Action Potential–Induced Dendritic Calcium Dynamics Correlated With Synaptic Plasticity in Developing Hippocampal Pyramidal Neurons

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INTRODUCTION

Intracellular free calcium plays crucial roles in synaptic plasticity such as long-term potentiation (LTP; Bliss and Collingridge 1993; Yuste and Tank 1996). Induction of LTP is known to be regulated age-dependently. LTP hardly occurs in hippocampal CA1 pyramidal cells during the first postnatal week, whereas robust LTP can be induced after the second postnatal week (Baudry et al. 1981; Dudek and Bear 1993; Figurov et al. 1996; Harris and Teyle 1984). Such age-dependent difference in susceptibility of LTP might be based on developmental changes of postsynaptic calcium dynamics. Recent studies by calcium imaging revealed that N-methyl-D-aspartate (NMDA) receptors and voltage-dependent calcium channels (VDCCs) contribute to synaptically induced calcium accumulations in the dendrites of CA1 pyramidal cells (Malinow et al. 1994; Miyakawa et al. 1992; Perkel et al. 1993; Regehr and Tank 1990, 1992; Regeh et al. 1989). In addition, calcium imaging techniques were able to show that action potentials cause large dendritic calcium influx through VDCCs (Christie et al. 1995; Jaffe et al. 1992; Spruston et al. 1995) and play critical roles in induction of LTP (Magee and Johnston 1997). In the present report, we made use of calcium imaging to investigate postnatal development of intracelluar calcium dynamics in proximal apical dendrites of the rat CA1 pyramidal cells. Action potential-induced calcium increase was adopted to compare calcium dynamics across different age groups for the following reasons. First, individual action potentials are known to cause a constant calcium increase under physiological conditions (e.g., Spruston et al. 1995). Second, critical roles played by action potentials in LTP have recently been supported (Magee and Johnston 1997). Third, action potentials may be potentially useful for quantitative comparison because of their all or none nature. To our knowledge, this paper provides the first direct evidence of developmental enhancement of dendritic calcium increases in hippocampal neurons in situ.

METHODS

Wistar rats (P4–20) anesthetized with ether were decapitated and the brains were dissected in cold artificial cerebrospinal fluid (ACSF) consisting of (in mM) 124 NaCl, 3.4 KCl, 1.3 KH2PO4, 26 NaHCO3, 2.0 MgSO4, 2.5 CaCl2, and 20–40 μM glucose saturated with 95% O2:5% CO2 (Kato 1993; Kato and Yoshinura 1993). Hippocampal slices (200 μm thick for whole cell recordings or 300 μm for field potential recordings) were prepared with a Micrisslicer (DTK-1000; Dosaka EM, Kyoto, Japan) and allowed to recover in ACSF at room temperature for 30 min. Each slice was transferred into a submerged-type recording chamber continuously circulated with ACSF at 30°C. The glucose concentration in ACSF was raised up to 40 μM to increase viability of neurons near the surface of slices during the dissection and slicing. No noticeable differences in electrophysiological properties were detected for recordings obtained in ACSF containing either 10 or 40 μM glucose.

Whole cell patch-clamp recordings were made from CA1 pyramidal cells near the surface of slices. Patch electrodes (8–10 MΩ) were filled with an internal solution containing (in mM) 122.5 K-gluconate, 17.5 KCl, 5 NaCl, 1 MgCl2, 10 HEPES, 0.2 EGTA, 2.5 μM Na3 ATP, and 1 μM fura-2 (Doyindo, Kumaamoto, Japan) (pH 7.3). After whole cell recordings were established by visual guidance under an upright microscope (Axioskop FS, Zeiss, Germany) with a ×63 water-immersion objective (Achromplan 63X/0.90W, Zeiss), fura-2 was loaded for 15 min and membrane potentials were recorded in the current-clamp mode (I = 0) with an amplifier (Axopatch 200A, Axon Instruments, CA). The data were low-pass-filtered at 2–5 kHz and digitized at 2–10 kHz with an A/D interface (Digidata 1200, Axon Instru-
membrane potentials. Recordings were obtained from the neurons which had sufficiently negative resting membrane potentials (see Results) without spontaneous action potentials. To evoke excitatory postsynaptic potentials (EPSPs) or action potentials synaptically, four consecutive pulses were given at 5 Hz to the Schaffer collateral fibers by a bipolar tungsten stimulation electrode placed in the stratum radiatum. To evoke action potentials intrasomatically, four brief depolarizing currents were injected at 5 Hz through the patch electrode, except for experiments described in Fig. 2G. The intensities of these stimulations were adjusted to be just suprathreshold. Theta-burst stimulation (TBS; 10 bursts at 5 Hz, with each burst consisting of 4 pulses at 100 Hz) was delivered by the stimulation electrode, where the intensities were adjusted to elicit a single action potential by the initial burst. Before finishing each experiment, a large and prolonged depolarization (+0.2 nA, 800 ms) was given to generate strong calcium increases and confirm that calcium signals were within a measurable range during test sessions. For blockade of VDCCs, CdCl₂ (250 μM; Nacalai, Kyoto, Japan) was added to KH₂PO₄-free ACSF. For blockade of ryanodine receptors, ruthenium red (20 μM; Nacalai) was added to the internal solution. For recording calcium currents, tetrodotoxin (TTX; 1 μM, Alomone Labs, Jerusalem, Israel), 4-aminopyridine (4-AP, 0.5 mM, RBI, MA), and tetroethylammonium chloride (TEA; 20 mM, RBI) were added to the ACSF, in which the concentrations of NaCl and CaCl₂ were reduced to 100 and 1 mM, respectively. Patch electrodes were filled with another internal solution containing (in mM) 130 Cs-glucuronate, 5 CsCl, 5 NaCl, 2 MgCl₂, 10 HEPES, 0.5 EGTA, 2.5’-ATP Na⁺, and 1 fura-2 (pH 7.3). Calcium currents induced by step-depolarizations from a holding potential of −50 mV to −50, −30, −10, and 10 mV for 200 ms were recorded in the voltage-clamp mode. Pipette capacitance and whole cell capacitance were compensated. Series resistance (15–36 MΩ) was also compensated to ≥70%. For field potential recordings, recording electrodes (2–5 MΩ) filled with 2.5 M NaCl were placed in the stratum radiatum. Test pulses were delivered every 12 s with the intensity adjusted to be 50–75% of threshold for population spikes and two trains of TBS at the interval of 20 s were given to induce LTP. In simultaneous recordings of calcium transients and field EPSPs, a patch electrode was withdrawn from the whole cell-clamped neuron after fura-2 was sufficiently loaded for ≥15 min. Before the electrode withdrawal, the stimulation intensity was adjusted to elicit a single action potential by the initial burst of TBS. Then another electrode for field potential recording was placed within 50 μm from the apical dendrite of the neuron, and the simultaneous recordings were started when the field responses became stable.

Fura-2 in neurons was excited by single- (380 nm) or dual- (360 and 380 nm) wavelength illumination, and fluorescence images on the basis of emission lights passing a 520 nm filter were captured with an intensified charge-coupled device (I-CCD) camera. The intensity of excitation lights and the sensitivity of I-CCD camera were controlled by the RatioArc and RatioVision systems (Attofluor, MD). The settings of this optical system were never changed through the entire course of experiments. Images were acquired in “static ratio” imaging mode; one image based on 360 nm excitation was captured at the beginning of each trial, and consecutive images based on 380 nm excitation were captured at video-rate (30 Hz) during the same trial. The images were stored with a rewritable optical disk recorder (LQ-4100A; Panasonic, Osaka, Japan) and digitized for off-line analysis. Regions of interest (ROIs), rectangles of 12 × 10 pixels (5 × 4 μm), were placed at 10 μm intervals along apical dendrite from soma-dendrite boundary, and the fluorescence intensities in each ROI were routinely subtracted and photobleaching of fura-2 was corrected. In the experiments by single-wavelength excitation, we defined ΔF₃80₃₆₀ as the index to estimate relative changes of intracellular calcium concentration. F₃6₀ is the averaged fluorescence intensity based on 360 nm excitation, obtained for 1 s before the stimulation. ΔF is the difference from F₃₆₀ to fluorescence intensity excited at 380 nm at a given time. In the experiments by dual-wavelength excitation, we defined ΔF₃₆₀₃₈₀ as the index to estimate absolute changes of the calcium concentration. F₃₆₀ is the fluorescence intensity based on 360 nm excitation at the beginning of each trial. ΔF₃₆₀ is identical to ΔF as described above. With calcium concentration increasing, the intensity of 360 nm-excited fluorescence decreases, whereas that of 360 nm-excited fluorescence remains unchanged (Grynkiewicz et al. 1985). Therefore increases in calcium ions will be expressed as positive values in both indexes. We did not determine the absolute concentration of intracellular calcium, given the apparent difficulty of accurate calibration in slice preparations. Student’s t-test or analysis of variance (ANOVA) was applied for statistical comparison. Data in text and figures were expressed as means ± SE, unless otherwise stated.

RESULTS

Subthreshold EPSPs, which were not summed up but isolated, elicited only small calcium transients in the proximal dendrite of CA1 pyramidal cells from 3-wk-old rats (Fig. 1, A and B). Once action potentials were generated, dendritic calcium transients became larger (Fig. 1C), in agreement with earlier studies (Christie et al. 1996; Magee et al. 1995). Synaptically and intrasomatically evoked action potentials induced dendritic calcium increases to much the same extents (P15–18, n = 8; data not shown), and here we examined proximal dendritic calcium increases induced by intrasomatically evoked action potentials during the first few postnatal weeks (Fig. 1, D–F). The calcium increases were measured at the proximal regions (0–50 μm from soma-dendrite boundary) of apical dendrites, but not at the more distal dendritic shaft and the branches. We presumed that the proximal regions are less prone to influences because of the difference in morphological parameters such as dendritic length and branching number from one developmental stage to another (Pokorny and Yamamoto 1981), and therefore are more suitable for quantitative comparison across developmental stages than are the distal regions.

Proximal dendritic calcium increases were very small at the first postnatal week (P4–6). At the second postnatal week they started to develop and reached a plateau level by P16–18 (Fig. 1D). The developmental enhancement was not simply linear but looked sigmoidal; almost no change was seen at the first postnatal week and prominent augmentation followed during the second and third weeks (Fig. 1E). There were significant augmentations observed at all the distances (one-way ANOVA, P < 0.001 at 0 μm, P < 0.001 at 10 μm, P < 0.001 at 20 μm, P < 0.001 at 30 μm, P < 0.001 at 40 μm, P < 0.001 at 50 μm; Fig. 1F). The baseline ratios (restF₃₆₀/F₃₈₀), measured at the resting states before stimulation, showed no significant difference among four age-groups from P4 to P18 (one-way ANOVA, P > 0.4; Fig. 1F, inset). The resting membrane potentials in these neurons (in mV, mean ± SD) were 52.5 ± 2.6 for P4–6, 57.8 ± 2.1 for P8–10, 61.4 ± 3.4 for P12–14, and 61.9 ± 4.5 for P16–18. We used relatively high concentration of fura-2 to improve the S/N ratio, which could have critically blurred calcium transient owing to its buffering effects (Helmchen et al. 1996) and might have possibly affected the present results. To exclude this possibility, we examined the developmental change of calcium increase with 200 μM fura-2. At this concentration also, the proximal dendritic calcium increases were larger at the late developmen-
expected from the developmental increase in membrane area, action potentials were greatly reduced by 250 μM CdCl₂ at 30 μM distant from the soma, for 4 age-groups: P4–6 (n = 8), P8–10 (n = 8), P12–14 (n = 8), and P16–18 (n = 8). The proximal dendritic calcium increases induced by 4 synaptic potentials at 5 Hz were evoked for 1 trial of calcium imaging. Traces of membrane potential from 5 trials are superimposed. Scale bars: 100 ms and 10 mV. Bottom, relative changes of intracellular calcium concentration against time and distance from the soma. Calcium transients were estimated by averaging fura-2 signals over the 5 trials. Stimulation started at 0 s. C: dendritic calcium transients induced by 4 intrasomatically evoked action potentials at 5 Hz. Top and bottom diagrams described in B. D: dendritic calcium transients induced by 4 intrasomatically evoked action potentials at 5 Hz, measured at 30 μM distant from the soma, for 4 age-groups: P4–6 (n = 8), P8–10 (n = 8), P12–14 (n = 8), and P16–18 (n = 8). E: age-dependency of dendritic calcium increases. Peak values of the calcium increase in all the neurons analyzed in D (n = 32) are plotted against the age, with each dot representing an individual neuron. A sigmoidal curve is fitted tentatively by using Boltzman function. Calcium increases were kept small during the first postnatal week and gradually developed at the second and third postnatal weeks. F: development of dendritic calcium increases at different distances from the soma. Calcium increases became enhanced during development at roughly a similar rate at all the distances. F: inset, ratios (rest/F360/F380) means and SD) of 380 nm-excited fluorescence intensity (F380) at the resting states to 360 nm-excited one (F360) acquired immediately before the measurement of F380.

We attempted to explain underlying mechanisms involved in the developmental enhancement of proximal dendritic calcium increases. First, to examine whether the amounts of calcium influx are developmentally augmented, calcium currents and intracellular calcium accumulations induced by step-depolarization were simultaneously recorded under the voltage-clamp condition in situ. Both calcium currents and calcium accumulations induced by depolarization were much greater at the late developmental stage (P15–18) than the early developmental stage (P4–7) (Fig. 2, A and B). The maximal values of calcium currents were significantly larger at the late developmental stage than the early at −10 and 10 mV (t-test, P < 0.001 at −10 mV, P < 0.005 at 10 mV; Fig. 2C). As expected from the developmental increase in membrane area, whole cell capacitance increased during the development (27.8 ± 5.2 for the early and 51.3 ± 2.7 for the late (in pF), t-test, P < 0.005), and current densities at −10 mV, obtained by normalizing to the capacitance, were also significantly greater at the late stage than the early (11.4 ± 4.5 for the early and 26.0 ± 3.4 for the late (in pA/pF), t-test, P < 0.05). The intracellular calcium accumulations were also significantly larger at the late developmental stage than the early (t-test, n = 6 in both, P < 0.05 at −30 mV, P < 0.002 at −10 mV, P < 0.002 at 10 mV; Fig. 2D). The extents of calcium currents and intracellular calcium accumulations were significantly correlated (γ=0.75, P < 0.01; Fig. 2E). The calcium accumulations were larger at the late developmental stage than the early at each of 0–50 μm distances (t-test, n = 6 in both, P < 0.001 at 0 μm, P < 0.001 at 10 μm, P < 0.005 at 20 μm, P < 0.005 at 30 μm, P < 0.005 at 40 μm, P < 0.005 at 50 μm; Fig. 2F). These recordings of calcium currents in situ were consistent with the previous studies in acutely dissociated neurons (Kortezaas and Wadman 1997; Thompson and Wong 1991).

Second, we considered whether calcium-induced calcium release (CICR), calcium buffering, and calcium sequestration contribute to developmental change of the calcium dynamics (Blaustein 1988). The calcium transients were not changed by...
intracellular application of 20 μM ruthenium red, a ryanodine receptor blocker, in our experimental conditions (t-test, n = 8 in both, ≥ P > 0.1), suggesting no major participation of CICR in developmental changes of the calcium dynamics. The decay of calcium transients were significantly slower at the early developmental stage than the late (t-test, n = 8 in both, P < 0.001; Fig. 2G). This slower decay at the early stage may be due to stronger calcium buffering or weaker calcium sequestration. It is unlikely that calcium buffering is stronger at the early stage than the late, because calcium-binding proteins including calbindin, acting as endogenous calcium buffers, generally increase during development (e.g., Rami et al. 1987) and that we routinely used the same concentration of fura-2, which may work as exogenous calcium buffer. Hence, calcium sequestration seems to be strengthened during development. This alone, however, could not bring about a developmental enhancement of action potential-induced calcium increases.

Third, somatic action potentials were compared at the early and late developmental stages as shown in Fig. 2H. In agreement with the previous reports (e.g., Costa et al. 1991), the amplitude became larger during development (72.4 ± 0.7 mV for the late (n = 3), t-test, P < 0.005) and the width at half height shorter (2.5 ± 0.2 ms for the early (n = 3) and 1.7 ± 0.1 ms for the late (n = 3), t-test, P < 0.02). Because the calcium increases were voltage-dependent, enlargement of the amplitude is thought to
enhance the calcium increase during the development. However, dendritic rather than somatic action potentials should have more relevance to dendritic calcium increases. It is yet to be determined whether backpropagation of dendritic action potentials could undergo developmental changes. In summary, increases in the voltage-dependent calcium conductances have been shown to contribute to the developmental enhancement of proximal dendritic calcium increases, and moreover it is possible that developmental changes in other ion channels such as dendritic sodium and potassium channels are involved.

In the hippocampal CA1 region, TBS-induced LTP does not appear until the second postnatal week (Dudek and Bear 1993; Figurov et al. 1996). Indeed, in our own slice preparations, TBS-induced LTP remarkably developed during the second and third postnatal weeks (109.5 ± 7.7% for P8–10, n = 10) and at the third postnatal week (173.6 ± 10.2% for P18–20, n = 8). Five consecutive field EPSP slopes were averaged for each data point. Robust LTP was induced only at the third postnatal week. Developmental enhancement of proximal dendritic calcium transients and field EPSPs at the beginning of the second postnatal week (P10). Top: dendritic calcium transient during TBS (the 10 bursts were generated at the timing indicated by (OE)). Middle: averaged traces of field EPSPs before (left) and 25 min after (right) the TBS delivery. Scale bars: 10 ms and 0.5 mV. Bottom: change of field EPSP slopes induced by TBS. Calcium increase was small and no LTP was induced. C: simultaneous recordings of dendritic calcium transients and field EPSPs at the third postnatal week (P16). Top: superimposed traces of membrane potential during TBS. Scale bars: 250 ms and 10 mV. Middle: TBS-induced calcium transients at 0–30 μm distant from the soma were estimated by averaging fura-2 signals over the 4 trials. E: TBS-induced dendritic calcium transients at the late developmental stage (P16). Top and bottom diagrams shown in D. There was large difference between the calcium transients in D and E, although overshooting action potentials were similarly evoked. F: scatter plot of calcium increases, measured at 50 μm distant from the soma, against mean number of action potentials elicited during TBS at the early (P4–7, n = 7) and the late developmental stages (P15–18; n = 7). Although various numbers of action potentials were generated during TBS, the calcium increases were always larger at the late developmental stage than the early. G: calcium increases as a function of distance from the soma at the early and the late developmental stages.
induced calcium increases were developmentally enhanced in CA1 pyramidal neurons if synapses can be induced even during the first postnatal week if synapses are functional only a few days after the birth (Durand et al. 1996), the amount of VDCC-mediated calcium component may put restrictions on susceptibility to LTP induction. We showed that the time course of developmental enhancement of VDCC-mediated calcium increases in the proximal apical dendrite resembles that of TBS-induced LTP (Fig. 1E, and see also Figurov et al. 1996). Thus developmental enhancement of action potential-induced proximal dendritic calcium increases may play a crucial role for maturation of calcium-dependent functions such as synaptic plasticity in hippocampal neurons.

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REFERENCES


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