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Modulation of neuronal calcium signaling by neurotrophic factors

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Abstract

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin (NT) family, is emerging as a key mediator of activity-dependent modifications of synaptic strength in the central nervous system. Because of the well-established role of post-synaptic elevations in concentrations of free Ca^{2+} ions ($[\text{Ca}^{2+}]_i$) in synaptic plasticity, we investigated the hypothesis that BDNF exerts its neuromodulatory effects on hippocampal pyramidal neurons by enhancing dendritic $[\text{Ca}^{2+}]_i$ transients mediated by voltage-dependent Ca^{2+} channels (VDCCs) during the firing of back-propagating action potentials. Simultaneous whole-cell recording and microfluorometric Ca^{2+} imaging were performed in CA1 pyramidal neurons from hippocampal organotypic slice cultures treated with BDNF for 2–4 days in vitro. Our observations indicate that long-term exposure to BDNF does not affect $[\text{Ca}^{2+}]_i$ transients in apical dendrites mediated by influx through L-type VDCCs during trains of back-propagating action potentials evoked by direct depolarizing current injections. These results suggest that, despite BDNF's profound effects on hippocampal synaptic plasticity, and of L-type Ca^{2+} channels on neuronal gene transcription, the role of BDNF in cellular models of hippocampus-dependent learning and memory does not involve modulation of voltage-gated dendritic Ca^{2+} signaling mediated by L-type channels in apical dendrites of CA1 pyramidal neurons.

Keywords

BDNF; Ca^{2+} imaging; Ca^{2+} channels; CA1; Hippocampus; Pyramidal neuron; TrkB

1. Introduction

Various models of activity-dependent synaptic plasticity postulate the existence of extracellular signaling molecules that enhance or stabilize synchronously active synapses. Neurotrophins (NTs) are postulated to play such a neuromodulatory role, since their production and release are regulated by neuronal activity. Furthermore, NTs have profound effects on several signaling pathways in central neurons, which have been shown to be necessary for the induction and maintenance of long-term changes in synaptic strength, such as long-term potentiation (LTP, Thoenen, 1995; McAllister et al., 1999; Poo, 2001). Because the spatio-temporal patterns of transient elevations of the intracellular concentration of free Ca^{2+} ions ($[\text{Ca}^{2+}]_i$) are known to be crucial for synapse development and plasticity (Zucker, 1999), and since NTs are also involved in these processes, a likely mechanism of NT action is the modulation of $[\text{Ca}^{2+}]_i$ patterns in pre- and/or post-synaptic neuronal compartments.

NTs are secretory proteins known to regulate neuronal survival and differentiation (Barde, 1989). Four NTs have been identified in mammals, and are widely expressed in the CNS: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5 (Lewin and Barde, 1996). These proteins exert their effects by binding to high-affinity cell membrane receptors. These receptors, members of the *trk* family of protooncogenes, are tyrosine kinases related to insulin and epidermal growth factor receptors. NGF binds to TrkA, BDNF and NT-4/5 to TrkB, and NT-3 to TrkC (Chao, 1992). Some of the tyrosine kinase-dependent signaling pathways activated by NTs are predicted to directly evoke transient elevations of $[Ca^{2+}]_i$ in neurons. However, experimental evidence of such NT-mediated Ca^{2+} signaling is sparse, and limited to embryonic cells maintained in primary cultures. In addition, most reported measurements of NT-induced changes in $[Ca^{2+}]_i$ were conducted without simultaneous electrophysiological recordings or membrane voltage control, making it difficult to distinguish NTs' direct effects on $[Ca^{2+}]_i$ transients from voltage-dependent $[Ca^{2+}]_i$ changes triggered by plasma membrane depolarization.

To begin addressing the modulation of dendritic Ca^{2+} signaling by BDNF in hippocampal neurons, we performed simultaneous whole-cell recordings and optical imaging of Ca^{2+} -sensitive dyes in CA1 pyramidal neurons from serum-free controls and BDNF-treated slice cultures during short trains of back-propagating action potentials (APs) evoked by direct current injection into the soma. Dendritic Ca^{2+} influx in CA1 pyramidal neurons depends on the generation and spread of Na^+ -dependent, back-propagating APs that activate different types of voltage-gated Ca^{2+} channels (Jaffe et al., 1992; Miyakawa et al., 1992; Spruston et al., 1995). Our observations indicate that long-term exposure to BDNF does not affect $[Ca^{2+}]_i$ transients in proximal apical dendrites and cell bodies mediated by dihydropyridine-sensitive L-type Ca^{2+} channels during trains of back-propagating APs. These results suggest that, despite BDNF's profound effects on hippocampal synaptic plasticity, and of L-type Ca^{2+} channels on neuronal gene transcription, the role of BDNF in cellular models of hippocampus-dependent learning and memory does not involve modulation of voltage-gated dendritic Ca^{2+} signaling mediated by L-type channels in the proximal apical dendrites and somas of CA1 pyramidal neurons.

2. Experimental procedures

2.1. Organotypic slice cultures

Hippocampi from postnatal-day seven rats were dissected after rapid decapitation under sterile conditions, and transverse slices (~500 μ m thick) were prepared with a custom-made wire-slicer fitted with 20 μ m thick gold-plated platinum wire (California Fine Wire Corporation). Hippocampal slices were cultured on Millicell-CM filter inserts (Millipore) in a 36°C, 5% CO_2 , 99% relative humidity incubator (Forma Scientific), as previously described (Pozzo-Miller et al., 1993). To control for unknown concentrations of growth factors and hormones in the culture media, the concentration of horse serum (Gibco BRL) was reduced to 10% at 4 days in vitro (div.), and again reduced to 5% 24 h later. After 24 h in 5% horse-serum media, slices were placed in a defined serum-free medium (Neurocellular II, Biofluids) containing B-27 supplement (Gibco BRL) for additional 24 h. On the seventh day in vitro, slices were treated with human recombinant BDNF (250 ng/ml, gift from AMGEN) in serum-free medium for 2–4 days, or maintained in serum-free medium as controls. The culture media were completely exchanged every 3 days, and slices were used for electrophysiological experiments between 9 and 11 div.

2.2. Simultaneous whole-cell recording and Ca^{2+} imaging

Beginning with 9 div., hippocampal slice cultures were transferred to an immersion chamber and perfused with artificial cerebrospinal fluid (ACSF) at room temperature that contained (in

mM): 124 NaCl, 2 KCl, 1.3 MgSO₄, 1.24 KH₂PO₄, 17.6 NaHCO₃, 2.5 CaCl₂, 10 D-glucose, 310–320 mOsm, and was equilibrated with 95% O₂/5% CO₂. Whole-cell recordings were performed with patch pipettes pulled from thin-wall glass capillaries that contained (in mM): 120 K⁺-gluconate, 17.5 KCl, 10 NaCl, 2 Mg-ATP, 0.2 Na-GTP, 10 Na-HEPES, 0.25 hexapotassium salt of bis-fura-2 (K_d = 525 nM, Molecular Probes), 280–290 mOsm, pH 7.2 (final resistance 4–6 MΩ). CA1 pyramidal neurons were visualized with infrared-DIC optics in a fixed-stage upright microscope (Zeiss Axioskop FS) using a water-immersion 63X objective (0.9NA, Zeiss Achroplan) and a CCD video camera (C2400-C77, Hama-matsu). Whole-cell intracellular recordings were performed in the current-clamp mode of an Axoclamp 2B amplifier (Axon Instruments). Electrical signals were filtered at 2 kHz and digitized at 10 kHz for display and analysis using an ITC-18 digitizing interface (Instrutech Corporation). Short bursts of 10–20 action potentials were evoked by injection of a train of brief (5 ms) depolarizing current pulses via the recording patch electrode into the soma. The AMPA receptor non-competitive antagonist GYKI (20 μM, RBI) was added to the ACSF to prevent excessive network activation in the slice during action potential discharge in the neuron under study. In experiments designed to block L-type voltage-gated Ca²⁺ channels, the dihydropyridine derivatives nifedipine or nimodipine (20 μM dissolved in 0.01% DMSO, RBI) were added to the ACSF.

Following 15–20 min of whole-cell access, the Ca²⁺-sensitive fluorescent dye bis-fura-2 included in the whole-cell patch pipette (250 μM) had diffused into the dendritic tree well beyond 200–400 μm from the soma and reached a steady-state in the soma and proximal dendrites. Bis-fura-2 was then alternately excited with 357 or 380 nm light (12 nm bandwidth) using a Xe lamp and a rapid (~2ms) galvanometric scanner-mounted grating (Polychrome II, TILL Photonics), and its emission at 510nm was detected with a digital, cooled CCD camera (Quantix, Photometrics) equipped with a back-illuminated chip (EEV57, 512 pixels × 1024 pixels) operating in frame-transfer mode. Representative subarrays of 100 pixels × 200 pixels were read out in ~10ms using 3 × 1 on-chip pixel binning; this read-out speed allowed frame rates of ~20–33 frames per second when using exposure times between 20 and 40 ms per image frame. Synchronized electrical and optical recordings, and control of the intracellular amplifier and galvanometric monochromator were all simultaneously performed using a single G3 Macintosh (Apple) computer running custom-written software (courtesy of Dr. Takafumi Inoue, University of Tokyo, Japan).

Free cytosolic Ca²⁺ concentration is directly proportional to the ratio of the fluorescence intensities obtained from two different Ca²⁺-sensitive excitation wavelengths, usually 340 and 380 nm for fura-2 (Grynkiewicz et al., 1985). When the isosbestic (Ca²⁺-insensitive) wavelength is used as the numerator to calculate the ratio of fluorescence intensities, then a single wavelength protocol can be used (Neher and Augustine, 1992; Pozzo-Miller et al., 1999), as follows: image frames at 357 nm excitation (Ca²⁺-insensitive wavelength, or isosbestic point, calibrated in our system) were acquired before and after a fast sequence of 380nm excitation frames (Ca²⁺-sensitive wavelength) to generate pixel-by-pixel ratio images. Illumination of the sample with UV light was limited to a maximum of ~1 s during the acquisition of image sequences of 32 frames. Small (~10 pixels × 10 pixels) regions of interests (ROIs) were defined within dendrites and somas, as well as over a region on the slice outside of the dye-filled cell for background subtraction. Peak Ca²⁺ concentration levels during somatic current injections and afferent synaptic stimulations were estimated by subtracting the basal pre-stimulus 357/380 ratio (R_b) from the same ratio at the peak of the response (R_p), and referred to as $\Delta R = R_p - R_b$. The proportion of dendritic Ca²⁺ transients mediated by L-type Ca²⁺ channels was estimated from the 357/380 ratio during an AP train in the presence of nifedipine (ΔR_{nife}), and expressed as a percentage of the 357/380 ratio during a similar AP train before drug application (ΔR_{ctl}), such as $\text{block}(\%) = \Delta R_{nife} \times 100 / \Delta R_{ctl}$. All data are

expressed as the mean $\pm$ S.E.M. Statistical differences were assessed using Student's *t*-test, $P < 0.05$ was considered significant.

3. Results

Brain-derived neurotrophic factor (BDNF), signaling through its plasma membrane tyrosine kinase receptor TrkB, activates several intracellular signaling cascades (Segal and Greenberg, 1996) which may directly elicit Ca^{2+} release from intracellular stores via IP3 production, and/or indirectly modulate Ca^{2+} influx through voltage-gated Ca^{2+} channels or NMDA receptors via enhanced protein phosphorylation of specific subunits. Our proposed model of BDNF modulation of neuronal Ca^{2+} signaling is illustrated in Fig. 1. To begin addressing the modulation of dendritic Ca^{2+} signaling in hippocampal neurons by BDNF, we focused on Ca^{2+} influx into proximal apical dendrites and somatic regions during short trains of back-propagating action potentials (APs) evoked by depolarizing current injection.

Whole-cell intracellular recordings were performed under current-clamp from visually identified CA1 pyramidal neurons from 9 to 11 days in vitro (div.) hippocampal slice cultures using patch pipettes containing the fluorescent Ca^{2+} indicator bis-fura-2 (250 μM in K^{+} -gluconate intracellular solution). Simultaneous fluorescence digital imaging of dye-filled neurons was restricted to proximal apical dendrites (up to 100 μm from the soma). No significant differences ($P > 0.05$) were found between CA1 pyramidal neurons from serum-free controls ($n = 5$) and slices treated with BDNF (250 ng/ml) for 2 to 4 div. ($n = 5$) with respect to resting membrane potentials (-62 ± 1 mV versus -63 ± 2 mV, control versus BDNF), input resistances (103 ± 17 M Ω versus 87 ± 7 M Ω , control versus BDNF), or resting intracellular Ca^{2+} concentrations (357/380 ratio = $0.15 \pm .02$ versus 0.14 ± 0.03 , control versus BDNF).

3.1. BDNF does not affect Ca^{2+} transients evoked by back-propagating action potentials in CA1 pyramidal neuron dendrites

Figure 2 shows the transient elevations of $[\text{Ca}^{2+}]_i$ in the proximal apical dendrites of CA1 pyramidal neurons (shown at left) in serum-free control and BDNF-treated slices, evoked by a train of 10 APs at 20 Hz elicited by short (5 ms) depolarizing current injections into their somas via the patch pipette. To prevent excessive network activity in the slice during depolarizing current injections into the cell under study, the AMPA receptor non-competitive antagonist GYKI (20 μM) was routinely added to the extracellular recording solution. Calcium concentration changes within the areas marked by the regions-of-interest (ROIs) in the fluorescent images (380 nm excitation, Fig. 2, left) were acquired at 20–33 frames per second simultaneously with recordings of membrane potential in the current-clamp mode, and are plotted as a function of time (Fig. 2, traces at right). Rapid Ca^{2+} elevations within proximal apical dendrites occur immediately after the firing of the first APs, and reach a maximum level by the end of the 10 AP train. Virtually equal spatio-temporal patterns of transient elevations of Ca^{2+} concentration were observed in CA1 neurons from serum-free controls ($n = 5$) and BDNF-treated slices ($n = 5$). In addition, no significant differences were observed in the peak dendritic Ca^{2+} concentrations evoked by these trains of 10 APs at 20 Hz between neurons from serum-free controls ($\Delta R = 0.033 \pm 0.007$, $n = 5$) and BDNF-treated slices ($\Delta R = 0.025 \pm 0.005$, $n = 5$, *t*-test, $P > 0.05$). These results suggest that long-term exposure to BDNF does not affect voltage-gated Ca^{2+} entry in proximal apical dendrites of CA1 pyramidal neurons in postnatal hippocampal slices.

3.2. BDNF does not affect Ca^{2+} transients mediated by L-type Ca^{2+} channels during back-propagating AP firing in CA1 pyramidal neuron dendrites and somas

Due to their clustering at the soma and in the base of proximal dendrites, high-threshold L-type voltage-gated Ca^{2+} channels are the most likely route of Ca^{2+} entry in those regions during back-propagating APs (Ahlijanian et al., 1990; Westenbroek et al., 1990). To test if the proportion of Ca^{2+} entry mediated by L-type channels was different between serum-starved and BDNF-treated neurons, we used the L-type channel blocker nifedipine (20 μM) and measured the magnitude of the blockade of Ca^{2+} transients during APs trains (Fig. 3). The magnitude of the L-type component of the dendritic and somatic Ca^{2+} transients observed here is in good agreement with similar Ca^{2+} measurements in CA1 neurons in acute slices (Christie et al., 1995), and in CA3 neurons in slice cultures (Elliott et al., 1995). Nifedipine reduced dendritic Ca^{2+} transients during APs trains by 19% (serum-free, $n = 5$) and 26% (BDNF-treated, $n = 5$), while somatic Ca^{2+} transients were reduced by 22 and 20% in serum-free and BDNF-treated neurons, respectively (Fig. 4). Therefore, there were no significant differences ($P > 0.05$) between serum-free controls and BDNF-treated neurons in the amount of L-type channel block by nifedipine. Similar results were obtained using another dihydropyridine L-type channel blocker, ni-modipine (not shown). These results strongly suggest that long-term exposure to BDNF does not affect dendritic Ca^{2+} entry mediated by L-type Ca^{2+} channels during the firing of back-propagating APs in CA1 pyramidal neurons.

4. Discussion

We have presented evidence that long-term BDNF treatment of hippocampal slice cultures does not affect proximal dendritic and somatic Ca^{2+} influx in CA1 pyramidal neurons mediated by activation of dihydropyridine-sensitive L-type voltage-gated Ca^{2+} channels during back-propagating action potential firing. These observations have implications for the role of BDNF in synaptic plasticity, since they rule out a major contributing pathway of Ca^{2+} influx into primary dendrites of CA1 pyramidal neurons that has been implicated in the generation of dendritic Ca^{2+} signals during coincident pre- and post-synaptic firing. In addition, our observations are relevant to synaptic plasticity because voltage-gated Ca^{2+} influx mediated by L-type Ca^{2+} channels plays a fundamental role in neuronal gene transcription (Mermelstein et al., 2000), and, thus, may also modulate the late phase of LTP (Kandel, 2001).

Some of the signaling pathways activated by NTs in neurons are proposed to directly elicit transient elevations in the intracellular concentration of Ca^{2+} ions (Segal and Greenberg, 1996). However, experimental evidence of such NT-mediated Ca^{2+} signaling is sparse, and limited to embryonic neurons in primary culture. The published data to date are rather controversial because measurements of changes in intracellular Ca^{2+} concentration were done without simultaneous electrophysiological measurements or experimental control of membrane voltage, making it difficult to isolate the role of plasma membrane depolarization in triggering Ca^{2+} entry. NGF has been reported to increase $[\text{Ca}^{2+}]_i$ in PC12 cells (Pandiella-Alonso et al., 1986), most likely via the activation of PLC- γ (Vetter et al., 1991), leading to IP_3 formation and subsequent Ca^{2+} release from intracellular stores (Berridge, 1998). BDNF has also been reported to induce rapid phosphorylation of PLC- γ , thereby increasing IP_3 levels and evoking Ca^{2+} release from intracellular stores (Widmer et al., 1992; Widmer et al., 1993; Zirrgiebel et al., 1995). At *Xenopus* neuromuscular junctions in culture, the enhancement of synaptic transmission by BDNF is accompanied by an increase in $[\text{Ca}^{2+}]_i$ within pre-synaptic terminals (Stoop and Poo, 1996). In embryonic hippocampal neurons in culture, BDNF induces elevations of $[\text{Ca}^{2+}]_i$ (Berninger et al., 1993; Canossa et al., 1997; Finkbeiner et al., 1997; Marsh and Palfrey, 1996). BDNF also potentiates spontaneous $[\text{Ca}^{2+}]_i$ oscillations in cultured hippocampal neurons (Sakai et al., 1997). However, due to the well-established enhancement of neurotransmitter release by the NTs (Berninger and Poo, 1996; Tyler and Pozzo-Miller,

2001; Tyler et al., 2002), cultured hippocampal neurons will increase their firing rates leading to increases in $[Ca^{2+}]_i$ via voltage-gated influx. These neurons will then exhibit potentiated $[Ca^{2+}]_i$ oscillations evoked by enhanced network activity driven by a higher rate of action potential firing in the presence of BDNF; in fact, tetrodotoxin (TTX) completely blocked these $[Ca^{2+}]_i$ oscillations (Sakai et al., 1997). Similarly, the blocking effect of the NMDA receptor (NMDAR) antagonist D,L-APV on the spontaneous $[Ca^{2+}]_i$ oscillations most likely reflects impaired influx via voltage-dependent Ca^{2+} channels by reducing post-synaptic depolarization, since the same effect was observed after application of the AMPA/kainate receptor antagonist CNQX (Sakai et al., 1997). Therefore, these reports of $[Ca^{2+}]_i$ elevations induced by BDNF can only be accounted for by action potential driven voltage-dependent Ca^{2+} influx. However, those $[Ca^{2+}]_i$ elevations evoked by NT application in cultured hippocampal neurons were reduced, but not completely blocked, in Ca^{2+} -free extracellular media, suggesting that the signaling pathway also involved mobilization of Ca^{2+} from intracellular stores (Canossa et al., 1997; Finkbeiner et al., 1997; Marsh and Palfrey, 1996). In addition, our results indicate that L-type Ca^{2+} channel-mediated Ca^{2+} -elevations in primary dendrites and somas are not affected by BDNF, suggesting that enhanced Ca^{2+} oscillations are a secondary effect of BDNF acting on quantal neurotransmitter release at pre-synaptic terminals (Tyler and Pozzo-Miller, 2001; Tyler et al., 2002).

Postranslational modifications of ion channels by NTs via protein phosphorylation may provide yet another molecular mechanism of their action at central synapses. NTs have been shown to enhance voltage-gated ionic currents (Lesser et al., 1997), including Ca^{2+} currents (Levine et al., 1995; Baldelli et al., 1999; Baldelli et al., 2000). Our results are in good agreement with recent reports that indicated BDNF has no effect on L-type Ca^{2+} channels in embryonic hippocampal and spinal motoneurons in vitro, but selectively enhances N-, P/Q-, and R-type Ca^{2+} channels, while NGF and NT-3 selectively up-regulate L-type Ca^{2+} channels (Baldelli et al., 1999; Baldelli et al., 2000). It is worth noting that the non-L-type Ca^{2+} channels modulated by BDNF have a predominately pre-synaptic localization and are involved in neurotransmitter release (Wu and Saggau, 1997), supporting the view that BDNF enhances pre-synaptic release by the modulation of Ca^{2+} levels in pre-synaptic terminals (Berninger and Poo, 1996; Tyler and Pozzo-Miller, 2001; Tyler et al., 2002). L-type Ca^{2+} channels, on the other hand, are mainly associated with neuronal activity at the somatic level leading to gene transcription (Mermelstein et al., 2000); it is, thus, likely that modulation of non-L-type Ca^{2+} channels by BDNF could be related to synaptic plasticity, while L-type Ca^{2+} channel modulation by other NTs may contribute to more classic neurotrophic effects, such as neuronal survival and differentiation through modulation of gene transcription (Mermelstein et al., 2000).

NT modulation of $[Ca^{2+}]_i$ also appears to be important in adult synaptic plasticity in the hippocampus. BDNF has been shown to induce a long-lasting potentiation of excitatory synaptic transmission in the CA1 region of acute hippocampal slices (Kang and Schuman, 1995), as well as in the dentate gyrus in vivo (Messaoudi et al., 1998). In the dentate gyrus, this BDNF-induced LTP requires activation of an extracellular signal-regulated kinase coupled to induction of the immediate early gene, Arc (Ying et al., 2002). While this suggests involvement of TrkB signaling through Ras-ERK in BDNF-LTP, recent evidence also suggests an important role for transient elevations in Ca^{2+} concentration. BDNF-LTP in the dentate gyrus in vivo is associated with activation of two Ca^{2+} -regulated protein kinases, Ca^{2+} /calmodulin-dependent protein kinase II (CaMK-II) and elongation factor-2 kinase, also known as CaMK-III (Kanhema et al., 2001). In addition, BDNF-induced potentiation in CA1 synapses of adult hippocampal slices in vitro is attenuated by compounds that block voltage-dependent Ca^{2+} channels, or mobilization of Ca^{2+} from intracellular stores (Kang and Schuman, 2000).

While our results suggest that BDNF has no effects on $[Ca^{2+}]_i$ transients elicited by a train of 10 APs at 20 Hz in CA1 pyramidal neuron dendrites, there are a few issues related to this conclusion that merit additional discussion. Firstly, our experiments were conducted in cultured slices exposed to BDNF for 2–4 days in vitro, thus, we are not addressing the potential acute modulation of voltage-gated Ca^{2+} transients by BDNF. Secondly, we focused our attention on Ca^{2+} transients elicited by short trains of 10 APs at 20 Hz because this pattern resembles the slow component of hippocampal theta rhythm and induces strong LTP in hippocampal slices. A third issue is that blockade of L-type channels by dihydropyridines reduced somatic and dendritic $[Ca^{2+}]_i$ transients during back-propagating APs by only about 20%. NMDA receptors could also contribute to dendritic Ca^{2+} signals, if activated by residual or spontaneously released glutamate during AP firing. However, the potential NMDA receptor activation by synaptically released glutamate is minimized because network activation during AP firing was reduced by blocking AMPA receptors with the non-competitive antagonist GYKI (20 μ M). On the other hand, there is always potential activation of NMDA receptors by ambient glutamate when the voltage-dependent Mg^{2+} block is relieved by the dendritic back-propagating AP. However, it has previously been reported that application of the NMDA receptor antagonist CPP to hippocampal slices during back-propagating APs evoked by somatic current injection did not reduce $[Ca^{2+}]_i$ transients in dendrites or spines of CA1 pyramidal neurons (Sabatini and Svoboda, 2000). This lends support to the suggestion that NMDA receptor-mediated $[Ca^{2+}]_i$ entry did not contribute significantly to the Ca^{2+} signals observed in our experiments. Non-L-type VDCCs could also contribute to the observed $[Ca^{2+}]_i$ transients. Sabatini and Svoboda (2000) reported that application of ω -conotoxin MVIIC, which blocks N-, P-, and Q-type VDCCs, did not affect dendritic $[Ca^{2+}]_i$ transients in CA1 pyramidal neurons during back-propagating APs, even though synaptic potentials were reduced by over 90%, indicating that pre-synaptic Ca^{2+} influx was blocked by the toxin. This result differs markedly from previous findings in cortical neurons, which indicated N- and P-type (as well as L- and R-type) VD-CCs did contribute significantly to $[Ca^{2+}]_i$ influx into layer V pyramidal neuron proximal dendrites (Markram et al., 1995), suggesting variability in dendritic Ca^{2+} channel types expressed by cortical and hippocampal pyramidal neurons. On the other hand, Christie et al. (1995) reported that high-threshold VDCCs were primarily responsible for Ca^{2+} influx into somata and proximal dendrites of CA1 neurons during back-propagating action potentials. The contribution of low-voltage-activated T-type VDCCs is minimized because these channels should be inactivated at the resting membrane potentials (–60 to –68 mV) of the cells used in our experiments. Considering that neither N-, P-, Q-, and T-type VDCCs, nor NMDA receptors appear to contribute significantly to the dihydropyridine-insensitive $[Ca^{2+}]_i$ transients during back-propagating APs in CA1 pyramidal neuron dendrites, it seems likely that R-type VDCCs could play a dominant role (Sabatini and Svoboda, 2000).

Our results indicate that, despite its profound effects on hippocampal synaptic transmission and plasticity, the role of BDNF in cellular models of hippocampal-dependent learning and memory does not involve the modulation of voltage-gated dendritic Ca^{2+} signaling mediated by L-type channels in the somas or proximal regions of apical dendrites of CA1 pyramidal neurons. On the other hand, L-type Ca^{2+} channel modulation by other NTs may contribute to more classic neurotrophic effects, such as neuronal survival and differentiation through modulation of gene transcription. Although NTs have been shown to induce $[Ca^{2+}]_i$ elevations by themselves, the specific mechanism/s involved, the route of Ca^{2+} entry, and the consequences for synaptic plasticity are not yet known. Therefore, the delineation of the pre- and post-synaptic mechanisms triggering those $[Ca^{2+}]_i$ elevations is fundamental to the understanding of how, NTs modulate synaptic plasticity.

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Fig. 1.

Hypothesized regulation of the different routes of Ca²⁺ influx and/or release from stores by NTs, and its effects on intracellular Ca²⁺ levels. (A) CA1 pyramidal neuron at rest (left). Intracellular calcium concentration is represented following the standard convention utilized to display data from imaging experiments using Ca²⁺-sensitive fluorescent dyes (blue to red, low to high Ca²⁺ levels, respectively). The diagram to the right is an expanded view of an excitatory synapse on a dendritic spine framed by a box in the apical dendrite at left. (B) Pre-synaptic release of excitatory neurotransmitter from afferent fibers depolarizes the post-synaptic membrane, eliciting Ca²⁺ elevations by influx through voltage-dependent Ca²⁺ channels and NMDA receptors. Activation of metabotropic glutamate receptors also contributes to Ca²⁺ rises by release from IP₃-sensitive intracellular stores. Ca²⁺-induced

Ca^{2+} release from ryanodine stores further amplifies dendritic Ca^{2+} elevations. (C) Our model proposes that, in addition to evoke Ca^{2+} changes by itself, BDNF enhances dendritic Ca^{2+} elevations through other routes evoked by synaptic activity. BDNF binding induces autophosphorylation of TrkB receptors, and subsequent activation of the PLC- γ /IP₃ signaling pathway, leading to Ca^{2+} release from intracellular stores. Activated TrkB receptors also trigger tyrosine phosphorylation of voltage-dependent Ca^{2+} channels and NMDA receptors, enhancing Ca^{2+} influx into spines and dendrites.

Fig. 2.

Voltage-dependent Ca^{2+} elevations in CA1 pyramidal neurons during back-propagating APs are not affected by BDNF treatment. No differences in the spatio-temporal pattern or in the peak amplitude of voltage-dependent Ca^{2+} transients were observed between serum-free controls and BDNF-treated slices. A train of 10 back-propagating APs (middle right traces) was evoked by short depolarizing current pulses (bottom right traces) delivered by the recording patch pipette in the soma. The square pulses shown in the top right traces represent the TTL signal output of the digital camera during image frame exposures used to synchronize optical and electrophysiological recordings. Top left, fluorescence image (380 nm excitation) of a CA1 pyramidal neuron from a serum-free control slice. Bottom left, CA1 pyramidal neuron from a BDNF-treated slice culture (250 ng/ml for 48 h). Topmost right traces show intracellular Ca^{2+} concentration levels calculated from the dotted ROIs shown in the fluorescence images at left after background-subtraction from a ROI over the slice but outside the dye-filled cell. Membrane voltage and current traces (at 10 kHz) were acquired synchronously with Ca^{2+} concentration traces (at 10 ms frame interval), and are shown with the same time base.

Fig. 3. Voltage-dependent Ca^{2+} elevations in CA1 pyramidal neurons evoked by 10 short depolarizing current pulses are partially sensitive to the L-type Ca^{2+} channel blocker nifedipine. The top panel shows a fluorescence image (380 nm excitation) of a representative CA1 pyramidal neuron from a BDNF-treated slice, and the traces below show the effect of the L-type Ca^{2+} channel blocker nifedipine on the Ca^{2+} elevations during a train of 10 back-propagating APs. The order of the bottom traces is the same as in Fig. 2 (top to bottom, Ca^{2+} profile from the ROIs in the fluorescence image, expose camera signal TTL, action potentials in membrane voltage, injected current).

Fig. 4.

The proportion of nifedipine block is not different between neurons from serum-free controls and BDNF-treated slices. Summary bar graphs of all experiments on the proportion of L-type Ca^{2+} channel block of Ca^{2+} transients during trains of 10 back-propagating APs. Nifedipine blocked between 19 and 26% of dendritic and somatic Ca^{2+} transients, and there were no differences between CA1 pyramidal neurons from serum-free controls and BDNF-treated slices (t -test, $P > 0.05$).