

PROPERTIES OF NEURONAL INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS/ Ca^{2+} -RELEASE CHANNELS

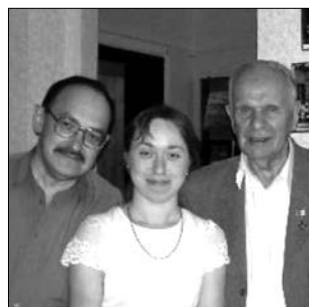
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The photo shows P.G. Kostyuk together with Dr. Marchenko S. and Dr. Fedorenko O.

Sergii Marchenko. Ph.D., D.Sc., is a leading scientist at the Bohomolets Institute.

Olena Fedorenko. Ph.D. was a junior scientist at the Bohomolets Institute, currently at the Université de Picardie Jules Verne, Amiens, France.

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Introduction

Inositol 1,4,5-trisphosphate receptors (InsP_3Rs) are a major type of intracellular Ca^{2+} release channels localized predominantly in the endoplasmic reticulum. Notwithstanding numerous investigations, there is a significant controversy on the basic properties of these receptors (compare e.g. Bezprozvanny, 1991, 2002; Marchenko et al., 2005 and Mak et al., 1998; Foscett et al., 2007). There is also a long-standing controversy on the role of the nuclear envelope in the regulation of nuclear Ca^{2+} (Marchenko, Thomas, 2006). To address these issues, we have studied native InsP_3Rs in the nuclear membrane of mammalian cells. The nuclear envelope is the only part of the endoplasmic reticulum readily accessible for patch-clamp recording.

Very high levels of expression of InsP_3Rs were reported for some types of neurons. Investigation of neuronal nuclei is hampered by the cellular heterogeneity of the brain where neurones comprise no more than 10% of the cells. We have developed methods for isolation of cell nuclei from identified types of central neurons (Marchenko et al. 2005). Here we report some recent data on the properties of neuronal InsP_3Rs .

Nuclear InsP_3Rs in different neurons

We have studied InsP_3Rs in the nuclear membranes of cerebellar Purkinje neurons and pyramidal neurons from CA1 and CA3 areas of hippocampus of the rat

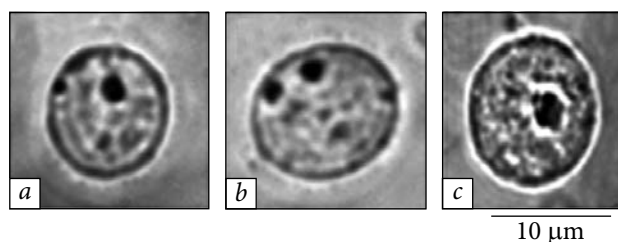


Fig. 1. Cell nuclei isolated from pyramidal neurons of CA1 (a) and CA3 (b) areas of the hippocampus and cerebellar Purkinje neurons (c)

(Fig. 1). Numerous InsP_3 -activated channels were recorded from the *inner* nuclear membrane of Purkinje and CA1 pyramidal neurons. No (Purkinje neurons) or very few (CA1 pyramidal neurons) InsP_3 Rs were found in the outer nuclear membrane. In contrast, only two (out of 48) patches of the outer nuclear membrane of CA3 pyramidal neurons contained InsP_3 -activated channels and no InsP_3 Rs were detected in their inner nuclear membrane. Also no InsP_3 Rs were found in the nuclear membrane of granule neurons (Marchenko et al., 2005). These data suggest that (1) the pattern of expression of InsP_3 Rs in the nuclear membrane follows the general level of expression of the receptors in particular cells; (2) In neurons with high levels of expression of InsP_3 Rs (Purkinje and CA1 pyramidal neurons) they are predominantly localized in the inner nuclear membrane suggesting an active role of the nuclear Ca^{2+} store in regulation of nuclear Ca^{2+} in these neurons (Marchenko & Thomas, 2006; Zhang et al., 2009). In neurons with low levels of InsP_3 R expression (CA3 pyramidal and cerebellar granule neurons), any role of the nuclear envelope as a Ca^{2+} store is questionable. Therefore the mechanism of nuclear Ca^{2+} regulation may differ in different cells.

InsP_3 -activated channels recorded from the nuclear membrane of different neurons had similar properties and apparently belong to type 1 of InsP_3 Rs, the main type of these receptors expressed in neurons. They all were poor selective Ca^{2+} channels with similar conductance (about 356 pS in symmetric KCl solution) and kinetics. InsP_3 Rs demonstrated clear voltage-dependence being inhibited by negative potentials at the luminal side of the membrane. The last property of InsP_3 Rs allowed us to confirm their localization in the inner or the outer nuclear membrane. The nuclear membrane also contained numerous large-conductance cationic channels. The density and localization of these channels closely correlated with those of InsP_3 Rs.

Ca^{2+} -dependence of neuronal InsP_3 Rs

InsP_3 Rs are activated by simultaneous binding of two agonists, InsP_3 and Ca^{2+} . The regulation of the receptors by Ca^{2+} evokes much controversy. It has initially been reported that under steady-state conditions and saturating concentration of InsP_3 cerebellar InsP_3 Rs incorporated into artificial lipid bilayers are activated by Ca^{2+} at low concentration and inhibited by higher Ca^{2+} concentration (Bezproz-

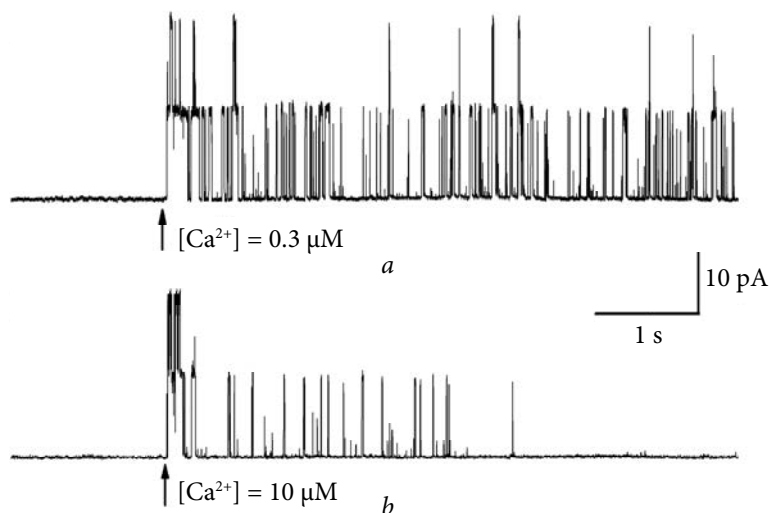


Fig. 2. Single channel activity of InsP_3Rs in the internal nuclear membrane of cerebellar Purkinje neurones. The receptors were activated by fast (<1 ms) application of $0.3 \mu\text{M}$ (a) or $10 \mu\text{M}$ (b) Ca^{2+} and $10 \mu\text{M}$ InsP_3 . The initial peak response of InsP_3Rs was not significantly inhibited by large concentrations of Ca^{2+} , but at concentrations $\geq 1 \mu\text{M}$ Ca^{2+} evoked about complete time-dependent desensitization of the receptors

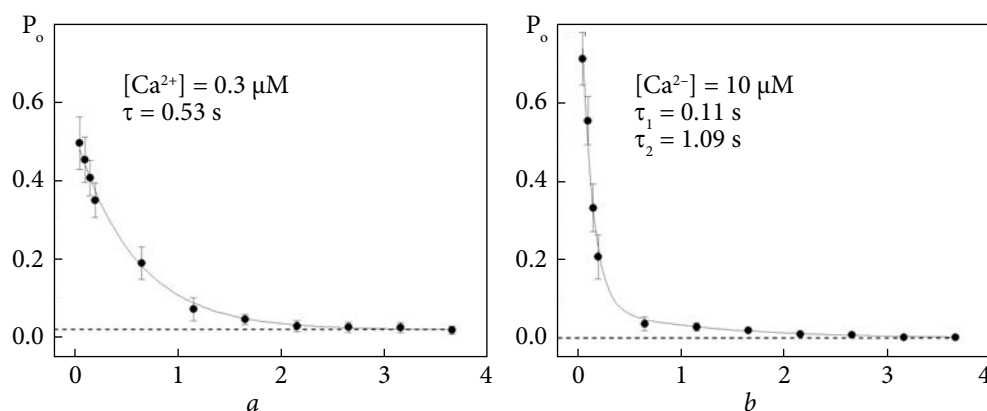


Fig. 3. Time and Ca^{2+} -dependence of InsP_3Rs . Rise in Ca^{2+} concentration increases the peak, but at concentrations $>0.2 \mu\text{M}$ decreases the steady-state P_o of InsP_3 -activated channels. Kinetics of desensitization of InsP_3Rs is accelerated at higher Ca^{2+} concentrations

vanny et al., 1991). In contrast, in recordings from the outer nuclear membrane of *Xenopus* oocytes, Mak et al. (1998) found that at $[\text{InsP}_3] > 33$ nM, Ca^{2+} does not inhibit InsP_3Rs in the physiological range of intracellular Ca^{2+} concentrations. The reason of this controversy has not yet clarified.

In the inner nuclear membrane, the agonist-binding loci of InsP_3Rs face the nucleoplasm and respectively bath solution in excised patches of the membrane.

This allowed us to use a concentration-clamp technique to study nonstationary kinetics of InsP_3 -activated channels in the inner nuclear membrane of cerebellar *Purkinje* neurons.

Fast ($<1 \mu\text{s}$) application of Ca^{2+} in the presence of saturating concentrations of InsP_3 (2–10 μM) evoked fast activation of InsP_3Rs (Fig. 2).

When $[\text{Ca}^{2+}]$ was below 1 μM , the initial burst was followed by a noticeable steady-state activity of the channels (Fig. 2, *a*). The probability of the open state (P_o) of the channel was higher at the initial than at the steady-state phase of response. For example, when Ca^{2+} concentration was 0.3 μM the average P_o of InsP_3Rs during first 100 ms of the response was 0.49 ± 0.7 , but 4 s after the channel activation it reduced to 0.02 ± 0.01 (Fig. 3, *a*). When $[\text{Ca}^{2+}]$ was above $\sim 1 \mu\text{M}$, InsP_3Rs almost completely desensitized within few seconds after the agonist application, but the initial peak response was high (Fig. 3, *b*). Therefore steady-state activity of InsP_3 -activated channels had bell-shaped dependence whereas the peak response was not inhibited by high (up to 50 μM) concentrations of Ca^{2+} .

These data make it possible to explain seeming contradictions between "bell-shaped" (Bezprozvanny et al., 1991) and "ligand tuning" (Mak et al., 1998) hypotheses of regulation of InsP_3Rs by Ca^{2+} . In experiments of Foskett's group the agonists were applied to InsP_3Rs through patch pipettes during formation of gigaseal contact with the outer nuclear membrane of *Xenopus* oocytes. The author reported that InsP_3Rs irreversibly desensitized after a short period of activity, the phenomenon that we did not observed in our experiments and which certainly is not an intrinsic property of InsP_3Rs *in situ*. Therefore, no steady-state channels activity was recorded in those experiments, and Ca^{2+} -dependence reported by Mak et al. refers to transient activity of the channel after application of the agonists. This agrees with our observation that Ca^{2+} at concentrations above 1 μM does not inhibit the peak activity of the channels. At the same time, InsP_3Rs underwent time-dependent desensitization. The rate of the desensitization increased with rise in Ca^{2+} concentration which at concentrations $>1 \mu\text{M}$ led to about complete inhibition of InsP_3Rs (Fig. 3) and accounts for bell-shaped Ca^{2+} -dependence of the steady-state activity of InsP_3Rs . The Ca^{2+} and time-dependent desensitization of InsP_3Rs may be a major mechanism of shaping transient Ca^{2+} signals, such as Ca^{2+} puffs, spikes and oscillations.

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