Cell Calcium 52 (2012) 422–432

Contents lists available at SciVerse ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Developmental profile of localized spontaneous Ca^{2+} release events in the dendrites of rat hippocampal pyramidal neurons

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ARTICLE INFO

Article history:
Received 6 April 2012
Received in revised form 3 July 2012
Accepted 2 August 2012
Available online 28 August 2012

Keywords:
Rat
Pyramidal neuron
Hippocampus
Dendrite
Calcium release
Development

ABSTRACT

Recent experiments demonstrate that localized spontaneous Ca^{2+} release events can be detected in the dendrites of pyramidal cells in the hippocampus and other neurons (J. Neurosci. 29 (2009) 7833–7845). These events have some properties that resemble ryanodine receptor mediated "sparks" in myocytes, and some that resemble IP_3 receptor mediated "puffs" in oocytes. They can be detected in the dendrites of rats of all tested ages between P3 and P80 (with sparser sampling in older rats), suggesting that they serve a general signaling function and are not just important in development. However, in younger rats the amplitudes of the events are larger than the amplitudes in older animals and almost as large as the amplitudes of Ca^{2+} signals from backpropagating action potentials (bAPs). The rise time of the event signal is fast at all ages and is comparable to the rise time of the bAP fluorescence signal at the same dendritic location. The decay time is slower in younger animals, primarily because of weaker Ca^{2+} extrusion mechanisms at that age. Diffusion away from a brief localized source is the major determinant of decay at all ages. A simple computational model closely simulates these events with extrusion rate the only age dependent variable.

1. Introduction

Calcium concentration ($[Ca^{2+}]_i$) changes regulate physiological processes in all cells. In neurons, with the complex arborization and specializations in both dendrites and axons, it is clear that the location of these changes is critical. Ca^{2+} entry through voltage gated Ca^{2+} channels (VGCCs) in the presynaptic terminals and boutons regulates transmitter release. Ca^{2+} entry through postsynaptic ligand-gated receptors, especially NMDA receptors, regulates the induction of some forms of synaptic plasticity and other physiological processes. Widespread Ca^{2+} entry following backpropagating action potentials (bAPs) also contributes to some forms of plasticity. The role of Ca^{2+} release from internal stores in neurons has been less understood, with little data on the underlying mechanisms, spatial distribution, or functions of these changes under physiological conditions.

One form of Ca^{2+} release in pyramidal neurons results from synaptic activation of metabotropic glutamate receptors (mGluRs). This release occurs either as large amplitude propagating waves [1–3] or as smaller Ca^{2+} entry near spines [4]. Localized Ca^{2+} release, mediated either by inositol 1,4,5-trisphosphate (IP_3) receptors ("puffs") or by ryanodine (RyR) receptors ("sparks") have been described in a variety of preparations including neurons [5–8]. Two forms of localized Ca^{2+} release have been detected in dendrites. In slice cultures, localized Ca^{2+} release, associated with GABAergic synapses, has been shown to modulate the extension of dendritic processes and may contribute to other aspects of neuronal development [9]. In acute slices recent experiments indicate that faster Ca^{2+} release events occur spontaneously in dendrites [10], presynaptic terminals [11,12], and cell bodies [11]. Spontaneous Ca^{2+} release events in dendrites occur primarily near branch points and their frequency can be modulated by changes in membrane potential and weak mGluR mediated synaptic trains [10]. The specific functions of these Ca^{2+} release events are not clear.

To help understand the significance of the Ca^{2+} release events in dendrites we examined their properties in CA1 pyramidal neurons from animals of different ages. Most properties were similar but two parameters changed with age. The amplitudes were larger and the decay times were slower in younger animals. The increase in decay time with age was primarily due to the stronger combined effect of plasma membrane, Na/Ca exchange, and SERCA pumps in older animals. The small changes in intrinsic properties

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http://dx.doi.org/10.1016/j.ceca.2012.08.001

with age suggest that these Ca^{2+} release events may have a general signaling role in neurons and are not just important during development.

2. Methods

2.1. Whole-cell recording and stimulation

Transverse hippocampal slices (300 μm thick) from Sprague–Dawley rats of different ages (P3–P80) were prepared as previously described [1,12]. Animals were anaesthetized with isoflurane and decapitated using procedures approved by the Institutional Animal Care and Use Committees of New York Medical College and the Marine Biological Laboratory. For older animals just before decapitation the heart was perfused with ice cold solution artificial cerebrospinal fluid (ACSF) composed of (mM): 80 NaCl, 2.5 KCl, 0.29 $CaCl_2$, 7 $MgCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 75 sucrose, 10.1 glucose, 1.3 ascorbate and 3 pyruvate. Slices were cut in the same solution. They were incubated for at least 1 h in solution consisting of (mM): 124 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10.1 glucose, 1.3 ascorbate and 3 pyruvate, bubbled with a mixture of 95% O_2–5% CO_2, making the final pH 7.4. Normal ACSF composed of (mM): 124 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10.1 glucose, was used for recording.

Submerged slices were placed in a chamber mounted on a stage rigidly bolted to an air table and were viewed with a 60× water-immersion lens in an Olympus BX50WI microscope mounted on an *X–Y* translation stage. Somatic whole-cell recordings were made using patch pipettes pulled from 1.5 mm outer diameter thick-walled glass tubing (1511-M, Friedrich and Dimmock, Millville, NJ, USA). Tight seals on CA1 pyramidal cell somata were made with the 'blow and seal' technique using video-enhanced DIC optics to visualize the cells [13]. For most experiments the pipette solution contained (mM): 145 potassium gluconate, 4 NaCl, 4 Mg-ATP, 0.3 Na-GTP, 14 Na-phosphocreatine, and 10 Hepes, pH adjusted to 7.3 with KOH. Final osmolarity was 297 mOs. This solution was supplemented with either 25 or 50 μM OGB-1 (Oregon Green BAPTA-1, a high affinity calcium indicator; Molecular Probes, Eugene, OR, USA). Except during experiments measuring the buffering capacity (see below) we usually waited at least 15 min after membrane rupture before making measurements. Temperature in the chamber was maintained between 29 and 33 °C.

2.2. Dynamic $[Ca^{2+}]_i$ measurements

Time-dependent $[Ca^{2+}]_i$ measurements from different regions of the pyramidal neuron were made as previously described [10,14]. We used two cameras for our measurements. In earlier experiments we used a high spatial resolution (512 × 512), Quantix 57 CCD camera (Photometrics, Tucson, AZ), with 5 × 5 pixel binning to read out at 40 Hz. This camera was adequate to measure Ca^{2+} release event amplitudes and most decay times but was too slow to measure rise times. It was controlled by a custom program (WDI) written in our laboratory. For later experiments we used a RedShirtImaging (Atlanta, GA) NeuroCCD-SMQ CCD camera, controlled by their Neuroplex software. The camera has 80 × 80 pixels and was read out at 500 Hz. A custom program (SCAN) was used to analyze and display the data from both cameras. After checking that both cameras produced similar results the data were combined to generate the distributions shown in the paper.

We measured fluorescence changes of OGB-1 with excitation at 494 ± 10 nm and emission at 536 ± 20 nm. $[Ca^{2+}]_i$ changes are approximated as $\Delta F/F$ where F is the fluorescence intensity when the cell is at rest and ΔF is the change in fluorescence during activity. Corrections were made for indicator bleaching during trials with events and spikes by subtracting a linear fit to the signal measured under the same conditions when the cell was not stimulated, after normalizing the unstimulated trace to the same value at the start of the trial, i.e. every point in the unstimulated trace was multiplied by the ratio of the mean of the F values for the first five sampled points (10 ms) of the two traces.

To examine the spatial distribution of postsynaptic $[Ca^{2+}]_i$ changes we selected pyramidal neurons that were in the plane of the slice and close to the surface. In these neurons when we used the 60× lens we could examine $[Ca^{2+}]_i$ increases over a range of 95 μm with the NeuroCCD-SMQ camera and 140 μm with the Quantix 57 camera. Increases in different parts of the cell are displayed using either selected regions of interest (ROIs) or a pseudo 'line scan' display [15].

2.3. Measurement errors

Since the Ca^{2+} release events were highly localized and fast there were several potential sources of measurement error that needed to be considered. The first issue was choosing the location and size of the region of interest (ROI) to measure the Ca^{2+} transients. The smallest pixel size with the NeuroCCD-SMQ camera was 1.2 μm^2, using the 60× water immersion lens. It is possible that the diameter of the dendrite and the size of the event initiation zone are less than 1.2 μm. Therefore, even without considering light scattering through the slice tissue and the possibility of out of focus elements, each pixel probably detected signals from areas larger than the source of the Ca^{2+} release events. Consequently, it is likely that there were spatial inhomogeneities within the chosen ROIs. Similarly, the time course of the Ca^{2+} release transients probably differed for sites within the ROI, especially at short times before diffusion smoothed the transients. This problem was compounded by the fact that it was often necessary to select ROIs larger than one pixel in order to detect signals with sufficient S/N to make quantitative measurements. If the ROIs extend beyond the dendrite boundaries then background tissue fluorescence affects the measurements of peak transient amplitudes and a correction for this autofluorescence should be applied. Supplementary Fig. S1 illustrates this problem. Choosing a larger ROI resulted in smaller measured peak amplitudes (Fig. S1A). Choosing ROIs with horizontal or vertical binning (Fig. S1B and C) affected the amplitude but not as much as square binning. Since horizontal binning affects the amplitude of bAP transients and Ca^{2+} release events in a similar manner (both require the same autofluorescence correction) we found that the ratio of the measured Ca^{2+} release event amplitude to the measured bAP signal amplitude was relatively insensitive to changes in horizontal binning (Fig. S1D), making this ratio less subject to error. Similarly, we found that binning affected the measured decay times of the event transients (Fig. S2). The best choice was to use horizontal binning (Fig. S2B) since it minimized the effects of spatial inhomogeneities in the event transient. To measure the decay time (FDHM; full duration at half maximum amplitude) of the spike evoked transient we used vertical binning since the decay time was approximately constant along the dendrite.

To improve the S/N of some of the measurements we digitally filtered the event transients with a 3-point or 5-point smoothing algorithm. The 3-point filter replaced each point (sampled at 500 Hz) with a weighted average (1,2,1) of the point and the points before and after. The 5-point filter replaced the point with a weighted average (1,2,3,2,1) of the point and its nearest two neighbors on each side.

Since the S/N of many Ca^{2+} release event signals was not high and the traces were noisy it was not clear if these filters affected event parameters. To examine this issue we applied the filters to averaged Ca^{2+} release events, which had higher S/N. This test