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BDNF upregulation rescues synaptic plasticity in middle-aged ovariectomized rats

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Abstract

Brain-derived neurotrophic factor (BDNF) has emerged as a possible broad-spectrum treatment for the plasticity losses found in rodent models of human conditions associated with memory and cognitive deficits. We have tested this strategy in the particular case of ovariectomy. The actin polymerization in spines normally found after patterned afferent stimulation was greatly reduced, along with the stabilization of long-term potentiation, in hippocampal slices prepared from middle-aged ovariectomized rats. Both effects were fully restored by a 60-minute infusion of 2 nM BDNF. Comparable rescue results were obtained after elevating endogenous BDNF protein levels in hippocampus with 4 daily injections of a short half-life ampakine (positive modulator of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate [AMPA]-type glutamate receptors). These results provide the first evidence that minimally invasive, mechanism-based drug treatments can ameliorate defects in spine plasticity caused by depressed estrogen levels.

Keywords: Hippocampus; Spines; Actin; LTP, Theta bursts; Ampakine; Estrogen

1. Introduction

Appropriately patterned afferent activity causes rapid reorganization of the dendritic spine cytoskeleton, as evidenced by intense actin polymerization (Kramár et al., 2006; Lin et al., 2005; Okamoto et al., 2004), along with marked changes in spine and synapse morphology in adult hippocampus (Chen et al., 2007; Honkura et al., 2008; Matsuzaki et al., 2004; Rex et al., 2009; Yang et al., 2008). Recent studies suggest that at least some of these effects occur during learning and are critical to the encoding of long-term memory (Fedulov et al., 2007). There are also reasons to suspect that defects in spine plasticity are important contributors to memory and cognitive problems in certain neuropsychiatric disorders (Kaufmann and Moser, 2000). Consonant with this idea, activity-driven polymerization of spine actin is disturbed in animal models of early-stage Huntington's disease (Lynch et al., 2007), a condition associated with memory impairments of varying degrees of severity. Deficits in spine cytoskeletal reorganization are also observed in ovariectomized (OVX) rats not given estrogen replacement (Kramár et al., 2009b). A substantial body of evidence links rapid changes in spine actin networks to long term potentiation (LTP) (Bramham, 2008; Fukazawa et al., 2003; Kramár et al., 2006; Krucker et al., 2000; Okamoto et al., 2004; Rex et al., 2009) and, as expected from this, the spine defects found in Huntington's disease and OVX rodents are accompanied by a failure of LTP consolidation (Kramár et al., 2009b; Lynch et al., 2007).

The loss of plasticity in the OVX cases raised the possibility that estrogen engages actin signaling at synapses. We tested this idea and found that the steroid promotes rapid actin filament assembly within adult spines by stim-

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ulating the small GTPase RhoA, which then drives the phosphorylation, and thus inactivation, of the actin severing protein cofilin (Kramár et al., 2009b). Induction of LTP by theta pattern stimulation also phosphorylates cofilin via RhoA-dependent kinase (Rex et al., 2009), so it appears that estrogen regulates spine plasticity through actions on a learning-related enzyme cascade.

The above conclusion has significance with regard to the development of strategies for treating memory problems commonly reported to accompany the loss of estrogen (Devi et al., 2005; Phillips and Sherwin, 1992; Wegesin and Stern, 2007). Recent work indicates that the RhoA to cofilin signaling targeted by estrogen is also acutely modulated by factors released during the brief periods of repetitive afferent activity used to induce LTP (for review, Kramár et al., 2009a). Thus, it is conceivable that manipulation of these other factors, such as increasing TrkB signaling (Chen et al., 2010) can be used to offset the loss of estrogen's positive influence on spine cytoskeletal reorganization and LTP. We have explored this possibility by measuring the effects of brain-derived neurotrophic factor (BDNF), an established and potent positive modulator of LTP, on ovariectomy-induced defects in actin filament assembly and LTP consolidation. More directly relevant to the clinical problem of estrogen-related memory loss, we tested if elevating forebrain BDNF levels with the short half-life ampakine CX929 rescues spine plasticity in hippocampal slices from OVX rats. Ampakines, via positive modulation of central α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-receptors, enhance excitatory transmission in the forebrain and thereby increase BDNF expression (Lauterborn et al., 2003). CX929 was chosen for the study because of its short half-life (<15 minutes) and past work showing that it upregulates BDNF and restores LTP in diverse rodent models (Rex et al., 2006; Simmons et al., 2009).

2. Methods

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with protocols approved by the Institutional Animal Care and Use Committee of the University of California at Irvine. This includes efforts to minimize animal suffering and numbers of rats used in the work described.

2.1. Slice preparation and recording

Studies employed Long Evans rats (Charles River) that were OVX at 9–10 months old (retired breeders) and allowed to age for at least 6 months before testing. Methods were slightly modified from those described previously (Kramár et al., 2006). Briefly, acute hippocampal slices were prepared and maintained in an interface recording chamber containing preheated artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 124 NaCl, 3 KCl, 1.25 KH_2PO_4, 1.5 $MgSO_4$, 2.5 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose. Slices were continuously perfused with this solution at a rate of 1.75–2 mL/minute while the surface of the slices were exposed to warm, humidified 95% O_2/5% CO_2. Recordings began following at least 1.5 hours of incubation.

Field excitatory postsynaptic potentials (fEPSPs) were recorded from CA1b stratum radiatum using a single glass pipette (2–3 MΩ). Stimulation electrodes (twisted nichrome wires, 65 μm) were positioned at 2 sites (CA1a and CA1c) in the apical Schaffer collateral-commissural projections to provide convergent activation of CA1b pyramidal cells. Pulses were administered in an alternating fashion to the 2 electrodes at 0.05 Hz using a current that elicited a 50% maximal response. After establishing a 10–20 minute stable baseline, LTP was induced with 10 "theta" bursts, each burst consisting of 4 pulses at 100 Hz with an interburst interval of 200 ms (i.e., theta burst stimulation or TBS). The stimulation intensity was not increased during TBS. In some slices, test compounds (see below) were introduced into the infusion line by switching from control aCSF to drug-containing aCSF prior to induction. Data were collected and digitized by NAC 2.0 Neurodata Acquisition System (Theta Burst Corporation, Irvine, CA USA) and stored on a disk.

2.2. BDNF application

The perfusion system used for BDNF application during field recordings was employed as previously described (Kramár et al., 2004). In some experiments, BDNF was inactivated by boiling for 10 minutes before experimentation.

2.3. In situ phalloidin labeling

To assess filamentous actin (F-actin) within individual dendritic spines, we used a recently developed in situ labeling technique which entails applying fluorescent-tagged phalloidin, a mushroom toxin that penetrates the cell membrane (Bernstein et al., 1998; Geeraert et al., 2003) and binds to F-actin, to living tissue before fixation (Kramár et al., 2006; Lin et al., 2005; Rex et al., 2007). Epifluorescence wide field microscopy was then used to quantify dendritic spine levels of fluorescent-labeled F-actin in association with treatments and the various stages of LTP. Specifically, Alexa568-phalloidin (6 μM, Tocris BioScience, Ellisville, MI, USA) was applied topically (2 μL) from a micropipette every 5 minutes for 20 minutes either at the end of the experiment in slices that received low-frequency stimulation (LFS) or 60 minutes after the delivery of 10 theta bursts. Slices were then fixed in 4% paraformaldehyde (12–18 hours), cryoprotected in 20% sucrose/0.1 M phosphate buffer (PB) for 1 hour at 4 °C and sectioned on a freezing microtome at a thickness of 20 μm (parallel to the broad surface of the slice). Sections were collected in phosphate buffer, mounted onto Superfrost slides (Fisher Scien-