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.

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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; Foskett 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

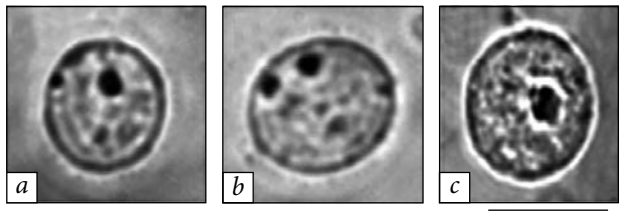

Fig. 1. Cell nuclei isolated from pyramidal neurons of CA1 (*a*) and CA3 (*b*) areas of the hippocampus and cerebellar Purkinje neurons (*c*)

(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) $InsP_3Rs$ 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 $InsP_3Rs$ were detected in their inner nuclear membrane. Also no $InsP_3Rs$ were found in the nuclear membrane of granule neurons (Marchenko et al., 2005). These data suggest that (*1*) the pattern of expression of $InsP_3Rs$ 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 $InsP_3Rs$ (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 $InsP_3R$ 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 $InsP_3Rs$, 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. $InsP_3Rs$ demonstrated clear voltage-dependence being inhibited by negative potentials at the luminal side of the membrane. The last property of $InsP_3Rs$ 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 $InsP_3Rs$.

Ca^{2+}-dependence of neuronal $InsP_3Rs$

$InsP_3Rs$ 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 $InsP_3Rs$ incorporated into artificial lipid bilayers are activated by Ca^{2+} at low concentration and inhibited by higher Ca^{2+} concentration (Bezproz-

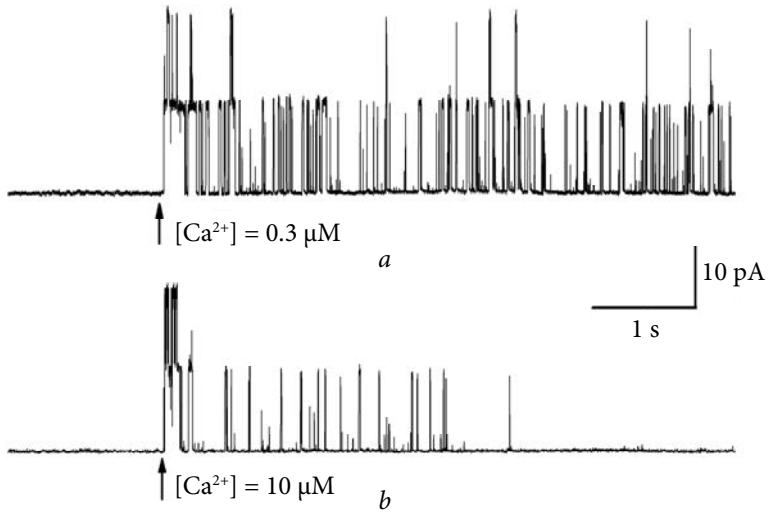

Fig. 2. Single channel activity of $InsP_3Rs$ in the internal nuclear membrane of cerebellar Purkinje neurones. The receptors were activated by fast (<1 ms) application of 0.3 μM (*a*) or 10 μM (*b*) Ca^{2+} and 10 μM $InsP_3$. The initial peak response of $InsP_3Rs$ was not significantly inhibited by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

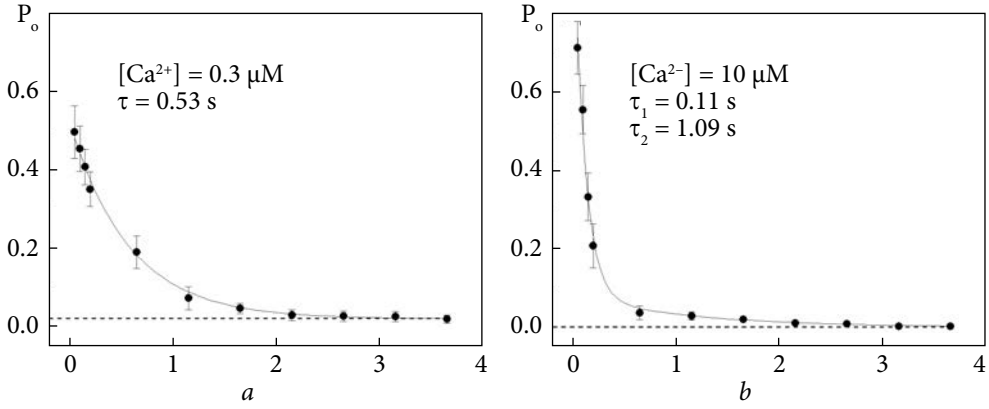

Fig. 3. Time and Ca^{2+}-dependence of $InsP_3Rs$. Rise in Ca^{2+} concentration increases the peak, but at concentrations >0.2 μM decreases the steady-state P_o of $InsP_3$-activated channels. Kinetics of desensitization of $InsP_3Rs$ is accelerated at higher Ca^{2+} concentrations

vanny et al., 1991). In contrast, in recordings from the outer nuclear membrane of Xenopus oocytes, Mak et al. (1998) found that at [$InsP_3$] >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 μs) 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 [Ca^{2+}] was below 1 μM, the initial burst was followed by a notiseble 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 [Ca^{2+}] was above ~1 μM, $InsP_3Rs$ almost completely desensetized 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 μM 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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