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Ca^{2+} Signaling in Cerebellar Purkinje Neurons—Editorial

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Abstract Tight regulation of calcium (Ca^{2+}) dynamics is critical for all neurons. Ca^{2+} is a major mediator of cellular excitability, synaptic plasticity, and regulation of transcription, amongst others. Recent years have seen major developments in terms of understanding the roles of Ca^{2+} signals in the cerebellar circuitry, especially for Purkinje neurons and granule cells. The unique morphology of Purkinje neurons serves as a platform to unravel the secrets of Ca^{2+} homeostasis in cerebellar microcircuits. This special issue covers recent advances in Ca^{2+} signaling and imaging, and highlights the importance of spatiotemporal compartmentalization underlying Ca^{2+} dynamics. Sorting out the pieces of the puzzle of homeostatic regulation of Ca^{2+} remains an instrumental step to start rational therapies of Ca^{2+} deregulation.

Keywords Calcium · Purkinje neuron · Signaling · Circuits · Imaging

This issue of *The Cerebellum* summarized and updates, both by review articles and original articles, the role of Ca^{2+} signaling in synaptic function of the Purkinje neuron and the molecular processes involved. Ca^{2+} is essential to the normal function of all CNS neurons [1]. Ca^{2+} is a key mediator of cellular excitability as well as an important second messenger. As a mediator of excitability, Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs) located on the plasma membrane plays a prominent role in defining the electrical properties of the neuron, communicating electrical signals initiated by synaptic events, and regulating the activities of membrane ion channels. As a second messenger, Ca^{2+} instructs molecular pathways that regulate biochemical homeostasis and initiate activity-dependent plastic changes that enable functions such as memory and learning.

Our understanding of Ca^{2+} signaling and the various roles played by Ca^{2+} in CNS neurons is now quite advanced. Studies of the cerebellar Purkinje neurons have been instrumental to this understanding. The key role played by Purkinje neurons is derived in part from its unique morphological characteristics including the large size of the soma and dramatic dendritic arbor, which enabled high-resolution electrophysiological, anatomical, biochemical, and Ca^{2+} imaging studies at the single-cell level. In addition, Purkinje neurons play a central role in cerebellar function. Thus, understanding processes such as Ca^{2+} signaling and Ca^{2+} dynamics that underlie the physiological function of the Purkinje neuron is basic to an understanding of cerebellar function.

The Purkinje neuron is the primary neuron of the cerebellar cortex and the only output neuron of the cortical region of the cerebellum. The Purkinje neuron functions as an important integrator of excitatory input signals from other brain regions. These excitatory signals are transmitted to the Purkinje neurons via excitatory synapses from the parallel fibers of the cerebellar granule neurons and climbing fibers of the inferior olivary neurons. Inhibitory interneurons of the molecular layer of the cerebellum (e.g., basket cells, stellate cells) and innervation from other

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Purkinje neurons play a contributing role in the integration process. These excitatory and inhibitory synaptic events interact with the intrinsic electrophysiological properties of the Purkinje neurons that underlie the high-frequency baseline firing that is characteristic of Purkinje neurons to shape the final output signal. The output signal from the Purkinje neuron is transmitted via inhibitory synaptic events to the neurons of the cerebellar nuclei, which communicate the information to downstream and upstream circuits. As is discussed in the articles in this issue, Ca^{2+} signaling plays a key role in all steps of the input-to-output translation process.

Ca^{2+} signaling in CNS neurons is determined by morphological features and molecular components of the neurons and varies in detail across neuronal types. In this issue, Empson and Knopfel describe the various molecules that are essential for normal Ca^{2+} signaling in the Purkinje neuron and the role played by these molecules in the synaptic function of the Purkinje neuron [2]. A number of molecular components are involved, including: (a) plasma membrane ion channels (e.g., VGCCs, cation channels) that allow controlled Ca^{2+} influx (Fig. 1); (b) G-protein-coupled receptors (e.g., metabotropic glutamate receptor 1), also localized to the plasma membrane, that activate signal transduction pathways linked to Ca^{2+} release from intracellular Ca^{2+} stores located in the endoplasmic reticulum; (c) membrane channels on the endoplasmic reticulum (IP3 receptors, ryanodine receptors) that control Ca^{2+} flux from Ca^{2+} stored within the endoplasmic reticulum; (d) Ca^{2+}-binding proteins (e.g., calbindin, parvalbumin) that regulate free Ca^{2+} levels in the cytosol by binding to Ca^{2+} and also act as Ca^{2+} transporters; and (d) Ca^{2+} pumps that regulate cytosolic Ca^{2+} levels by extruding Ca^{2+} from the cytosol into the endoplasmic reticulum or across the plasma membrane to the extracellular space. These molecules are highly expressed in the soma and expansive dendrites of the Purkinje neuron.

The Cerebellar Classic paper included in this issue describes studies of Purkinje neurons by Llinas and Sugimori that were instrumental to an understanding of dendritic excitability and the role of Ca^{2+} signaling in dendritic function. These studies showed that Purkinje neuron dendrites are excitable and can generate a variety of active responses that are mediated by Ca^{2+} influx through VGCCs including action potentials [3, 4]. Later studies identified the particular VGCCs involved in action potential generation as the P/Q subtype VGCCs [4]. More recently, Purkinje neuron dendrites were also shown to express T-type VGCCs as discussed in the review article by Isope, Hildebrand, and Snutch in this issue [5]. T-type VGCCs contrast with the P/Q VGCCs in that they are activated at hyperpolarized potentials and rapidly inactivate. T-type VGCCs are inactive at the depolarized potentials that are necessary to activate P/Q VGCCs. Thus, dendritic expression of T- and P-type VGCCs enable Ca^{2+} influx over a range of membrane potentials with distinct spatial and temporal characteristics. Both T- and P/Q-type VGCCs are localized to the dendritic spines that mediate the excitatory synaptic input from parallel fibers. The T-type VGCCs can be activated by small synaptic events that would not be sufficient to raise the membrane potential to levels needed to activate P/Q VGCCs. Interestingly, both T- and P-type VGCCs are regulated by metabotropic glutamate receptor I (mGluR1), a G-protein-coupled receptor that is expressed in abundance in Purkinje neurons. mGluR1 activates a signal transduction pathway that induces Ca^{2+} release from intracellular stores and plays an important role in synaptic plasticity, a topic that is discussed in several articles in this issue.

Classically, Purkinje neurons have been a favored model for studies of cellular and synaptic physiology, and many fundamental concepts were developed from studies of Purkinje neurons. One of the most important contributions was an understanding of the mechanisms responsible for long-term depression (LTD), a form of synaptic plasticity that results in a reduction in the magnitude of excitatory synaptic events. LTD of excitatory transmission to the Purkinje neuron is induced by co-activation of parallel fiber and climbing fiber input and is highly dependent on Ca^{2+} signaling in the dendritic spines. Several articles in this issue cover various aspects of this synaptic property. The article by Finch, Tanaka, and Augustine [6] provides an overview of LTD and underlying mechanisms, whereas the article by Goto and Mikoshiba [7] focuses on the role of the IP3 receptor, a protein that was isolated from the cerebellum by Mikoshiba and colleagues and initially called P_{400} [8]. Later work by Mikoshiba and colleagues confirmed that P_{400} was a functional IP3 receptor and cloned the gene for this receptor (type 1 IP3R) [9, 10]. The article by Schmidt, Arendt, and Eilers [11] outlines the importance of endogenous Ca^{2+} buffers (parvalbumin, calbindin) in dendritic function and in the regulation of the spread of LTP within the dendritic structure. Their studies show that there is a steep gradient in intracellular Ca^{2+} between active and inactive dendritic branches resulting from an interplay between diffusion and extrusion. Ca^{2+} buffering and its relationship to the firing patterns of Purkinje neurons are also addressed in the article by Anwar, Hong, and De Schutter [12], who discuss several models of Ca^{2+} dynamics and how effectively they can simulate the physiological events generated by the Purkinje neurons. Their studies show that the inclusion of Ca^{2+} buffers, Ca^{2+} pumps, and Ca^{2+} diffusion in the dynamic model provides a more realistic picture of the contribution of Ca^{2+}-activated K^+ channels to the Ca^{2+} spike shape in dendrites.

Homeostatic control of Ca^{2+} signaling is essential for normal neuronal function, as either an excessive or insufficient cytosolic Ca^{2+} level can lead to abnormal function and cell death. Synaptic events and intrinsic firing are the main

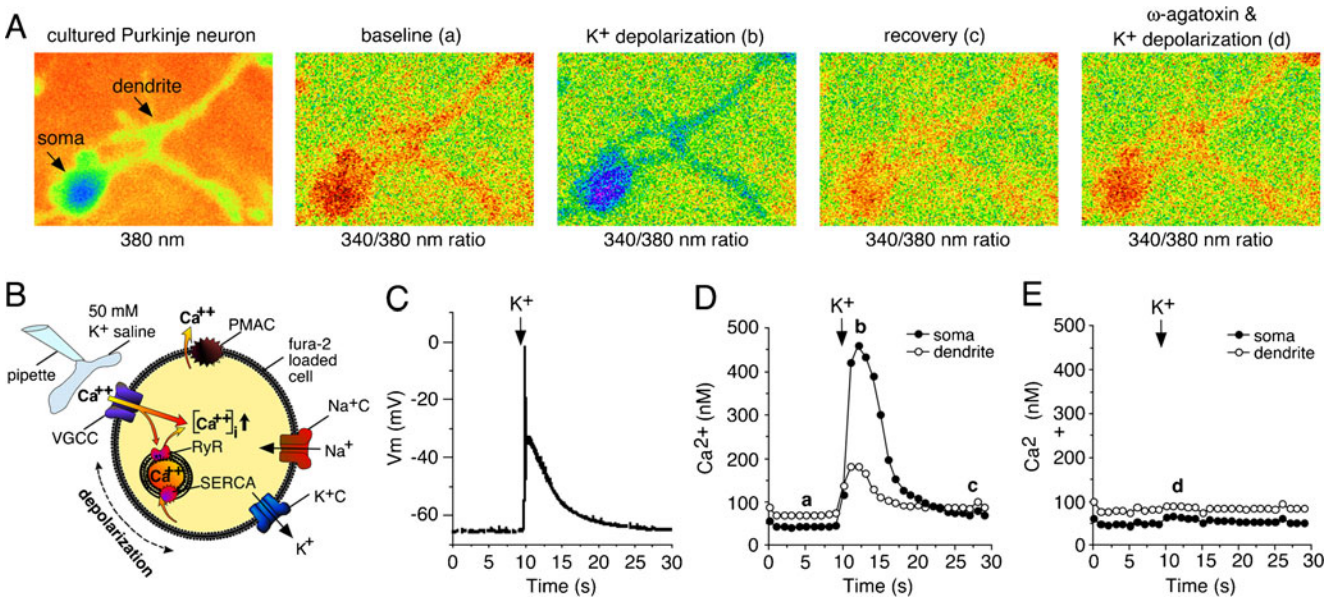

Fig. 1 Ca^{2+} signal evoked by membrane depolarization in the somatic and dendritic region of a cultured Purkinje neuron. **A** Digitized fluorescent images in pseudocolor showing the relative level of intracellular Ca^{2+} in a cultured Purkinje neuron loaded with the Ca^{2+}-sensitive dye fura-2. The fura-2 dye load is shown in the 380-nm image. Both the soma and dendrites are filled with dye. Other images show (*left* to *right*) the ratio of the 340/380-nm measurement under baseline conditions, at the peak of the response to stimulation with a brief (1 s) pulse of K^+ saline (at the *arrow*) to depolarize the neuron, after recovery from the K^+ depolarization and during a subsequent application of K^+ after addition of the P-type Ca^{2+} channel blocker ω-agatoxin to the recording chamber. The K^+ saline (50 mM) was prepared by substituting KCl for NaCl in physiological saline. The culture method and experimental protocols were as described previously [15]. Graphs in **D** and **E** show the quantification of the Ca^{2+} signals produced by K^+ depolarization under control conditions (**D**) and in the presence of ω-agatoxin (**E**). ω-Agatoxin completely blocked the Ca^{2+} signal consistent with a primary role for P-type VGCCs in the Purkinje neurons [4]. In **D** and **E**, the letters *a*, *b*, *c*, or *d* indicate the time when the 340/380 images were collected. A representative recording of the membrane depolarization that elicited the Ca^{2+} signal in **D** is shown in the graph in **C**. A diagram showing the various Ca^{2+} signaling molecules involved in the Ca^{2+} response to K^+ depolarization is shown in **B**. K^+ depolarizes the membrane potential resulting in activation of voltage-gated Ca^{2+} (*VGCCs*), Na^+ (*Na^+C*), and K^+ (*K^+C*) channels. Ca^{2+} influx through the VGCCs increases the intracellular Ca^{2+} concentration, which in turn activates ryanodine receptors (*RyR*) on the endoplasmic reticulum and release of Ca^{2+} from intracellular stores. The membrane potential recovers to baseline levels as the K^+ diffuses from the cell, resulting in closure of the voltage-gated channels. The intracellular Ca^{2+} levels are returned to baseline by Ca^{2+} pumps that transport the Ca^{2+} to the intracellular stores (*SERCA*) or pump it out of the cell (*PMAC*)

mechanisms responsible for activation of VGCCs and Ca^{2+} influx into the cytosol of Purkinje neurons via the plasma membrane (Fig. 1). Ca^{2+} release from intracellular stores controlled by IP3 and ryanodine receptors also contributes to cytosolic Ca^{2+}. Alterations that affect this influx can disrupt Ca^{2+} homeostasis. Thus, if the firing pattern of Purkinje neurons is altered, Purkinje neuron function and, consequently, cerebellar function are likely to be altered. Studies discussed in the article by Kasuma and Bezprozvanny in this issue illustrate the linkage between dysregulated Ca^{2+} signaling in the Purkinje neuron and the pathogenesis of spinocerebellar ataxias (SCA), a heterogeneous group of neurodegenerative disorders [13]. SCAs in humans and animal models are characterized by ataxia and cerebellar pathology including cell death. In SCA2, a polyglutamine expansion coded by a CAG repeat in the coding region of the ATXN2 gene underlies the disease. The ATXN2 gene codes for a protein called ataxin-2. Experiments show that an important consequence of the altered ataxin-2 protein is enhanced Ca^{2+} release from intracellular stores, which is produced by an interaction between the altered ataxin-2 protein and IP3 receptor. Altered Ca^{2+} signaling is also found in other SCAs. For example, polyglutamine expansion occurs in the P/Q VGCC in SCA6 resulting in abnormal function of this channel and altered Ca^{2+} influx. In other SCAs, altered levels of mRNA for Ca^{2+} signaling proteins have been identified. The authors propose that altered Ca^{2+} signaling is a key factor in the pathogenesis of many SCAs and can occur through a variety of mechanisms.

Studies by Ovspian and Friel described in this issue [14] also demonstrate the consequence of altered Ca^{2+} signaling to Purkinje neuron function. In this case, the altered Ca^{2+} signaling occurs in inhibitory interneurons that provide control of Purkinje neuron firing. Their studies utilize the learner mutant mouse model in which there is a mutation in the P/Q VGCC that results in loss of function. This mutation resulted in enhanced inhibitory synaptic input to the Purkinje neuron

from the molecular layer interneurons. This effect involved enhanced release of Ca^{2+} from intracellular Ca^{2+} stores in the presynaptic terminals of the inhibitory interneurons. As a consequence of the increased inhibition, the spontaneous firing properties of the Purkinje neurons were altered, an effect likely to disrupt normal cerebellar function.

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