic concentration of Ca^{2+} is low and $InsP_3Rs$ are in a conformation with low affinity for $InsP_3$, but the activating stimuli trigger the rise in the intracellular Ca^{2+} concentration and the production of $InsP_3$ from the plasma membrane. These low concentrations of the two agonists activate one $InsP_3R$ leading to a rapid localized Ca^{2+}flux called "blip" (Parker and Yao, 1996). At the next, "elementary", level Ca^{2+} signals, so called "puffs", arise from the concerted opening of multiple $InsP_3R$ channels. It has been demonstrated that $InsP_3Rs$ are initially randomly distributed in the membranes, but low concentrations of $InsP_3$ cause them to aggregate rapidly and reversibly into clusters (Yao et al., 1995). There is no agreement about the number of $InsP_3Rs$ in a cluster, it was proposed that from four (Rahman,

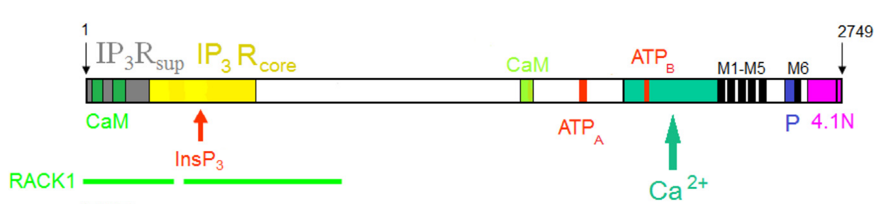

Fig. 1. Domain structure of $InsP_3R1$. $InsP_3R_{sup}$ and $InsP_3R_{core}$ domains, CaM, RACK1, IRBIT and 4.1 N binding sites, two ATP (A and B) binding sites, the Ca^{2+} sensor region, the M1–M6 transmembrane domains and the pore-forming region (P) are shown.