available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

BDNF enhances dendritic Ca^{2+} signals evoked by coincident EPSPs and back-propagating action potentials in CA1 pyramidal neurons

*Lucas Pozzo-Miller**

Department of Neurobiology and Civitan International Research Center, University of Alabama at Birmingham, 1825 University Blvd. Birmingham, AL 35294-2182, Alabama, USA

ARTICLE INFO

Article history:
Accepted 21 May 2006
Available online 22 June 2006

Keywords:
BDNF
Ca^{2+} imaging
CA1
Hippocampus
NMDA receptor
Pyramidal neuron
Synapse
TrkB

ABSTRACT

BDNF, a member of the neurotrophin family, is emerging as a key modulator of synaptic structure and function in the CNS. Due to the critical role of postsynaptic Ca^{2+} signals in dendritic development and synaptic plasticity, we tested whether long-term exposure to BDNF affects Ca^{2+} elevations evoked by coincident excitatory postsynaptic potentials (EPSPs) and back-propagating action potentials (bAPs) in spiny dendrites of CA1 pyramidal neurons within hippocampal slice cultures. In control neurons, a train of 5 coincident EPSPs and bAPs evoked Ca^{2+} elevations in oblique radial branches of the main apical dendrite that were of similar amplitude than those evoked by a train of 5 bAPs alone. On the other hand, dendritic Ca^{2+} signals evoked by coincident EPSPs and bAPs were always larger than those triggered by bAPs in CA1 neurons exposed to BDNF for 48 h. This difference was not observed after blockade of NMDA receptors (NMDARs) with D,L-APV, but only in BDNF-treated neurons, suggesting that Ca^{2+} signals in oblique radial dendrites include a synaptic NMDAR-dependent component. Co-treatment with the receptor tyrosine kinase inhibitor k-252a prevented the effect of BDNF on coincident dendritic Ca^{2+} signals, suggesting the involvement of neurotrophin Trk receptors. These results indicate that long-term exposure to BDNF enhances Ca^{2+} signaling during coincident pre- and postsynaptic activity in small spiny dendrites of CA1 pyramidal neurons, representing a potential functional consequence of neurotrophin-mediated dendritic remodeling in developing neurons.

1. Introduction

Different models of activity-dependent synaptic development and plasticity postulate the existence of extracellular signaling molecules that enhance or stabilize synchronously active synapses. Neurotrophins have been postulated to play such role because their synthesis and release are regulated by neuronal activity, they activate prominent signaling pathways in neurons, and they are necessary for dendritic development and long-term changes in synaptic strength (McAllister et al., 1999; Poo, 2001; Tyler et al., 2002a; Bramham and Messaoudi, 2005). Because the spatiotemporal pattern of transient Ca^{2+} elevations is fundamental for neuronal development and synaptic plasticity (Zucker, 1999; Spitzer et al., 2004), their modulation represents a potential target for neurotrophin action (McCutchen et al., 2002; Amaral and Pozzo-Miller, 2005).

* Fax: +1 205 934 6571.
E-mail address: lucaspm@uab.edu.

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.05.067

The extensive dendrites of central neurons are the principal sites for excitatory synaptic input mediated by ligand-gated receptors, in addition to expressing several voltage-gated conductances that endow them with active properties (Johnston et al., 1996). The ensuing complex interactions between afferent synaptic activity and intrinsic excitability in active dendrites make them substrate of substantial computational power (Hausser and Mel, 2003). During trains of action potentials, CA1 pyramidal neurons display widespread dendritic Ca^{2+} transients caused by Na^{+}-dependent back-propagating action potentials (bAPs) that in turn activate voltage-gated Ca^{2+} channels (Jaffe et al., 1992; Spruston et al., 1995). On the other hand, Ca^{2+} signals evoked by synaptic activation of NMDA receptors (NMDAR) in CA1 pyramidal neurons are initially restricted to dendritic spines (Petrozzino et al., 1995; Emptage et al., 1999; Mainen et al., 1999; Pozzo-Miller et al., 1999; Kovalchuk et al., 2000; Nimchinsky et al., 2004). Of relevance to associative models of synaptic plasticity, Ca^{2+} transients evoked by coincident excitatory postsynaptic potentials (EPSPs) and bAPs within individual spines of hippocampal and neocortical pyramidal neurons exhibit a supralinear relationship (Yuste and Denk, 1995; Koester and Sakmann, 1998; Nevian and Sakmann, 2004). Such supralinearity of spine Ca^{2+} signals is thought to originate from the simultaneous activation of NMDAR during the EPSP and the removal of their Mg^{2+} block by the back-propagating spike (Schiller et al., 1998). These features of highly branched, spiny dendrites with active conductances have been recently related to several rules of compartmentalized cellular excitability, dendritic integration, as well as synaptic and intrinsic plasticity (Goldberg and Yuste, 2005; Johnston et al., 2003; Polsky et al., 2004; Schaefer et al., 2003).

In the present report, we present evidence that long-term BDNF exposure enhances Ca^{2+} signals in spiny dendrites during coincident pre- and postsynaptic activity. In control CA1 pyramidal neurons, brief trains of paired EPSPs and bAPs evoked Ca^{2+} transients in oblique radial branches of the main apical dendrite that were similar to those evoked by bAPs alone. On the other hand, Ca^{2+} signals evoked by coincident EPSPs and bAPs were always larger than those triggered by bAPs in CA1 neurons exposed to BDNF. The NMDAR antagonist D,L-APV prevented this effect, indicating that either direct Ca^{2+} influx through synaptic NMDAR in spines, or voltage-gated influx through Ca^{2+} channels activated by the NMDAR-mediated depolarization contributes to dendritic Ca^{2+} elevations. Consistent with the activation of Trk receptors, coincubation with the receptor tyrosine kinase inhibitor k-252a prevented the effect of BDNF on dendritic Ca^{2+} signals evoked by coincident pre- and postsynaptic activity.

2. Results

The aim of this study was to examine whether long-term BDNF exposure modulates dendritic Ca^{2+} elevations evoked by coincident pre- and postsynaptic activity in CA1 pyramidal neurons maintained in hippocampal slice cultures (Fig. 1A). Slice cultures were kept in serum-free media as controls, exposed to BDNF for 48 h (250 ng/mL) in the absence and presence of the receptor tyrosine kinase inhibitor k-252a (200 nM) or exposed to k-252a alone (200 nM, 48 h). The Ca^{2+} indicators bis-fura-2 (250 μM) or OGB-1 (100 μM) were included in the whole-cell recording pipette, and simultaneous fluorescence imaging was restricted to secondary spiny dendrites after they branched from the main apical primary dendrite (50–100 μm from the soma; Fig. 1A). All data were obtained from a total of 39 CA1 pyramidal neurons. Neurons from all treatment groups had similar values of resting membrane potential (control-57 ± 2 mV, n = 10; BDNF-60 ± 1 mV, n = 10; BDNF + k252a-55 ± 1 mV, n = 4; k252a-54 ± 1 mV, n = 4; ANOVA $P > 0.05$). In addition, BDNF did not affect the resting Ca^{2+} concentration in these secondary spiny dendrites (control bis-fura-2 357/380 nm ratio = 0.15 ± 0.02, n = 4; BDNF 0.14 ± 0.03, n = 4; t test $P > 0.05$).

Fig. 1A shows a montage of fluorescence images of a representative CA1 pyramidal neuron loaded with OGB-1 through the somatic whole-cell electrode. Measurements of Ca^{2+}-indicator fluorescence intensity were obtained from secondary spiny dendrites after branching from the main apical primary dendrite, which sometimes included the so-called radial oblique dendrites (Frick et al., 2003; Losonczy and Magee, 2006). Intracellular Ca^{2+} elevations were evoked by one of the following protocols (Fig. 1B): (i) a 20-Hz train of 5 subthreshold EPSPs (5–10 mV in amplitude each) evoked by afferent fiber stimulation with an extracellular electrode positioned parallel to the Schaeffer collaterals in CA1 *stratum radiatum*, always within 100 μm of *stratum pyramidale*, and within 20 μm of the dendrites of the dye-filled cell; (ii) a 20-Hz train of 5 back-propagating action potentials (bAPs) triggered by short (5 ms) depolarizing current pulses via the somatic recording electrode; (iii) a 20-Hz train of 5 coincident EPSPs and bAPs, where each bAP was triggered after a 10-ms delay from the extracellular afferent stimulation. Fig. 1C shows Ca^{2+} transients within the color-coded ROIs in Fig. 1A in response to a train of subthreshold EPSPs (left), a train of 5 bAPs (middle), or a train of 5 coincident EPSPs-bAPs (right). In all the experiments described here, the imaging was performed in the absence of BDNF or k-252a, which were present only in the culture media during 48 h in vitro, but not in the recording ACSF. Neurons that fired complex spikes, especially during coincident trains of EPSP-bAP, were discarded and not further studied to avoid the confounding effect of an additional source of voltage-dependent Ca^{2+} influx, i.e., regenerative Ca^{2+} spikes (Hoffman et al., 2002).

2.1. *Dendritic Ca^{2+} signals evoked by coincident EPSP-bAP trains were larger than those evoked by bAPs alone, but only in BDNF-treated neurons*

The amplitude of dendritic Ca^{2+} signals evoked by trains of 5 EPSPs, 5 bAPs, or 5 coincident EPSP-bAP pairings were not significantly different between BDNF-treated and control neurons (Table 1; ANOVA $P > 0.05$). We next wondered how much bigger were dendritic Ca^{2+} signals evoked by coincident EPSP-bAP trains compared to those triggered by bAPs alone. In other words, what was the contribution of the EPSPs to the coincident EPSP-bAP train in terms of Ca^{2+} signals in secondary apical dendrites. To this aim, the ratio of the