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Developmental regulation of hippocampal long-term depression by cofilin-mediated actin reorganization

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ABSTRACT

Long lasting synaptic plasticity involves both functional and morphological changes, but how these processes are molecularly linked to achieve coordinated plasticity remains poorly understood. Cofilin is a common target of multiple signaling pathways at the synapse and is required for both functional and spine plasticity, but how it is regulated is unclear. In this study, we investigate whether the involvement of cofilin in plasticity is developmentally regulated by examining the role of cofilin in hippocampal long-term depression (LTD) in both young (2 weeks) and mature (2 months) mice. We show that both total protein level of cofilin and its activation undergo significant changes as the brain matures, so that although the amount of cofilin decreases significantly in mature mice, its regulation by protein phosphorylation becomes increasingly important. Consistent with these biochemical data, we show that cofilin-mediated actin reorganization is essential for LTD in mature, but not in young mice. In contrast to cofilin, the GluA2 interactions with NSF and PICK1 appear to be required in both young and mature mice, indicating that AMPAR internalization is a common key mechanism for LTD expression regardless of the developmental stages. These results establish the temporal specificity of cofilin in LTD regulation and suggest that cofilin-mediated actin reorganization may serve as a key mechanism underlying developmental regulation of synaptic and spine plasticity.

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1. Introduction

Plastic changes at glutamatergic synapses are critically important for both brain development and function, and deficits in these changes are responsible for a wide range of neurodevelopmental and neuropsychiatric disorders. Long-lasting synaptic plasticity, including long-term potentiation (LTP) and depression (LTD), a key mechanism for memory formation, involves changes in both synaptic morphology and electrical transmission (also referred to as morphological and functional plasticity respectively) (Bliss and Collingridge, 1993; Malenka and Bear, 2004; Lamprecht and LeDoux, 2004; Alvarez and Sabatini, 2007; Bourne and Harris, 2008). Although extensive studies exist to elucidate the molecular mechanisms underlying these two forms of plasticity individually, how these changes interact and are coordinated during synaptic remodeling remains poorly understood.

In the CA1 region of the hippocampus, where the molecular mechanisms underlying LTP and LTD are most extensively studied, the induction of both functional and morphological changes requires activation of NMDA receptors (NMDARs) and subsequent Ca^{2+}-dependent signaling processes (Bliss and Collingridge, 1993; Malenka and Bear, 2004; Lamprecht and LeDoux, 2004; Alvarez and Sabatini, 2007; Bourne and Harris, 2008). Although AMPA glutamate receptors (AMPARs), the principal mediator of fast excitatory synaptic transmission, are a major target of these

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signaling processes that express and maintain functional changes (Malinow and Malenka, 2003; Bredt and Nicoll, 2003; Collingridge et al., 2004; Malenka and Bear, 2004; Shepherd and Huganir, 2007; Lüscher and Huber, 2010; Collingridge et al., 2010), the actin cytoskeleton, the predominant structural component of the dendritic spine, is believed to be the main determinant that governs morphological plasticity (Luo, 2002; Cingolani and Goda, 2008; Asrar and Jia, 2013). In this regard, we and others have previously shown that Rho GTPase-activated protein kinases, including p21-activated kinases (PAK1 and 3), Rho-kinase 2 (ROCK2) and LIM-domain containing kinase (LIMK1), are critically involved in both LTP, LTD and associated spine plasticity (Meng et al., 2002, 2005, 2004; Asrar et al., 2009; Zhou et al., 2009; Huang et al., 2011; Bosch et al., 2014; Todorovski et al., 2015; Nishiyama and Yasuda, 2015). Importantly, these kinases all target cofilin, a potent regulator of the actin cytoskeleton (Bernstein and Bamburg, 2010), suggesting that cofilin-mediated actin reorganization may serve as a common effector to mediate, and potentially coordinate functional and morphological changes during synaptic remodeling (Meng et al., 2003a; Jia et al., 2009; Asrar and Jia, 2013; Rust, 2015). However, how cofilin is regulated during plasticity remains largely unknown.

In this study, we investigated whether cofilin is regulated during mouse development, a process characterized by profound changes in both spine dynamics and functional plasticity, and if so, how this developmental regulation impacts its involvement in hippocampal LTD. We provide evidence that both cofilin expression/activity and its involvement in hippocampal LTD change dramatically as the brain matures, suggesting that cofilin-mediated actin reorganization may serve as a key mechanism underlying developmental regulation of synaptic plasticity.

2. Methods

2.1. Electrophysiology

Electrophysiological recordings were conducted at the Schaffer/Collateral pathway in the hippocampus as previously described (Meng et al., 2002, 2003; Zhou et al., 2011). Briefly, the mouse brains of either sex were quickly removed and sagittal 300–400 μm hippocampal slices prepared in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O_2/5% CO_2. ACSF contained (in mM): 120 NaCl, 3.0 KCl, 1.2 $MgSO_4$, 1.0 NaH_2PO_4, 26 $NaHCO_3$, 2.0 $CaCl_2$, and 11 D-glucose. These acute slices were recovered at 22–26 °C for at least 2 h, then transferred to a submersion chamber perfused with 95% O_2/5% CO_2 saturated ACSF with (for whole-cell recordings) or without (for field recordings) 100 μM picrotoxin. Perfusion flow rate was set at 2 ml/min. Hippocampal CA1 neurons were visualized using an infrared differential interference contrast microscope (Zeiss Axioscope or Olympus X51). Synaptic transmission was evoked at 0.05 Hz for field recordings and 0.1 Hz for whole-cell recordings and recorded with glass pipettes (3–4 MΩ) filled with either ACSF (for field responses) or the intracellular solution (for whole-cell response) containing (in mM) 130 $CsMeSO_4$, 5 NaCl, 1 $MgCl_2$, 0.05 EGTA, 10 HEPES, 3 Mg-ATP, 0.3 Na_3GTP, and 5 QX-314 (pH 7.25) (280–300 mOsm). For whole-cell experiments, cells were clamped at −65 mV throughout the experiment. Whole-cell series resistance was monitored throughout LTD experiments by applying a −3 mV step at the end of each response sweep and the experiment was excluded from analysis if resistance changed by more than 20%. For peptide infusion experiments, the lack of the effect of each peptide on basal synaptic responses was independently tested by conducting baseline recordings in the presence of the peptides for at least 1 h without LTD induction. Mice at the age of 2–3 weeks were defined as 'young mice' and 2–3 months as 'mature mice'. In all recordings using peptides or chemicals, control and experimental groups were tested alternately to minimize variables. Field EPSP LTD induction protocols used in this study were: low-frequency stimulation (LFS, 900 pulses at 1 Hz), paired-pulse LFS (PP-LFS, 900 pairs of pulses at 1 Hz with 50 ms pairing interval), NMDA chemical LTD (25 μM for 3 min) and DHPG chemical LTD (100 μM for 10 min). LTD of whole-cell EPSC recordings was induced by 300 pulses at 1 Hz (LFS) or 600 pairs of pulses at 1 Hz with 50 ms pairing interval (PP-LFS) or 100 μM DHPG perfusion for 10 min. Data acquisition and analysis were done using pClamp 10 software (Molecular Devices, USA). N in all figures represents the number of slices and at most two slices from each animal were used. All recording data were statistically evaluated with Student's *t*-test.

2.2. Slice treatment and biochemical assays

In order to correlate electrophysiological and biochemical data, protein lysates extracted from acutely prepared hippocampus were analyzed. The conditions for preparing and maintaining hippocampal slices were the same as for electrophysiological recordings. For each experiment, slices were recovered for at least 2 h at room temperature in 95% O_2/5%CO_2 saturated ACSF, then transferred to a treatment chamber for additional 30 min recovery before DHPG treatment. The treatment experiments were divided into two groups: slices removed immediately before DHPG application were used as untreated control and slices treated with 100 μM DHPG for 10 min as DHPG treated group. Hippocampal slices were frozen in dry ice/ethanol slurry and stored at −20 °C at the end of each treatment. Samples were lysed for 50 min in ice-cold lysis buffer containing (in mM) 20 Tris pH 7.5, 150 NaCl, 1 EDTA, 1 EGTA, 1% Triton X-100, 2.5 sodium pyrophosphate, 1 β-glycerophosphate, 1 Na_3VO_4, 20 NaF, 1 μg/ml leupeptin, 1 PMSF, and 0.5% protease inhibitor cocktail (Calbiochem) and phosphatase inhibitor (Roche). The supernatant was collected by centrifugation at 12,000 rpm (4 °C) for 10 min. For synaptosomal fraction preparation, hippocampi were isolated, homogenized in 0.32 M sucrose (in HBSS) and transferred to polycarbonate centrifuge tube for centrifugation at 1,000*g*, 4 °C for 10 min to remove the nuclear fraction. The supernatant (S1) was collected and centrifuged at 13,800*g* for 10 min to separate supernatant (S2) and pellet (P2) that contains microsomes/light membranes and crude synaptosomal fractions respectively. The P2 fraction was suspended in HBSS and subjected to discontinuous sucrose gradient (1.2 M, 1.0 M and 0.85 M in HBSS) centrifugation at 82,500*g* for 2 h at 4 °C. The synaptosomal fraction was then collected from the 1.0 M–1.2 M gradient and subsequently centrifuged at 150,000*g* for 30 min to obtain the synaptosomal pellet. The pellet was suspended overnight at 4 °C in sample buffer containing (in mM) 50 HEPES, 2 EDTA, 0.5% Triton, 20 NaF, 1 NaVO4 and 0.5% protease inhibitor cocktail (Calbiochem) and phosphatase inhibitor (Roche). The enrichment of synaptic proteins in synaptosomal fractions was verified using synaptic markers synapsin and PSD95. The total protein concentration of each sample was estimated by a BCA assay. For each experiment, protein samples (10 μg of total proteins) of each group were loaded on SDS gel. Proteins were separated on 12% SDS-PAGE separation gel and electrotransfered to a nitrocellulose filter. Filters were then blocked with 2% dry milk or 2% fetal bovine serum in TBST (20 mM Tris base, 9% NaCl, 0.1% Tween-20, pH 7.6) and incubated overnight at 4 °C with cofilin or p-cofilin antibodies in TBST. After washing and incubating with appropriate secondary antibodies, membranes were extensively washed with TBST and subjected to chemiluminescence signal detection using a Pierce HRP kit. Band intensity was analyzed using the AlphaEaseFC software. GAPDH was used as the protein loading control. Primary antibodies included