LECTURE

Dynamics of calcium and its roles in the dendrite of the cerebellar Purkinje cell

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Abstract. The calcium ion (Ca^{2+}) serves as an important cellular messenger with spatio-temporally highly dynamic patterns. Not only Ca^{2+} entering from the plasma membrane but also Ca^{2+} released from intracellular store sites play crucial roles in neurons as well as in other cell types. The cerebellar Purkinje cell shows a variety of spatio-temporal Ca^{2+} dynamics in its rich arborization, and the Ca^{2+} release mechanism mediated by IP_3 receptors and ryanodine receptors has been shown to be crucial for the induction of long-term depression, a form of synaptic plasticity, in this neuron. Purkinje cells in a model mouse line of human neurodegenerative disease, spinocerebellar ataxia type 1, showed abnormal Ca^{2+} release properties, which indicated tight regulation of Ca^{2+} dynamics in the wild type Purkinje cell. Quantitative analysis of the complex Ca^{2+} dynamics in the Purkinje cell dendrite is still in progress to elucidate the link between external stimuli and the resultant biochemical changes. (Keio J Med 52 (4): 244–249, December 2003)

Key words: calcium, cerebellar Purkinje cell, dendrite, IP3 receptor, Ca release

Introduction

In contrast to their simple structure and ubiquity, calcium ions (Ca^{2+}) are postulated to be potent intracellular signaling messenger molecules. Ca^{2+} concentration ($[Ca^{2+}]$) in the cytosol ($[Ca^{2+}]_i$) is strictly maintained at about 50–100 nM in the resting state. This low range of $[Ca^{2+}]_i$ is remarkable when compared to the $[Ca^{2+}]$ in the extra-cellular space (1–2 mM). The plasma membrane forms a tight barrier against this chemical gradient. When channel proteins on the plasma membrane are opened by several signaling mechanisms on stimuli, Ca^{2+} rushes into the cell from the outer space and $[Ca^{2+}]_i$ rises rapidly. Ca^{2+} is also stored intracellularly in certain kinds of organelles, namely the endoplasmic reticulum (ER) and mitochondria. Ca^{2+} stored in the ER is released upon stimulation through the ER membrance Ca^{2+} channels, namely inositol 1,4,5-trisphosphate receptor (IP_3R) and the ryanodine receptor. IP_3R opens when a second messenger molecule IP_3 binds to it, which is generated through the phospholipase C-activation pathway.[1] Both the IP_3R and ryanodine receptors are sensitive to cytosolic Ca^{2+} concentration,[2] which property adds a feature of ''Ca^{2+}-induced Ca^{2+} release'' to the functions of these receptors.[3] The ability of Ca^{2+} to stimulate the ryanodine receptor is modulated by cyclic ADP ribose in neurons[4–6] as well as in other tissues.[7] Since prolonged high $[Ca^{2+}]_i$ states lead to cytotoxic events,[8] extrusion mechanisms for Ca^{2+} immediately start to pump out the excess Ca^{2+} when cytosolic $[Ca^{2+}]$ is raised by these Ca^{2+} channels on the plasma and ER membranes. The extrusion mechanism for Ca^{2+} includes Ca^{2+}-binding proteins in the cytosol and Ca^{2+} pumps (*e.g.*, Ca-ATPase and Na^+/Ca^{2+} exchanger) on the plasma membrane, ER and mitochondria. $[Ca^{2+}]_i$ is thus strictly controlled, and $[Ca^{2+}]_i$ is actively increased to change the cell's state and its subsequent fate through affecting the activities of Ca^{2+}-dependent proteins. High $[Ca^{2+}]_i$ activates numerous Ca^{2+}-dependent enzymes, *e.g.*, calcineurin, calmodulin-dependent protein kinases, protein kinase C, and many other important proteins. For instance, calmodulin is activated by binding with up to four Ca^{2+} molecules and controls the activity of numerous channels and enzymes. Increase in $[Ca^{2+}]_i$ finally leads to fertilization, proliferation, exocytosis,

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muscle contraction, cell death, and numerous other consequences.[3] Thus Ca^{2+} regulation is very important from the clinical point of view. Mutations in Ca^{2+} channels on the plasma membrane cause a variety of symptoms, *e.g.*, migraine, ataxia and epilepsy, which form a part of channelopathy.[9] $[Ca^{2+}]_i$ needs to be increased for cellular functions, but it has to be brought back right away. Therefore a change of $[Ca^{2+}]_i$ is usually observed as a single transient or as multiple transients, the latter of which is sometimes referred to as Ca^{2+} oscillation or Ca^{2+} wave according to the pattern. Ca^{2+} oscillation in an egg at fertilization is a very well-known phenomenon that is crucial in commencing the initial steps of ontogeny.[10] The temporal pattern of Ca^{2+} oscillation has been shown to be important for the specificity of downstream targets for Ca^{2+} increase.[11]

In this lecture, I will focus on the significance and dynamics of $[Ca^{2+}]$ in the cerebellar Purkinje cell. The Purkinje cell in the cerebellar cortex is a neuron with a large dendritic arborization, which is very interesting in terms of Ca^{2+} dynamics. Various types of Ca^{2+} dynamics are known in the Purkinje cell dendrite. The Ca^{2+} spike is a regenerative Ca^{2+} influx that repeats periodically over the entire dendritic structure.[12] Widespread Ca^{2+} influx is observed on activation of one of the two excitatory synaptic connections, the climbing fiber input.[13,14] Activation of the other excitatory synapse, the parallel fiber-Purkinje cell synapse, results in Ca^{2+} increase in a relatively restricted area in the dendrite mediated by Ca^{2+} influx and Ca^{2+} release.[14–16] From a molecular biological point of view, many proteins involved in Ca^{2+} handling are abundantly expressed in the Purkinje cell. Calbindin D28k and parvalbumin are Ca^{2+}-binding proteins, rich in the Purkinje cell, that serve as Ca^{2+} buffers. Calsequestrin, a well-known low-affinity/high-capacity intraluminal ER protein, is not found in neurons other than the Purkinje cell.[17] Subtypes of ryanodine receptor are expressed.[18] In particular, the IP_3 receptor type 1 (IP_3R1) is predominantly expressed in the Purkinje cell.[19]

IP_3 receptor type 1 is required for Purkinje cell function

The molecular function and the expression pattern of IP_3 receptors had already been well-researched by the mid 1990's.[1] It had been shown that IP_3 receptors in the Purkinje cell certainly release Ca^{2+} from intracellular Ca^{2+} store sites in the neuron as well as in other cells by agonist stimulation[20] or by means of caged-IP_3 which releases active IP_3 molecules by UV-photolysis.[21] The molecular basis for the Ca^{2+} release by IP_3R through synaptic stimulation had been well established by this period. The excitatory synaptic transmitter, glutamate, activates a metabotropic type of glutamate receptor (mGluR),[22] which activates phospholipase C through trimeric G proteins, then IP_3 is produced. However, the physiological significance of the IP_3 receptor in the neuron was unclear. Ca^{2+} increase in the Purkinje cell dendrite was known to play an important role in the induction of a form of synaptic plasticity, long-term depression (LTD) at the parallel fiber-Purkinje cell synapse.[23,24] However, the amount of Ca^{2+} entering through Ca^{2+} channels on the plasma membrane was thought to suffice for the formation of LTD, since Ca^{2+} influx by synaptic activation was very large[13] and there had been no evidence showing that Ca^{2+} release actually occurred by synaptic activation. On the assumption that IP_3R should have some physiological roles in the Purkinje cell because of its Ca^{2+} releasing function and its abundant amount of expression, our group generated an IP_3R1-deficient mouse line by gene-targeting to understand its physiological functions.[25] Homozygous IP_3R1 deficient (IP_3R1-/-) mice had severe ataxia and tonic or tonic-clonic seizures and died by the weaning period. These neurological symptoms indicate that IP_3R1 is indeed an important molecule in the central nervous system. There was no obvious histological impairment in the IP_3R1-/- brain. Purkinje cells in cerebellar slice preparations from IP_3R1-/- mice showed normal electrophysiological properties, *e.g.*, Na^+ spike and Ca^{2+} spike firing, paired pulse facilitation of parallel fiber-Purkinje cell synapses, or paired pulse depression of the climbing fiber-Purkinje cell synapse.[25] Combined electrophysiological and imaging experiments showed that not Ca^{2+} influx by evoked depolarization but Ca^{2+} release evoked by flush photolysis of caged-IP_3 was lost in the IP_3R1-/- Purkinje cell.[26] All of these results indicate that the Purkinje cells in IP_3R1-/- mice develop without significant alteration in structure, excitability or synaptic connections except for the IP_3-induced Ca^{2+} release activity. LTD experiments were performed using these IP_3R1-/- Purkinje cells and it was revealed that LTD was totally lost in these IP_3R1-/- Purkinje cells.[26] To further confirm this result, another approach was taken. The monoclonal antibody 18A10 raised against IP_3R1 was shown to be a function-blocking antibody *in vitro*[27] and *in vivo*.[28] 18A10 was introduced into wild-type Purkinje cells in a slice through a recording patch pipette, and an LTD experiment was performed. LTD was blocked by 18A10.[26] Altogether, IP_3R1 was shown to be essential in the formation of LTD at the parallel fiber-Purkinje cell synapse. This finding was the earliest study giving solid evidence for a physiological role of IP_3R in the central nervous system.

Ryanodine receptor is also important in LTD

Ryanodine receptors are another class of Ca^{2+} release channel on the ER. Since ryanodine receptors are

also expressed in the Purkinje cell dendrite,[29] and they function as a Ca^{2+}-induced Ca^{2+} release channel in the Purkinje cell,[30] they were also thought to be one of the candidates responsible for the Ca^{2+} signaling in the LTD induction. To clarify this question, we performed LTD experiments in Purkinje cells in primary culture preparation with various blockers against ryanodine receptors. A high concentration of ryanodine, which keeps the ryanodine receptor/channel open and blocks the receptor activity, and ruthenium red, which inhibits the ryanodine receptor, blocked the LTD induction,[31] showing the possibility that ryanodine receptors are also involved in LTD induction. Though it is still unclear whether ryanodine receptors play a role in LTD induction in slice preparation, ryanodine receptors could work in concert with IP_3Rs by enhancing Ca^{2+} released by IP_3Rs or enhancing Ca^{2+} that entered the cytoplasm through Ca^{2+} channels on the plasma membrane. Together with the importance of IP_3R in LTD induction, Ca^{2+} release mechanisms have been shown to play an important role in neuronal function. This view posed a question: What is the difference between the Ca^{2+} dynamics generated solely by Ca^{2+} influx and those by the combination of Ca^{2+} influx and Ca^{2+} release? Ca^{2+} influx could have compensated for Ca^{2+} release with stronger stimulation, but it seemed not to be the case. The answer was thought to come from understanding the precise nature of the Ca^{2+} dynamics in various situations in the Purkinje cell dendrite.

Aberrant Ca^{2+} release in Purkinje cells of the SCA1 model mouse

A mutant mouse line provided an example showing tight regulation of the patterns in Ca^{2+} dynamics in the Purkinje cell dendrite. Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurological disorder in humans characterized by ataxia and brain stem dysfunction. Like several other neurodegenerative diseases, SCA1 is caused by the expansion of a CAG trinucleotide repeat resulting in an expanded polyglutamine tract in its gene product, ataxin-1.[32] In SCA1, the consistent neuropathological finding is loss of Purkinje cells in the cerebellar cortex and loss of neurons in the pontine nuclei.[33] Lines of transgenic mice that expressed the mutant form of the *SCA1* gene developed ataxia and Purkinje cell pathology. Importantly, the eventual development of ataxia is attributable not to cell death *per se* but to cellular dysfunction and morphological alterations that occur long before neuronal death.[34] It was found that several key neuronal genes are specifically altered in the Purkinje cells of the mutant cerebellum prior to any clinicopathologic manifestations.[35] Some of these genes are important for regulating intracellular Ca^{2+} dynamics. Among them, IP_3R1 and sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA2) are greatly reduced.[35] Therefore, it was of interest to study the electrophysiological and synaptic properties of SCA1 Purkinje cells, particularly those that affect stimulus-evoked $[Ca^{2+}]_i$ changes. Purkinje cells in SCA1 mice had Na^+ spikes and Ca^{2+} spikes, electrical responses of the parallel and climbing fibers, that were qualitatively similar to those recorded from wild type mice.[14] The membrane resistance of SCA1 Purkinje cells was 3.3 times higher than that of wild type cells which correlated with the 1.7 times smaller cell body size. mGluR-mediated parallel fiber-evoked Ca^{2+} release from intracellular stores was observed in SCA1 Purkinje cells as well as in wild type Purkinje cells, and it was slightly easier to evoke the mGluR-mediated $[Ca^{2+}]_i$ release in SCA1 Purkinje cells. In response to climbing fiber stimulation, most Purkinje cells in mutant and wild type mice had rapid widespread $[Ca^{2+}]_i$ changes, recovering in less than 200 ms. Some SCA1 Purkinje cells showed a slow, localized, secondary Ca^{2+} transient following the initial climbing fiber Ca^{2+} transient, which may reflect release of Ca^{2+} from intracellular stores. Recently climbing fiber stimulation was shown to activate IP_3 production[36] and to evoke Ca^{2+} release[37] in the Purkinje cell dendrites. The latter study was performed by enhancing the mGluR activity with a glutamate uptake inhibitor. The Ca^{2+} release observed in the SCA1 Purkinje cell is an exceptional case in that climbing fiber stimulation evoked apparent Ca^{2+} release without pharmacological enhancement. In conclusion, the basic physiological properties of the mutant Purkinje cells other than Ca^{2+} release were similar to those of wild type neurons, even with the dramatic alteration in their morphology. It was furthermore surprising that Ca^{2+} release activity was more hyperactive than depressed in spite of the down-regulation of Ca^{2+} handling molecules. This study conversely suggests that the Ca^{2+} release mechanism is tightly regulated in wild type Purkinje cells.

Ca^{2+} influx in the Purkinje cell dendrite

Ca^{2+} influx from the extra-cellular space and Ca^{2+} release from the intra-cellular Ca^{2+} store form a variety of Ca^{2+} dynamics in the Purkinje cell dendrite. This variation of Ca^{2+} dynamics may result in different consequences in the dendrite, *e.g.*, LTD or LTP in synaptic efficacy[38] and protein synthesis.[39] Furthermore, the Ca^{2+} increase events in the dendrite may be transmitted to the nucleus thereby affecting gene transcription patterns. A potent candidate for such information transmission mechanism from synapse to nucleus is the calmodulin (CaM)- Ca^{2+}/CaM-dependent protein kinase IV (CaMK IV)- cAMP-response element-binding protein (CREB; a transcription factor) pathway.[40] It

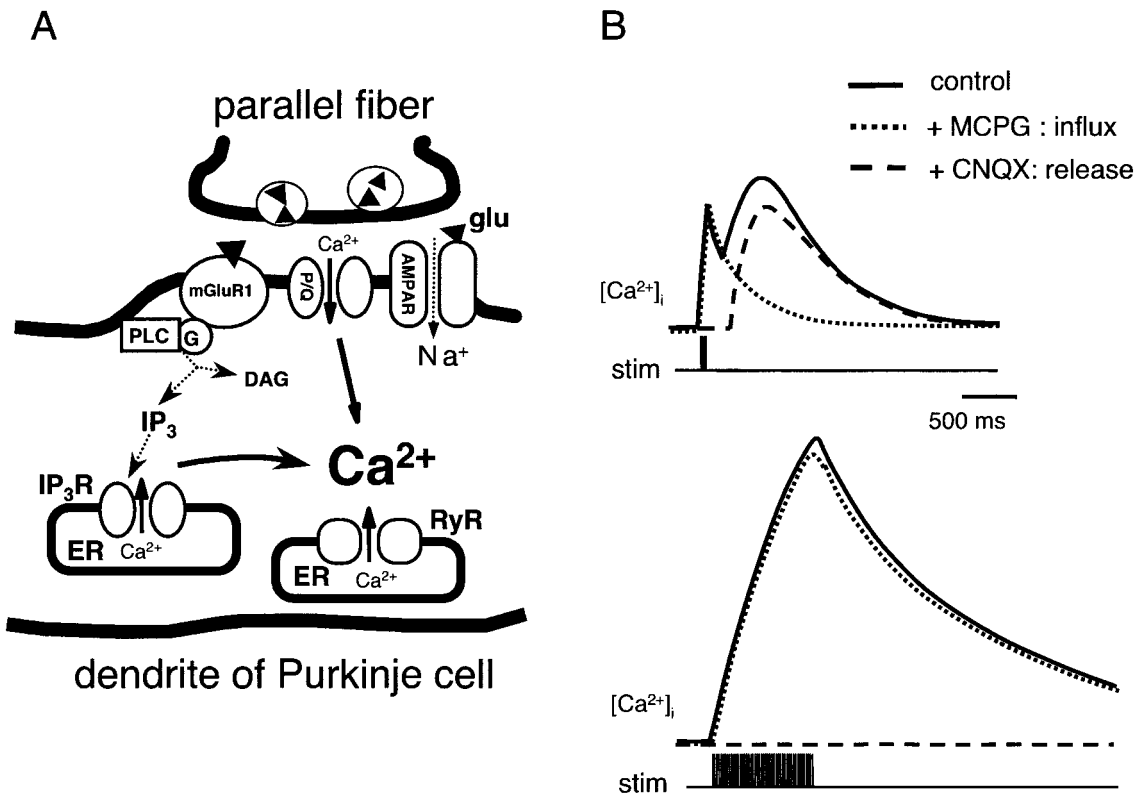

Fig. 1 Ca^{2+} dynamics in the Purkinje cell dendrite. A. Molecular mechanisms underlying Ca^{2+} dynamics in the parallel fiber-Purkinje cell synapse are shown. Excitatory synaptic transmitter, glutamate (glu; filled triangle), released from the parallel fiber terminal activates the AMPA-type glutamate receptor (AMPAR) and metabotropic glutamate receptor type 1 (mGluR1). AMPAR depolarizes the membrane potential by passing Na^+. The depolarization then opens the P/Q type voltage-gated Ca^{2+} channel (P/Q). Activated mGluR1 produces IP_3 and diacylglycerol (DAG) through the trimeric G protein (G) and phospholipase C pathway. IP_3 then opens IP_3R on the ER and Ca^{2+} is released from the ER. The ryanodine receptor (RyR) is another Ca^{2+} release channel on the ER which is activated by high $[Ca^{2+}]$. B. Time course of $[Ca^{2+}]_i$ in a small area of the Purkinje cell dendrite evoked by parallel fiber stimulation. Upper panel: when the parallel fiber is modestly stimulated (50 Hz × 5 times), Ca^{2+} transient shows a biphasic pattern. The first phase derives from Ca^{2+} influx and the second phase from Ca^{2+} release, since the first phase was blocked by CNQX, an AMPAR antagonist (broken line) and the second phase was blocked by MCPG, an antagonist against mGluR1 (dotted line).[15] Lower panel: when the parallel fiber is intensively stimulated (50 Hz × 50 times), the Ca^{2+} transient becomes very large in intensity and monotonous. Ca^{2+} influx mostly contributes to this large Ca^{2+} increase, because CNQX diminished the Ca^{2+} transient but MCPG affected it little.[47] The ordinate of the plots ($[Ca^{2+}]_i$) are scaled. Stimulation patterns are indicated at bottoms of the plots (stim).

has been shown that the activation of CREB depends on the stimulation pattern and types of Ca^{2+} influx pathway.[40–42] Thus it is important to describe the pattern of Ca^{2+} dynamics in the dendrite before discussing the relationship between Ca^{2+} changes and biological consequences. So far, numerous studies have been performed on the Ca^{2+} dynamics in this particular neuron. The dynamic change of Ca^{2+} in the Purkinje cell dendrite was measured optically by means of Ca^{2+}-sensitive dyes in the late 1980's by the pioneering attempts of Ross's[43] and Llinas's groups.[44] Since then, with refinement of the technique, various aspects of Ca^{2+} dynamics in Purkinje cell dendrite have been described. A Ca^{2+} spike was found to be a synchronized Ca^{2+} transient over the entire dendrite of the Purkinje cell.[12] Climbing fiber input induces widespread Ca^{2+} transients similar to a Ca^{2+} spike while parallel fiber input results in a local Ca^{2+} increase.[13] In the late 1990's, spatial resolution of the imaging technique was improved by means of the confocal microscope to resolve single spines on dendrites where parallel fibers from synapses. Ca^{2+} increase was observed in the spine.[45] Ca^{2+} release was measured by parallel fiber stimulation in fine dendrites (Fig. 1).[15,16] However, the current knowledge about Ca^{2+} dynamics in this particular neuron is insufficient in the quantitative point due to technical difficulties in estimating absolute $[Ca^{2+}]$ in small structures such as neuronal dendrites,[46] which is essential to discuss the link between Ca^{2+} dynamics and the resultant biochemical consequences. To approach this issue, we started with a quantitative analysis of Ca^{2+} dynamics in the Purkinje cell dendrite.

Whole-cell patch clamp and imaging with fluorescent Ca^{2+} and Na^+ indicators were performed in mouse

Purkinje cells in slice preparation.[47] Membrane potentials, $[Ca^{2+}]$ and $[Na^{+}]$ were observed simultaneously. High frequency parallel fiber stimulation (50 Hz 3–50 times) depolarized Purkinje cells and caused a large increase in $[Ca^{2+}]$ at anatomically expected sites in dendrites for seconds that turned out to reach ~200 μM of Ca^{2+}. This amplitude of $[Ca^{2+}]_i$ was much higher than ever reported in the neuron by synaptic activation. The $[Ca^{2+}]$ increase was confined to a small area in the dendrite and sustained for a longer period of time as more stimuli were applied. However increase in $[Na^{+}]$ was sustained for tens of seconds and diffused away from the activated site. Ca^{2+} release was observed as a separate delayed peak to the preceding peak formed by Ca^{2+} influx at fewer parallel fiber stimuli (3–5 times), and the delayed peak was hidden by the initial Ca^{2+} influx at larger numbers of parallel fiber stimuli (Fig. 1). It was shown by pharmacological experiments that not Ca^{2+} release but rather Ca^{2+} influx contributes to most of the large Ca^{2+} increase brought about by 50 shocks of parallel fibers. Although it had already been reported that the AMPA-type glutamate receptor (AMPA receptor)[48] and the P/Q-type Ca^{2+} channel are major players at the postsynaptic side of the parallel fiber-Purkinje cell synapse, pharmacological interventions with this simultaneous Na^{+} and Ca^{2+} imaging study clearly demonstrated that the AMPA receptor and the P/Q-type Ca^{2+} channel exclusively pass Na^{+} and Ca^{2+} respectively, at the very distal end of the dendritic tree. Ca^{2+} concentration had a linear relationship with the number of parallel fiber stimuli. Thus, at a smaller number of parallel fiber stimuli, Ca^{2+} dynamics were comprised a complex of Ca^{2+} influx and Ca^{2+} release, and at intensive stimuli, Ca^{2+} dynamics became simple and followed the number of parallel fiber stimuli. Thus different patterns of parallel fiber input produce different patterns of Ca^{2+} dynamics, which difference could lead to different biochemical consequences. Observing changes in localizations and activities in Ca^{2+}-dependent proteins with such a variety of Ca^{2+} dynamics will link the variety of stimuli and the variety of cell responses and fates.

Conclusion

Ca^{2+} plays an important role as a messenger with very dynamic spatial-temporal patterns in the neuron as well as other cells. The fact that Ca^{2+} release is a requisite for the induction of LTD in cerebellar Purkinje cell provides an example of the complex nature of Ca^{2+} signaling. Different types and combinations of stimuli yield a variety of Ca^{2+} signaling patterns, which in turn lead to differential biochemical effects. To clarify this flow of information process, a precise description of the Ca^{2+} dynamics is indispensable.

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