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ESTIMATING INTRACELLULAR Ca^{2+} CONCENTRATIONS AND BUFFERING IN A DENDRITIC INHIBITORY HIPPOCAMPAL INTERNEURON

C. W. LIAO AND C. C. LIEN*

Institute of Neuroscience and Brain Research Center, National Yang-Ming University, 155, Section 2, Li-Nong Street, Taipei 112, Taiwan, Republic of China

Abstract—Calcium is known to regulate several phenomena like neuronal excitability and plasticity. Interestingly, the spatiotemporal profile of dendritic calcium depends on several processes, specific to each neuronal type. In this study, we investigated Ca^{2+} buffering and action potential (AP)-evoked Ca^{2+} signaling in the dendrites of anatomically identified oriens lacunosum-moleculare (O-LM) cells, a major type of dendrite-targeting interneurons in the hippocampal CA1 region, using a combination of whole-cell patch-clamp recording and fast Ca^{2+} imaging in acute rat brain slices. Cells were loaded with fluorescent Ca^{2+} indicators fura-2 or Oregon Green BAPTA-1 (OGB-1) via patch-clamping electrode, and the effect of fura-2 on AP-evoked dendritic Ca^{2+} transients was determined by ratiometric Ca^{2+} imaging. To estimate intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) and endogenous Ca^{2+}-binding ratio (κ_s) in the proximal dendrite, fluorescence signals were converted into $[Ca^{2+}]_i$ using the ratioing method and were analyzed on the basis of the "single compartment model." Resting $[Ca^{2+}]_i$ was 22±5 nM and the build-up of $[Ca^{2+}]_i$ during a single AP was up to 656±226 nM. Analysis of Ca^{2+} transients revealed that O-LM cells have a relatively low endogenous Ca^{2+}-binding ratio (κ_s): the κ_s was 20±8 estimated during fura-2 loading and 27 estimated under steady-state fura-2 concentrations, respectively. To further examine the spatial profile of dendritic Ca^{2+} transients, we measured somatic AP-evoked Ca^{2+} transients beyond proximal dendrites using OGB-1. Dendritic Ca^{2+} transients evoked by single APs or AP trains are not limited to regions close to the soma. The amplitude and decay of $[Ca^{2+}]_i$ associated with backpropagating APs are relatively independent of the distance from the soma. In sum, O-LM cells exhibit low endogenous Ca^{2+}-binding ratios and relatively distance-independent Ca^{2+} dynamics in the dendrites. These special features of Ca^{2+} signaling in O-LM cells may have important functional implications for both normal and pathological conditions.

Key words: GABAergic interneuron, Ca^{2+} binding ratio, fura-2, dendrite, Ca^{2+} imaging.

*Corresponding author. Tel: +886-2-2826-7325; fax: +886-2-2821-5307.
E-mail address: cclien@ym.edu.tw (C. C. Lien).

Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; bAPs, backpropagating action potentials; BC, basket cell; GluR, glutamate receptor; LTD, long-term depression; LTP, long-term potentiation; OGB-1, Oregon Green BAPTA-1; O-LM cell, oriens lacunosum-moleculare cell; ROI, region of interest; STDP, spike timing-dependent plasticity; VGCCs, voltage-gated Ca^{2+} channels.

Quantitative features of Ca^{2+} homeostasis in the neuronal cytoplasm are important because they determine the spatiotemporal profile of Ca^{2+} signaling that controls the development, plasticity, regeneration, and cell death in the nervous system (Zheng and Poo, 2007; Neher, 2008). These features include the dynamics of Ca^{2+} sources, Ca^{2+} buffering and diffusion. Intracellular Ca^{2+} buffering systems consist of Ca^{2+}-binding proteins, plasma membrane extrusion, and sequestration into intracellular organelles (Pottorf et al., 2000; Hartmann and Konnerth, 2005; Müller et al., 2007; Scheuss et al., 2006; Stocca et al., 2008). Although the spatiotemporal profile of dendritic Ca^{2+} dynamics varies greatly among different types of neurons (Lee et al., 2000; Kaiser et al., 2001; Goldberg et al., 2003, 2004; Goldberg and Yuste, 2005; Rozsa et al., 2004; Aponte et al., 2008), accumulating evidence supports the prevalent view that γ-aminobutyric acid-releasing (GABAergic) interneurons possess higher Ca^{2+} buffer capacities than those of principal neurons (Lee et al., 2000; Rozsa et al., 2004; Goldberg and Yuste, 2005; Aponte et al., 2008).

GABAergic interneurons consist of a heterogeneous population of cells (reviewed by Klausberger and Somogyi, 2008). At least two classes of functionally distinct interneurons are known to exist in the hippocampus (McBain and Fisahn, 2001; Jonas et al., 2004). Somatic inhibitory interneurons, such as fast-spiking basket cells (BCs), control the spike initiation of principal neurons via axonal innervations onto perisomatic areas of principal neurons (Cobb et al., 1995; Miles et al., 1996; Kraushaar and Jonas, 2000), whereas dendritic inhibitory interneurons, such as oriens lacunosum-moleculare (O-LM) cells, regulate dendritic Na^+ or Ca^{2+} spikes and synaptic plasticity by innervating dendritic domains of principal neurons (Miles et al., 1996; Pouille and Scanziani, 2004). These two distinct interneuron subtypes differ not only in their intrinsic properties, such as neurochemical contents, Ca^{2+}-binding proteins, ion channels, and transmitter receptors (Koh et al., 1995; Freund and Buzsáki, 1996; Martina et al., 1998; Lien et al., 2002; Pouille and Scanziani, 2004; Aponte et al., 2006), but also in their synaptic and network functions (Pouille and Scanziani, 2004; Klausberger et al., 2003; Klausberger and Somogyi, 2008). A recent study showed that fast-spiking parvalbumin-expressing BCs in the dentate gyrus exhibit efficient Ca^{2+} buffer capacity in the proximal dendrites (Aponte et al., 2008). In contrast to BCs and other types of GABAergic interneurons (Kaiser et al., 2001; Goldberg et al., 2003, 2004; Goldberg and Yuste, 2005; Rozsa et al., 2004), the Ca^{2+} handling properties of O-LM

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cells, a major type of dendritic inhibitory interneurons in the hippocampus, remain unexplored.

Understanding the Ca^{2+} buffering and Ca^{2+} load in distinct types of interneurons may be helpful for understanding the selective resistance or vulnerability of these neurons under pathophysiological conditions (Morin et al., 1998; Cossart et al., 2001; Sloviter et al., 2003). For instance, dendritic inhibitory interneurons (e.g., O-LM cells), but not somatic inhibitory interneurons (e.g., BCs in the CA1 region), preferentially degenerate in experimental temporal lobe epilepsy (Morin et al., 1998; Cossart et al., 2001; Maglóczky and Freund, 2005). Although the exact mechanism of this cell loss is unknown, accumulating evidence points to Ca^{2+} overload as the cause of differential vulnerability of O-LM cells (Woodhall et al., 1999; Gee et al., 2001). However, Ca^{2+} buffer capacity and changes of Ca^{2+} concentrations during spiking activities in O-LM cells remain unknown.

In this study, we combined whole-cell patch-clamp recording with ratiometric Ca^{2+} imaging at the dendrites using high-affinity Ca^{2+} indicators fura-2 and Oregon Green BAPTA-1 (OGB-1). A major goal of this study was to determine the endogenous Ca^{2+}-binding ratio and the AP-evoked Ca^{2+} concentrations in the absence of exogenous buffers in the proximal dendrites. A second major goal was to determine the spatial profile of dendritic Ca^{2+} transients evoked by backpropagating action potentials (bAPs) along the dendrites of O-LM cells. Our results revealed that O-LM cells have low endogenous Ca^{2+}-binding ratios which may contribute to the large $[Ca^{2+}]_i$ transients during APs. In addition, Ca^{2+} accumulation in the dendrite of O-LM cells during bAPs is relatively independent of the distance from the soma.

EXPERIMENTAL PROCEDURES

Preparation of hippocampal slices

Transverse hippocampal slices (300–350 μm thick) were prepared from male Sprague–Dawley rats [postnatal day 16 (P16)-P21] using a vibrating tissue slicer (DSK-1000, Dosaka, Kyoto, Japan) as described previously (Lien et al., 2002; Lien and Jonas, 2003). Animals were sacrificed by rapid decapitation without anesthesia in accordance with national and institutional guidelines. Experiments were approved by the Animal Care and Use Committee of National Yang-Ming University. Slices were sectioned in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 25 glucose, 2 $CaCl_2$, 1 $MgCl_2$. Following sectioning, slices were incubated in a holding chamber filled with the oxygenated (95% O_2−5% CO_2) solution containing (in mM): 87 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 10 glucose, 75 sucrose, 0.5 $CaCl_2$, 7 $MgCl_2$ at 34 °C for 25 min, and then at room temperature until used. During experiments, an individual slice was transferred to a submersion recording chamber and was continuously superfused with ACSF, bubbled with 95% O_2−5% CO_2.

Electrophysiology

Patch pipettes for recordings were pulled from borosilicate glass tubing (outer diameter 1.5 mm, inner diameter 0.86 mm; Harvard Apparatus, Holliston, MA, USA) and heat-polished before use. The pipette resistance ranged from 2 to 6 MΩ. Experiments were performed under visual control using an infrared differential interference contrast (IR–DIC) microscope (BX51WI, Olympus, Tokyo, Japan). O-LM cells in the stratum oriens/alveus of CA1 region were visually identified by the fusiform cell bodies and two horizontally oriented dendrites (Lien et al., 2002; Lien and Jonas, 2003). Cells exhibited pronounced sag responses during 1 s hyperpolarizing current pulse injection and modest fast-spiking patterns (~50 Hz) upon injection of depolarizing current pulses were used for further recording. Patch-clamp whole-cell recordings were made as described previously (Lien and Jonas, 2003; Lien et al., 2006), using a Multiclamp 700 B amplifier (Molecular Devices, Union City, CA, USA). Pipette capacitance and access resistance (10–30 MΩ) were compensated. Single APs in short bursts were evoked by single current pulses of 2 ms, with 2–3 nA. Oscillatory spikes were evoked by 5 Hz sinusoidal (8 s, 250–450 pA peak to peak) current pulses. Signals were low-pass filtered at 4 kHz (four-pole Bessel), and sampled at 10 kHz using a Digidata 1440 (Molecular Devices); data acquisition and pulse generation were performed using pClamp 10.2 (Molecular Devices). Recordings were made at 22–25 °C.

Fluorescence measurements with fura-2 and OGB-1

Ca^{2+} concentrations were measured using dual wavelength ratiometric method (Grynkiewicz et al., 1985). A polychromatic illumination system (polychrome V with 150 W Xenon lamp, bandwidth ~13 nm, TILL Photonics GmbH, Gräfelfing, Germany) was coupled to the epi-fluorescent port (BX-RFA, Olympus) of the microscope (Olympus BX51WI with a 60× water immersion objective, Olympus, Tokyo, Japan) via a quartz light guide and an epi-fluorescence condenser. The system provides fast switching (<1 ms) between the excitation wavelength (380 nm) and the Ca^{2+}-insensitive (isosbestic) excitation wavelength for fura-2 (362 nm in our system, comparable to 360 nm used by Neher and Augustine, 1992). Light intensity was attenuated by 75% using a neutral density filter (ND-25, Olympus, Tokyo, Japan) to reduce bleaching of the fluorescence dyes. The filter combination for excitation and emission consisted of a beam splitter (400DCLP, Chroma Technology Corp., Rockingham, VT, USA) and a long-pass emission filter (E420LPv2, Chroma Technology Corp). To investigate the spatial profile of dendritic $[Ca^{2+}]_i$ along dendrites, we used OGB-1 instead of fura-2 illuminated by a single wavelength of 494 nm through a filter combination for excitation and emission consisting of a dichroic mirror (DM505, Olympus, Tokyo, Japan) and a barrier filter (BA510IF, Olympus, Tokyo, Japan). Fluorescence was measured with a 16-bit frame-transfer electron-multiplying charged-coupled device (EM-CCD) camera (QuantEM 512SC, Photometrics, 10 MHz read out, a pixel size of 16 μm). The monochromator and image acquisition were controlled by a PC running MetaFluor software (Molecular Devices).

Images with full spatial resolution (512×512 pixel sizes) were taken with exposure times up to 2.5 s. For high-speed Ca^{2+} measurement (≥95 Hz), a rectangular region of interest (ROI), typically 2.7×5.3 μm (~10×20 pixel sizes), was defined over the proximal dendrites of O-LM cells at a distance of about 20 μm from soma (Fig. 1D). Fluorescence measurements were initiated 20 min after whole-cell recording was obtained, with the exception of the loading curve experiments (Fig. 2A) in which measurements were started before and immediately after break-in. Fluorescence traces had duration of 10–20 s and were separated by intersweep intervals of 20–40 s. Signals were corrected for background, which was obtained from a second ROI with identical size but shifted by 22–24 μm perpendicularly to the dendritic axis in comparison to the original ROI. Changes in background-subtracted fluorescence were expressed as $\Delta[Ca^{2+}]_i$ for fura-2 experiments or $\Delta F/F\% = [(F - F_{rest})/F_{rest}] \times 100\%$ for OGB-1/−5 N experiments.