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Reviews

Inositol 1,4,5-trisphosphate receptors in the endoplasmic reticulum: A single-channel point of view

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ABSTRACT

As an intracellular Ca^{2+} release channel at the endoplasmic reticulum membrane, the ubiquitous inositol 1,4,5-trisphosphate ($InsP_3$) receptor ($InsP_3R$) plays a crucial role in the generation, propagation and regulation of intracellular Ca^{2+} signals that regulate numerous physiological and pathophysiological processes. This review provides a concise account of the fundamental single-channel properties of the $InsP_3R$ channel: its conductance properties and its regulation by $InsP_3$ and Ca^{2+}, its physiological ligands, studied using nuclear patch clamp electrophysiology.

1. Introduction

The ubiquitously expressed inositol 1,4,5-trisphosphate ($InsP_3$) receptor ($InsP_3R$) is mostly localized to the endoplasmic reticulum (ER) membrane [1], where it functions as an ion channel to release Ca^{2+} stored in the ER lumen (Ca_{ER}^{2+}) into the cytoplasm to raise the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) when it is activated by its physiological ligand, $InsP_3$, generated in the cytoplasm as part of a signal cascade resulting from activation of specific plasma membrane receptors by various extracellular stimuli [2]. $InsP_3R$ thus plays a crucial role in the generation, propagation and regulation of cytoplasmic Ca^{2+} (Ca_i^{2+}) signals that regulate numerous physiological and pathophysiological processes. It is therefore not surprising that it has been the subject of intense study ever since its identification [3]. Many aspects of the $InsP_3R$ channel have been extensively reviewed recently, including its molecular structure [4–7] and its regulation by phosphorylation [8], redox reagents [9,10] and ATP [8]. Thus, this short review will focus on the more fundamental properties of the single $InsP_3R$ channel: its ion conductance properties and its regulation by its physiological ligands—$InsP_3$ and Ca^{2+}, especially those reported since our previous reviews of single $InsP_3R$ channel properties [11,12].

Studies of individual $InsP_3R$ channels began with the reconstitution of isolated and purified functional $InsP_3R$ channels in artificial planar lipid bilayers [13], which allows the reconstituted channel(s) to be studied with known and rigorously controlled ionic and ligand conditions on both its cytoplasmic and luminal sides [14]. Because of the intracellular localization of the $InsP_3R$, application of patch clamp electrophysiology to investigate behaviors of the $InsP_3R$ channel in a more native membrane environment was not feasible until isolated nuclei were used as surrogate for the ER [15,16] because of the continuity of the outer nuclear membrane with the ER [17]. Since isolated nuclei with intact outer nuclear membranes can be obtained with high success rates [18], nuclear patch clamping in the "on-nucleus" configuration (Fig. 1A) preserves the protein environment on the luminal side of the recorded $InsP_3R$ channel(s) while maintaining rigorous control of ligand and ionic conditions on both sides of the channel(s) [19]. Combining rapid perfusion techniques with nuclear patch clamping in luminal-side-out (lum-out) (Fig. 1B) or cytoplasmic-side-out (cyto-out) (Fig. 1C) configurations allows rapid (~ms), repeated (tens of times in one experiment) and reversible exchanges of the bath solution to study dynamic responses of $InsP_3R$ channel activity to abrupt changes in $InsP_3$ and Ca^{2+} concentrations on either side of the channel, as well as to compare the gating and conductance properties of the *same* channels in the *same* isolated membrane patches under different ionic environments [19]. Performing nuclear patch clamping on an isolated nucleus with its outer nuclear membrane stripped by chemical treatment [20,21] (Fig. 1D) can achieve the nucleoplasmic-side-out (nucleo-out)

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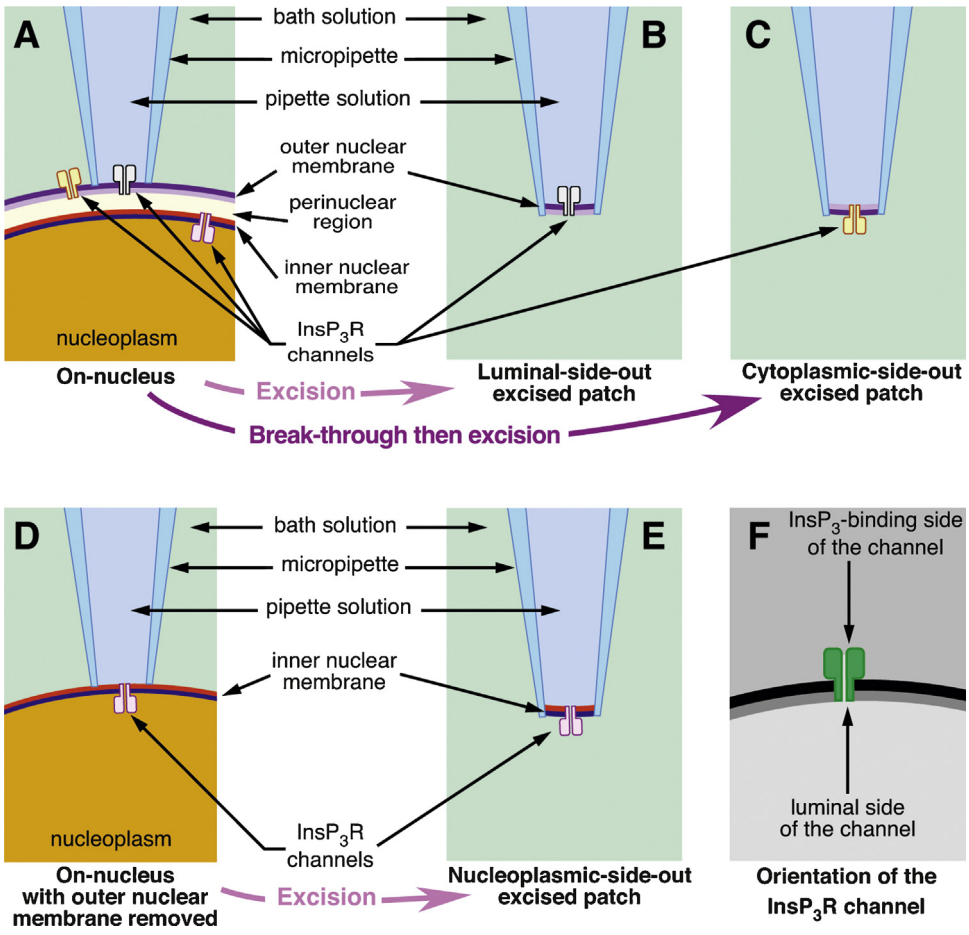

Fig. 1. Different configurations for nuclear patch clamping experiments. Schematic diagrams illustrating the orientation of $InsP_3R$ channels in nuclear membrane patches in various configurations of nuclear patch-clamp experiments. (A) On-nucleus configuration with outer nuclear membrane intact, (B) excised luminal-side-out configuration, (C) excised cytoplasmic-side-out configuration, (D) on-nucleus configuration with outer nuclear membrane removed, (E) excised nucleoplasmic-side-out configuration. (F) Diagram showing how the two aspects of the $InsP_3R$ channel are represented in this figure. Figure modified from [19].

configuration (Fig. 1E) to study $InsP_3R$ localized to the inner nuclear membrane [20,22].

Single-channel properties of the $InsP_3R$ channel have also been studied by applying whole-cell patch clamp techniques to chicken lymphocyte DT40 cells [23–29], in which $InsP_3R$ channels are localized to the plasma membrane at very low density (<5 channels/cell) [24]. However, the lipid and protein environments around these $InsP_3R$ channels in the plasma membrane are different from those around the ER channels, and conductance and ligand regulation of $InsP_3R$ channels in the two locations were significantly different [19]. Strictly speaking, $InsP_3R$ channels localized to the plasma membrane are acting as plasma membrane Ca^{2+} entry channels rather than intracellular Ca^{2+} release channels [23]. Ca^{2+} signals generated by $InsP_3R$ channels located in the ER near the plasma membrane in intact mammalian cells can also be studied using total internal reflection fluorescence (TIRF) microscopy [30]. By using a fast [Ca^{2+}] indicator dye and loading Ca^{2+} buffer (EGTA) into cells to rapidly sequester Ca^{2+} released by active $InsP_3R$ channels, spatial and temporal resolution of observed Ca^{2+} signals were sufficiently improved so that Ca^{2+} release by single $InsP_3R$ channels can be imaged. With such "optical patch-clamping", many individual channels can be monitored in their native environment in intact cells, thereby preserving the interaction between an active $InsP_3R$ channel with neighboring channels mediated by Ca^{2+} released by the active channel. However, to date, the temporal resolution and signal-to-noise ratio of electrophysiological patch clamping are still substantially superior to those of optical patch clamping. Thus, optical and electrophysiological patch clamping are mutually complimentary techniques to study $InsP_3R$-mediated Ca^{2+} signals.

Another factor besides the intracellular location of the $InsP_3R$ that complicates the study of $InsP_3R$ channels is the primary amino acid sequence diversity of the channels. Vertebrates have three isoforms of $InsP_3R$: types 1 ($InsP_3R$-1), 2 ($InsP_3R$-2) and 3 ($InsP_3R$-3) that are encoded by three separate genes and are ~60–80% homologous. Invertebrates have only one $InsP_3R$ that is most closely related to $InsP_3R$-1. Alternate splicing further enhances diversity of $InsP_3R$. $InsP_3R$-1 has three major splice regions (S1, S2 and S3), and a few minor ones. Mammalian neuronal cells express the S2+ variant while other peripheral cells express mainly the S2− form. $InsP_3R$-2 has at least one splice region. The invertebrate $InsP_3R$ also has multiple splice variants. Given that most vertebrate cells express multiple $InsP_3R$ isoforms in various levels, and $InsP_3R$ can form homo- and heterotetrameric channels, the diversity at the $InsP_3R$ channel level can be impressive indeed (see [11] and references therein).

To avoid possible complications arising from heterotetrameric channels, initial studies of $InsP_3R$ channel used cells or tissues that were known to express predominantly one $InsP_3R$ isoform [31], like cerebellum [32] for $InsP_3R$-1 S2+, *Xenopus* oocytes [15,16] for $InsP_3R$-1 S2− [33], ventricular cardiac myocytes [34] for $InsP_3R$-2, RIN-5F insolinoma cells [35] for $InsP_3R$-3, and insect *Spodoptera frugiperda* Sf9 cells [36] for invertebrate $InsP_3R$. Nevertheless, the possibility that these cells may express different splice variants of the same $InsP_3R$ isoforms cannot be ruled out. To address that