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Identification of new functions of Ca^{2+} release from intracellular stores in central nervous system

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Abstract

Ca^{2+} release from intracellular stores regulates muscle contraction and a vast array of cell functions, but its role in the central nervous system (CNS) has not been completely elucidated. A new method of blocking IP_3 signaling by artificially expressing IP_3 5-phosphatase has been used to clarify the functions of intracellular Ca^{2+} mobilization in CNS. Here I review two of such functions: the activity-dependent synaptic maintenance mechanism and the regulation of neuronal growth by spontaneous Ca^{2+} oscillations in astrocytes. These findings add new bases for better understanding CNS functions and suggest the presence of as yet unidentified neuronal and glial functions that are regulated by Ca^{2+} store-dependent Ca^{2+} signaling.

Keywords: Inositol 1,4,5-trisphosphate; IP_3 receptor; Ryanodine receptor; Synapse; Neuronal growth; Glia; Calcium signaling

The pioneering work of Setsuro Ebashi on the regulation of muscle contraction [1] led to the recognition of the physiological roles of Ca^{2+} release from the intracellular store in not only muscle contraction but also many other cell functions. The mechanism of Ca^{2+} release from the intracellular store in muscle cells (sarcoplasmic reticulum) was initially identified as the "Ca^{2+}-induced Ca^{2+} release" (CICR) mechanism, in which Ca^{2+} at micromolar concentrations activates the release of Ca^{2+} from the sarcoplasmic reticulum [2]. The molecule responsible for the CICR mechanism was subsequently identified as the ryanodine receptor (RyR). Three subtypes of RyR are expressed in a tissue-specific manner in mammals. Another type of Ca^{2+} release mechanism that is sensitive to inositol 1,4,5-trisphosphate (IP_3) was then discovered and its molecular basis was identified as the IP_3 receptor, which consists of three subtypes in mammals [3]. The IP_3-induced Ca^{2+} release mechanism was also found to be sensitive to Ca^{2+}, so that not only RyR but also IP_3R functions as the CICR mechanism [4,5]. Indeed, RyR and IP_3R are homologous molecules [6,7]. Thus, Ca^{2+} release from the intracellular store is mediated by a family of CICR channels consisting of three subtypes of RyR and three subtypes of IP_3R in mammals.

The intracellular Ca^{2+} release channels IP_3Rs and RyRs are also expressed in the brain in both neurons and glia. Although Ca^{2+} release mechanisms have been implicated in CNS functions such as certain forms of synaptic plasticity [8], their roles in the regulation of cell functions in the CNS remains to be elucidated In this review, I will discuss newly identified CNS functions that are regulated by Ca^{2+} mobilization from the intracellular store, by referring to our recent results [9,10].

IP_3 and Ca^{2+} signaling in cerebellum

Purkinje cells are the sole output neurons from the cerebellar cortex receiving two types of excitatory glutamatergic input: numerous inputs from parallel fibers or granule cell axons and a strong input from usually single climbing fibers originating from the inferior olive. Purkinje cells

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express both IP_3R and RyR. In particular, they have an extremely high type 1 IP_3R expression level. Indeed, the properties and roles of Ca^{2+} release mechanisms in neurons have been most extensively studied in parallel fiber-to-Purkinje cell (PF→PC) synapses.

The primary electrical transmission in PF→PC synapses is mediated by the postsynaptic ionotropic glutamate receptor, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor. Thus, the PF input generates excitatory postsynaptic current (EPSC) in PCs. Ca^{2+} imaging studies in PF→PC synapses have shown that physiologically plausible repetitive PF inputs generate two types of local Ca^{2+} signal in spines and dendrites receiving activated PF inputs [11,12]. The first one is generated by an influx of Ca^{2+} via voltage-dependent ion channels in response to AMPA-receptor-dependent depolarization. This is followed by a delayed Ca^{2+} signal that is dependent on the type 1 metabotropic glutamate receptor (mGluR1), which is coupled to phospholipase C via the G-protein to generate IP_3. The delayed Ca^{2+} response is blocked by the intracellular application of heparin, an inhibitor of IP_3R [11,12]. Thus, the mGluR1-dependent Ca^{2+} signal seems to be mediated by IP_3-induced Ca^{2+} release. We imaged IP_3 signals in fine dendrites of PCs using GFP-PHD, an IP_3 indicator [13]. PF stimulation at 50 Hz induced dendritic IP_3 signaling, and the magnitude of IP_3 signals was dependent on the number of pulses delivered to PFs up to about 20 pulses [14].

The CF input to PCs is also mediated by AMPA receptors and induces action potentials called complex spikes. Complex spikes generate Ca^{2+} influx via voltage-dependent Ca^{2+} channels and induce an increase in intracellular Ca^{2+} concentration in the entire dendritic tree of PCs. Although single complex spikes are not associated with IP_3 signals, repetitive CF stimulation at 1 Hz induces a slow increase in IP_3 concentration within PCs [15]. The physiological role of the CF-induced IP_3 signal remains to be identified.

The synaptic strength of PF→PC synapses undergoes long-term depression (LTD) when PF inputs are coupled with CF inputs for about 5 min [16]. Cerebellar LTD is considered to underlie certain forms of motor learning, such as vestibulo-ocular reflex [16]. It has been shown that Ca^{2+} release via IP_3R is essential for LTD induction, and that no LTD is observed in mutant mice in which PC spines are free of intracellular stores so that Ca^{2+} release within PC spines is absent, although the uncaging of caged Ca^{2+} induces LTD [17]. Because IP_3Rs require IP_3 and Ca^{2+} simultaneously for their activation [4,5], IP_3Rs may function as a coincidence detector of Ca^{2+} and IP_3 signals. Indeed, the pairing of the CF input (generating a Ca^{2+} signal throughout dendritic arborization in PCs) with the PF input (generating a local IP_3 signal) enhances Ca^{2+} release via IP_3Rs [18]. The coincidence detector property of IP_3Rs is considered to underlie the requirement of conjunctive PF and CF stimulations in cerebellar LTD.

Ca^{2+}-dependent maintenance of synaptic strength in cerebellum

The above results indicate that mGluR1-mediated IP_3-induced Ca^{2+} release is important for LTD induction. We then asked whether its role in LTD is the only one played by intracellular Ca^{2+} release in PF→PC synapses. To answer this question, IP_3-Ca^{2+} signaling in PCs was inhibited and the PF→PC synapse was examined to determine if there are any changes in synaptic functions. Because there is no specific drug that inhibits IP_3-induced Ca^{2+} release, IP_3 5-phosphatase (5ppase), which specifically hydrolyzes IP_3 to generate inositol 1,4-bisphosphate, was used [13,14].

5ppase was expressed in PCs by injecting the Sindbis virus encoding 5ppase into the mouse cerebellum, and cerebellar slices were prepared the following day [9]. The magnitude of EPSC (output) recorded in PCs in response to varying PF stimulus (input) intensity was measured to assess the synaptic strength of the PF→PC synapse. There was a significant reduction in the slope of the input–output relationship for the PF→PC synapse. On the other hand, the expression of mutant 5ppase, in which one of the arginine residues within the active center was replaced with alanine (R343A) to reduce the enzyme's activity [14], had no significant effect on synaptic strength. These observations indicate that synaptic strength decreases when IP_3-Ca^{2+} signaling is inhibited in PCs.

The mechanism underlying the reduced synaptic strength at the PF→PC synapse could be either reduced transmitter release from the presynaptic terminal or reduced postsynaptic glutamate sensitivity. The amplitude of quantal EPSC, which was generated by asynchronous vesicular release following PF stimulation in a bath solution in which Ca^{2+} was replaced with Sr^{2+}, was not altered. This result suggests that postsynaptic glutamate sensitivity remained constant. Paired-pulse ratio (PPR), which is often used in assessing presynaptic transmitter release probability in PF→PC synapses, increased, suggesting that the presynaptic function was reduced. The coefficient of variation of EPSCs, which is another measure of transmitter release probability, also increased in agreement with the notion that transmitter release probability decreases when IP_3-Ca^{2+} signal is blocked in PCs.

Taken together, the above results indicate that postsynaptic IP_3-Ca^{2+} signaling in PCs is necessary for the maintenance of presynaptic functions in terms of glutamate release. Therefore, it is likely that a retrograde messenger that maintains presynaptic functions is released from PCs. What then is the retrograde signal? The brain-derived neurotrophic factor (BDNF) is a neurotrophin that is strongly expressed in PCs [19–21]. Furthermore, TrkB, the BDNF receptor, is expressed in granule cells [22,23]. Thus, BDNF can be a potential retrograde messenger at the PF→PC synapse. Indeed, after chronic application of an anti-BDNF antibody to the cerebellar cortex *in vivo*, the PPR subsequently measured in cerebellar slices