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BDNF Induces Calcium Elevations Associated With I_{BDNF} , a Nonselective Cationic Current Mediated by TRPC Channels

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Abstract

Brain-derived neurotrophic factor (BDNF) has potent actions on hippocampal neurons, but the mechanisms that initiate its effects are poorly understood. We report here that localized BDNF application to apical dendrites of CA1 pyramidal neurons evoked transient elevations in intracellular Ca^{2+} concentration, which are independent of membrane depolarization and activation of *N*-methyl-D-aspartate receptors (NMDAR). These Ca^{2+} signals were always associated with I_{BDNF} , a slow and sustained nonselective cationic current mediated by transient receptor potential canonical (TRPC3) channels. BDNF-induced Ca^{2+} elevations required functional Trk and inositol-tris-phosphate (IP_3) receptors, full intracellular Ca^{2+} stores as well as extracellular Ca^{2+} , suggesting the involvement of TRPC channels. Indeed, the TRPC channel inhibitor SKF-96365 prevented BDNF-induced Ca^{2+} elevations and the associated I_{BDNF} . Thus TRPC channels emerge as novel mediators of BDNF-induced intracellular Ca^{2+} elevations associated with sustained cationic membrane currents in hippocampal pyramidal neurons.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a potent modulator of neuronal structure and function (Amaral et al. 2007; Bramham and Messaoudi 2005; Lu 2003; Poo 2001; Tyler et al. 2002). Because Ca^{2+} plays a critical role in these fundamental processes, it is significant that BDNF modulates intracellular Ca^{2+} levels. One of the signaling cascades activated by neurotrophin Trk receptors, the hydrolysis of phosphatidylinositol biphosphate (PIP_2) by phospholipase C gamma ($\text{PLC}\gamma$) leading to IP_3 formation, causes intracellular Ca^{2+} mobilization (Segal and Greenberg 1996). However, direct evidence of such neurotrophin-initiated Ca^{2+} signals is sparse, controversial, and mostly limited to embryonic cultured neurons. BDNF increased Ca^{2+} levels in cultured hippocampal (Berninger et al. 1993; Canossa et al. 1997; Finkbeiner et al. 1997; Li et al. 1998; Marsh and Palfrey 1996) and cortical neurons (Behar et al. 1997; Matsumoto et al. 2001; Mizoguchi and Nabekura 2003; Mizoguchi et al. 2002; Yang and Gu 2005; Zirrgiebel et al. 1995). In contrast, BDNF failed to affect Ca^{2+} levels in cultured cerebellar granule cells (Gaiddon et al. 1996; but see Jia et al. 2007; Numakawa et al. 2001) and in acute slices from visual cortex (Pizzorusso et al. 2000). BDNF also potentiated spontaneous Ca^{2+} oscillations in cultured hippocampal neurons (Numakawa et al. 2002; Sakai et al. 1997); however, this effect was due to enhanced network activity leading to voltage-dependent Ca^{2+} influx (Sakai et al. 1997). Also, BDNF increased Ca^{2+} levels within presynaptic terminals of cultured *Xenopus* neuromuscular junctions (Boulanger and Poo 1999; Stoop and Poo 1996). Unfortunately, nearly all published Ca^{2+} imaging studies of BDNF

actions on intracellular Ca^{2+} levels were done without simultaneous membrane voltage control, making it difficult to differentiate the contribution of voltage-gated and receptor-operated Ca^{2+} influx to the observed Ca^{2+} signals. In fact, most studies to date conclude that a significant fraction of the BDNF-induced Ca^{2+} elevations is sensitive to glutamate receptor antagonists (e.g., Yang and Gu 2005). It should be noted that dendritic and spine Ca^{2+} elevations induced by BDNF in hippocampal dentate granule cells were sensitive to voltage-gated Ca^{2+} channel blockers (Kovalchuk et al. 2002) and always associated with the membrane depolarization proposed to be mediated by $\text{Na}_v1.9$ channels (Blum et al. 2002; Kafitz et al. 1999).

The controversial state of our understanding of BDNF actions on intracellular Ca^{2+} levels prompted us to perform simultaneous whole cell recording and microfluorometric imaging in voltage-clamped neurons. We present evidence that localized BDNF application to apical dendrites of CA1 pyramidal neurons in hippocampal slice cultures evoked transient elevations in intracellular Ca^{2+} concentration, which are independent of voltage-gated Ca^{2+} channels and *N*-methyl-D-aspartate (NMDA) receptors. These Ca^{2+} signals were always associated with I_{BDNF} , a slow and sustained nonselective cationic current mediated by TRPC3 channels (Amaral and Pozzo-Miller 2007; Li et al. 1999). BDNF-induced Ca^{2+} elevations required functional Trk and IP_3 receptors, full intracellular Ca^{2+} stores, as well as extracellular Ca^{2+} , suggesting the involvement of TRPC channels. Indeed, the TRPC channel inhibitor SKF-96365 prevented BDNF-induced Ca^{2+} elevations and the associated I_{BDNF} . Thus TRPC channels emerge as novel mediators of BDNF-induced intra-cellular Ca^{2+} elevations in hippocampal pyramidal neurons.

METHODS

Organotypic slice culture

All procedures performed on experimental animals adhered to national and international guidelines for the ethical use of research animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Alabama at Birmingham. Briefly, hippocampi were dissected from anesthetized postnatal day 7–11 Sprague Dawley rats (Harlan, Indianapolis, IN, or Charles River, Wilmington, MA) and cut transversely into ~400- μm -thick slices using a custom-made wire-slicer fitted with 20- μm -thick gold-coated platinum wire (Pozzo-Miller et al. 1995). Hippocampal slices were individually plated on Millicell-CM filter inserts (Millipore; Billerica, MA) and cultured in 36°C, 5% CO_2 , 98% relative humidity incubators (Thermo-Forma; Waltham, MA). Slices were maintained in culture media (Neurobasal-A plus B27, Invitrogen; Carlsbad, CA) containing 20% equine serum for the first 4 days in vitro (div). To avoid the confounding effects of hormones and growth factors in the serum, its concentration was gradually reduced over a period of 48 h starting at 4 div (24 h each in 10% and 5% serum), as described (Tyler and Pozzo-Miller 2001). After a period of 24 h in serum-free media (Neurobasal-A plus B27), 7–10 div slices were used for simultaneous electrophysiology and Ca^{2+} imaging.

Simultaneous electrophysiology and Ca^{2+} imaging

Individual 7–10 div slices were transferred to a recording chamber mounted on a fixed-stage upright microscope (Zeiss Axioskop FS; Oberkochen, Germany) and continuously perfused (2 ml/min) with artificial cerebrospinal fluid (ACSF) at room temperature (24°C), containing (in mM) 124 NaCl, 2 KCl, 1.24 KH_2PO_4 , 1.3 MgSO_4 , 17.6 NaHCO_3 , 2.5 CaCl_2 , 10 glucose, and 29.2 sucrose (310–320 mosM); ACSF was bubbled with 95% O_2 –5% CO_2 (pH 7.4). Superficial CA1 pyramidal neurons were visualized with a water-immersion 40 $\times$ objective (0.9 NA) using IR-DIC microscopy. Simultaneous whole cell recording and microfluorometric Ca^{2+} imaging was performed as described (McCutchen et al. 2002; Petrozzino et al. 1995; Pozzo-Miller 2006; Pozzo-Miller et al. 1996, 1999). Briefly, unpolished patch pipettes

contained (in mM) 120 Cs-gluconate, 17.5 CsCl, 10 Na-HEPES, 4 Mg-ATP, 0.4 Na-GTP, 10 Na₂ creatine phosphate, and 0.2 mM fura-2 (or 0.5 mM bis-fura-2); 280–290 mosM; pH 7.2 (resistance 3–4 MΩ). Nominally calcium-free extracellular ACSF was prepared by replacing CaCl₂ with an equimolar concentration of MgCl₂. Some drugs were dissolved in DMSO (0.01%) and others directly into the ACSF or intracellular solution; vehicle controls using 0.01% DMSO were routinely performed yielding no effects on membrane currents or BDNF-induced responses. Membrane currents were recorded in the voltage-clamp mode at a holding potential of –65 mV using an Axoclamp 200B amplifier (Molecular Devices; Sunnyvale, CA), filtered at 2 kHz, and digitized at 10 kHz. Recordings were accepted only if access (series) resistance was ≤30 MΩ. CA1 neurons had whole cell capacitances of ~100 pF. Input resistance (R_i) was measured with hyperpolarizing voltage pulses (50 ms, –20 mV), and cells were discarded if any of those cell parameters (C_m , R_i , R_s) changed by ≥20% during the course of an experiment. All experiments were performed in the presence of TTX (0.5 μM) to block voltage-gated Na⁺ channels. As noted, some experiments included Cd²⁺ (200 μM) and D,L-2-amino-5-phosphonovaleric acid (D,L-APV, 50 μM) to block voltage-gated Ca²⁺ channels and NMDA receptors, respectively.

Fura-2 or bis-fura-2 (Molecular Probes; Carlsbad, CA) were alternatively excited at 360 and 380 nm using a monochromator (Polychrome-II, TILL Photonics; Munich, Germany), and its emission (>510 nm) filtered and detected with a frame-transfer cooled CCD camera (PXL-37, Roper Scientific; Duluth, GA); digital image pairs were acquired every 4 s (50-ms exposures for ~100 × 200 pixel sub-arrays, 1 × 1 binning). Background-subtracted fluorescence intensity measurements were obtained within regions of interest (ROIs) defined over apical primary and secondary dendrites, as well as over neuronal cell bodies. The average ratio of 360 and 380 nm fluorescence within each ROI was used as an estimate of intracellular Ca²⁺ concentration as these two parameters are directly proportional to each other (Grynkiewicz et al. 1985). Electrical and optical data were simultaneously acquired on a single G4 Macintosh computer (Apple; Cupertino, CA) running custom-written software (TIWorkBench, kindly provided Dr. T. Inoue, Waseda University, Japan). All of the chemicals used for these experiments were obtained from Sigma (St. Louis, MO), Calbiochem (San Diego, CA), or Tocris (Ellisville, MO).

Recombinant human mature BDNF (supplied by Amgen; Thousand Oaks, CA) was pressure-applied from glass pipettes (~5 MΩ) using a Picospritzer-III (Parker Hannifin; Cleveland, OH). An application pipette was positioned ~100 μm above the slice and ~200 μm away from the soma of the CA1 neuron under recording, aimed at its apical dendrites within stratum radiatum (~150 μm from the soma) and against the direction of ACSF perfusion flow. This arrangement produced a stream of BDNF solution that overshoots the cell under recording and flows back over the slice, already diluted in the ACSF. Application of glutamate (100 μM, 8 psi, 9 s) from similar pipettes was used to optimize this arrangement, yielding highly reproducible and stable transient membrane currents. In addition, food coloring was used to assess the spatial spreading of the applied solution over the slice. Pressure pulses of 30 psi lasting 25–30 s delivered a total volume of 2 μl of solution from ~5 MΩ glass pipettes. In most experiments, the pipette contained 100 μg/ml BDNF in 0.0001–0.1% bovine serum albumin (BSA). BDNF denatured by boiling (10 min; 100 μg/ml), BSA alone (0.0001 or 0.1%), and ACSF were used as pressure application controls, which yielded neither changes in membrane currents (Amaral and Pozzo-Miller 2007) nor intracellular Ca²⁺ levels.

Statistical analyses

All data are presented as means ± SE; the SD of the mean was used to calculate the coefficient of variance (CV = mean/SD), which is provided as a measure of consistency. All data were statistically analyzed using unpaired Student's *t*-test or ANOVA tests with the Prism software

package (GraphPad Software; San Diego, CA). Probability values lower than 0.05 were considered statistically significant (i.e., $P < 0.05$, <5% probability that the observations are due to chance). When lower than this cut-off value, the actual P values ≤ 4 decimal points are given in RESULTS (rather than just the statement “greater than” or “less than”) to provide readers with more detailed information regarding the outcome of the statistical analyses. Compromise power analyses were performed to determine the statistical power given the number of observations, sample means and SDs, using G*Power (Erdfeiler et al. 1996). These power analyses yielded values of statistical Power ($1 - \beta$; where β is the Type-II error) larger than 0.95 (i.e., 95% confidence of accepting the null hypothesis when is true).

The application of BDNF to CA1 pyramidal neurons evoked transient fura-2 ratio elevations that *preceded* I_{BDNF} with a mean amplitude of 1.26 ± 0.14 ($n = 3$; CV = 0.19); this value was five to six times the SD of the pre-BDNF baseline fura-2 ratio (0.89 ± 0.03 , $n = 7$, $P = 0.0043$ vs. peak amplitude; CV = 0.08). The peak of the sustained fura-2 ratio elevations that were *simultaneous* with I_{BDNF} reached a mean amplitude of 1.71 ± 0.09 ($n = 7$ of 7 cells; CV = 0.14), which was 17 times the SD of the pre-BDNF baseline fura-2 ratio (0.89 ± 0.03 , $n = 7$, $P < 0.0001$ vs. peak amplitude; CV = 0.08). Therefore the fura-2 responses to BDNF applications were always above “noise” fluctuations in fura-2 ratio values.

RESULTS

In the present experiments, we chose to locally apply BDNF from pipettes placed $\sim 100 \mu\text{m}$ above hippocampal slice cultures to avoid pressure and mechanical artifacts, and over s. radiatum dendrites $\sim 200 \mu\text{m}$ away from CA1 neuron cell bodies to reproduce the release profile of a paracrine neuropeptide (Lessmann et al. 2003). Under these conditions, a single 25- to 30-s application of BDNF activated I_{BDNF} , a delayed and slowly developing nonselective cationic conductance described originally in acutely dissociated pontine neurons (Li et al. 1999). I_{BDNF} was observed in every hippocampal CA1 pyramidal neuron from which we recorded (Amaral and Pozzo-Miller 2007), having a mean amplitude of 577.23 ± 41.07 pA ($n = 24$). Considering that I_{BDNF} was sensitive to the Ca^{2+} chelator bis-(*o*-aminophenoxy)-*N,N,N',N'*-tetraacetic acid (Amaral and Pozzo-Miller 2007), TRPC channel activity is enhanced by intracellular Ca^{2+} elevations (Clapham 2003), and plasma membrane TRPC channels mediating I_{BDNF} are permeable to Ca^{2+} ions (Li et al. 1999), we set out to perform simultaneous whole cell recording and microfluorometric imaging in voltage-clamped CA1 pyramidal neurons filled with Ca^{2+} indicators (200 μM fura-2 or 500 μM bis-fura-2 in the patch pipette).

Within 100 s of its application, BDNF evoked transient elevations in the 360/380 nm ratio in the targeted apical dendrites that *preceded* the onset of I_{BDNF} in three of seven cells tested in the presence of TTX (0.5 μM), despite the fact that every cell expressed I_{BDNF} (Amaral and Pozzo-Miller 2007). These initial fura-2 ratio elevations were restricted to apical dendrites $\sim 100 \mu\text{m}$ from the cell body and had peak amplitudes of 1.26 ± 0.14 ($n = 3$; CV = 0.19). To rule out the potential contribution of voltage-gated Ca^{2+} channels and NMDA receptors (NMDAR) to BDNF-induced Ca^{2+} elevations, Cd^{2+} (200 μM) and DL-APV (50 μM) were included in an additional set of experiments. In two of four cells, BDNF evoked transient fura-2 ratio increases that preceded I_{BDNF} in the absence of Ca^{2+} channel and NMDAR activity (peak amplitudes 1.05–1.6; Fig. 1A, expanded in B). The effects of BDNF were confirmed to be specific, as vehicle alone ($\leq 0.1\%$ BSA) or BDNF denatured by boiling were entirely ineffective (Amaral and Pozzo-Miller 2007).

The significant reduction of I_{BDNF} amplitude in the absence of extracellular Ca^{2+} (Amaral and Pozzo-Miller 2007) suggested that I_{BDNF} itself causes intracellular Ca^{2+} elevations. Indeed, the fura-2 ratio increased again during the sustained phase of I_{BDNF} but now simultaneously

throughout the somato-dendritic compartment (Fig. 1A). The peak of these sustained fura-2 ratio elevations occurred at 440 s from the onset of BDNF application and reached an average amplitude of 1.71 ± 0.09 ($n = 7$ of 7 cells; CV = 0.14). As with the initial fura-2 ratio elevations, voltage-gated Ca^{2+} channels and NMDARs did not contribute to these sustained BDNF-induced fura-2 signals, because they were not affected by Cd^{2+} (200 μM) and D,L-APV (100 μM ; peak amplitude 2.12 ± 0.35 , $n = 4$ of 4 cells, $P = 0.03$ vs. pre-BDNF baseline), respectively. Quantitative data for maximum fura-2 ratio elevations are summarized in Table 1.

The intracellular Ca^{2+} elevations induced by BDNF required a signaling pathway consistent with the activation of the Trk-IP₃R cascade, which was also necessary for the activation of the membrane conductance I_{BDNF} (Amaral and Pozzo-Miller 2007). First, the tyrosine kinase inhibitor k-252a (200 nM) (Knusel and Hefti 1992) completely prevented BDNF-induced Ca^{2+} signals (peak 0.86 ± 0.03 , $n = 6$, $P > 0.05$ vs. pre-BDNF baseline; Fig. 2A) as well as I_{BDNF} recorded in the same cells (5.57 ± 7.67 pA, $n = 6$, $P < 0.05$). Second, the IP₃R inhibitor xestospongine-C (1 μM intracellular) (Gafni et al. 1997) also completely blocked BDNF-induced Ca^{2+} elevations (peak: 0.89 ± 0.01 , $n = 3$, $P > 0.05$ vs. baseline; Fig. 2B) and I_{BDNF} in the same cells (14.17 ± 16.45 pA, $n = 3$, $P < 0.05$). Consistent with a requirement of IP₃R-dependent Ca^{2+} mobilization, pretreatment (30 min) with 1 μM thapsigargin (in 0.01% DMSO), which depletes intracellular Ca^{2+} stores by inhibiting SERCA pumps (Thastrup et al. 1990), also prevented BDNF-induced Ca^{2+} signals (peak: 0.9 ± 0.03 , $n = 3$, $P > 0.05$ vs. baseline; Fig. 3A) as well as I_{BDNF} in the same cells (0.24 ± 3.23 pA, $n = 3$, $P < 0.05$). Intriguingly, removal of extracellular Ca^{2+} also prevented BDNF-induced fura-2 ratio elevations (peak: 0.79 ± 0.03 , $n = 6$, $P > 0.05$ vs. baseline; Fig. 3B) and I_{BDNF} (9.97 ± 9.14 pA, $n = 6$, $P < 0.05$). Taken together, these observations demonstrate that Trk receptors, IP₃Rs, full intracellular Ca^{2+} stores and Ca^{2+} influx are all required for BDNF-induced Ca^{2+} elevations and membrane currents.

Collectively, the features of BDNF-induced Ca^{2+} signals in voltage-clamped CA1 pyramidal neurons resemble capacitative Ca^{2+} entry, a process thought to be mediated by Ca^{2+} -permeable TRPC channels (Clapham 2003; Mikoshiba 1997; Parekh and Putney 2005; Putney 2003). The imidazole SKF-96365, an inhibitor of store-operated Ca^{2+} entry in several cell types—e.g., human neutrophils, platelets and endothelial cells, HL-60 cells, rat thymic lymphocytes and thyroid FRTL-5 cells (Merritt et al. 1990) as well as in cells heterologously expressing TRPC3 channels (Zhu et al. 1998)—completely prevented I_{BDNF} in CA1 pyramidal neurons (Amaral and Pozzo-Miller 2007). Consistent with these observations, peak amplitudes of BDNF-induced Ca^{2+} signals after application of SKF-96365 (30 μM in 0.01% DMSO) were indistinguishable from baseline levels (0.88 ± 0.03 , $n = 4$, $P > 0.05$ vs. pre-BDNF baseline, Fig. 3C); SKF-96365 also prevented I_{BDNF} recorded in these same cells (0.53 ± 2.55 pA, $n = 4$, $P < 0.05$). Taken together, these results indicate that BDNF induces intracellular Ca^{2+} elevations through the activation of the IP₃ signaling cascade leading to TRPC channel activation.

DISCUSSION

Here we present novel insights into the immediate actions of BDNF on hippocampal neurons. First, BDNF caused intracellular Ca^{2+} elevations in CA1 pyramidal neurons under voltage-clamp and in the absence of voltage-gated Na^{+} and Ca^{2+} channels as well as NMDA receptor activation. Second, these Ca^{2+} signals were always associated with I_{BDNF} , a sustained nonselective cationic current mediated by TRPC3 channels (Amaral and Pozzo-Miller 2007; Li et al. 1999). Third, BDNF-induced Ca^{2+} elevations required IP₃ receptors, full intracellular Ca^{2+} stores and extracellular Ca^{2+} . Consistent with this last observation and the role of TRPC channels in BDNF-induced membrane currents, Ca^{2+} signals evoked by BDNF were sensitive to a TRPC/SOC inhibitor. We propose that BDNF binding to the TrkB receptor activates the

PLC γ pathway. PLC γ then hydrolyzes PIP $_2$ to IP $_3$; IP $_3$ binds to its receptor (IP $_3$ R) on the smooth endoplasmic reticulum (SER) and causes Ca $^{2+}$ to be released. TRPC3 channels are then activated and mediate Ca $^{2+}$ entry into the neuron.

It has been known for a while that BDNF elicits somatic Ca $^{2+}$ elevations in cultured hippocampal neurons (Berninger et al. 1993), but the mechanism(s) underlying these responses has remained elusive (Amaral and Pozzo-Miller 2005; Amaral et al. 2007; McCutchen et al. 2002). BDNF-induced somatic Ca $^{2+}$ elevations in cultured neurons were reduced—but not completely blocked—in the absence of extracellular Ca $^{2+}$ (Finkbeiner et al. 1997; Li et al. 1998), suggesting that both Ca $^{2+}$ influx and mobilization from intracellular stores contribute to the responses. Some features of these Ca $^{2+}$ signals resemble capacitative Ca $^{2+}$ entry (Putney 2003), a mechanism postulated to be mediated by some members of the TRPC channel subfamily (Birnbaumer et al. 1996; Mikoshiba 1997; Montell et al. 2002; but see Clapham 2003). Indeed TRPC3/6 channels mediate BDNF-evoked Ca $^{2+}$ signals in growth cones (Li et al. 2005) and somata (Jia et al. 2007) of cultured cerebellar granule cells, whereas xTRPC1, a *Xenopus* homo-logue of TRPC1, plays a similar role in BDNF-induced growth cone turning in vitro (Wang and Poo 2005). Consistently, the TRPC/SOC inhibitor SKF-96365 completely prevented BDNF-induced Ca $^{2+}$ responses and I_{BDNF} . It was originally reported that SKF-96365 also inhibited voltage-gated Ca $^{2+}$ channels in GH3 pituitary cells and rabbit ear-artery smooth muscle cells (Merritt et al. 1990); however, a broad-spectrum Ca $^{2+}$ channel blocker (i.e., 200 μM Cd $^{2+}$) did not affect I_{BDNF} or BDNF-induced Ca $^{2+}$ signals in CA1 pyramidal neurons in our experiments. Furthermore, siRNA-mediated TRPC3 knockdown, or intracellular application of anti-TRPC3 anti-bodies—but not anti-TRPC5—prevented the activation of I_{BDNF} in CA1 neurons (Amaral and Pozzo-Miller 2007). Thus our results suggest that ion channels containing at least TRPC3 subunits mediate I_{BDNF} and its associated Ca $^{2+}$ elevations.

It is worth noting that dendritic and spine Ca $^{2+}$ elevations induced by BDNF in hippocampal dentate granule cells were sensitive to voltage-gated Ca $^{2+}$ channel blockers (Kovalchuk et al. 2002) and always associated with fast and brief membrane depolarizations proposed to be mediated by Na $_v$ 1.9 channels (Blum et al. 2002; Kafitz et al. 1999). In addition, I_{BDNF} in pontine (Li et al. 1999) and CA1 pyramidal neurons (Amaral and Pozzo-Miller 2007) is markedly different from those faster and transient TTX-insensitive Na $^+$ current activated by TrkB ligands in several regions of the brain (Kafitz et al. 1999). Furthermore, fast BDNF-activated Na $^+$ currents were blocked by the Na $^+$ channel blocker saxitoxin (Blum et al. 2002), whereas I_{BDNF} in CA1 pyramidal neurons is not (Amaral and Pozzo-Miller 2007). It has been recently reported that brief and focal BDNF applications elicited fast and local Ca $^{2+}$ elevations near synaptic sites on apical dendrites of immature CA3 pyramidal neurons (slice cultures prepared from P0 to P2 rats and cultured for 3 div), which required the activation of voltage-gated Ca $^{2+}$ and Na $^+$ channels (Lang et al. 2007). In contrast with the observations presented here regarding more developed CA1 neurons (slice cultures prepared from P7 to P11 rats and cultured for 7–11 div), fast and local Ca $^{2+}$ elevations evoked by BDNF in immature CA3 neurons were unaffected by store depletion with CPA or inhibition of SOC/TRPC channels with SKF-96365 (Lang et al. 2007). It remains to be tested whether BDNF-induced Ca $^{2+}$ elevations in immature CA3 neurons are a secondary response to the membrane depolarization caused by activation of Na $_v$ 1.9 channels (Blum et al. 2002; Kafitz et al. 1999) as shown in dentate granule neurons (Kovalchuk et al. 2002).

Is there a role for these BDNF-induced membrane currents and Ca $^{2+}$ elevations? Because BDNF increases spine density in CA1 neurons (Alonso et al. 2004; Tyler and Pozzo-Miller 2001, 2003), it is tempting to speculate that sustained intracellular Ca $^{2+}$ elevations such as those induced by brief BDNF applications and mediated by TRPC channels trigger the cytoskeletal rearrangements necessary for dendritic spine remodeling and/or formation.

Indeed, siRNA-mediated TRPC3 knockdown as well as TRPC inhibitors prevented the increase in spine density by BDNF (Amaral and Pozzo-Miller 2007). Together with the observations that Ca^{2+} mobilization from intracellular stores (Korkotian and Segal 1999) and activation of group-I mGluRs (Vanderklish and Edelman 2002) also induce changes in spine form and promote spine formation, our results suggest a convergence of mGluR and TrkB signaling pathways on TRPC channels to engage a program of spine formation and/or maturation via Ca^{2+} -dependent, actin-based structural remodeling. In this view, TRPC channels emerge as novel effectors of BDNF-mediated dendritic remodeling through the activation of a sustained depolarization associated with intracellular Ca^{2+} elevations.

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FIG. 1. Brain-derived neurotrophic factor (BDNF) induces intracellular Ca²⁺ elevations that *precede* and are *simultaneous* with I_{BDNF} activation. *A*: representative example of BDNF-evoked Ca²⁺ signals in the presence of TTX, Cd²⁺ and D,L-2-amino-5-phosphonovaleic acid (D,L-APV). These Ca²⁺ elevations included a transient and localized dendritic signal during BDNF application (—) and a secondary widespread elevation associated with I_{BDNF} . The initial Ca²⁺ transient (*) is shown in *B* at an expanded time scale. *B*: expanded view of the initial Ca²⁺ elevation that preceded I_{BDNF} . Here and in the other figures, the fluorescence images (360 nm excitation) show the color-coded regions of interest (ROIs) plotted with the simultaneously recorded membrane currents.

FIG. 2.

BDNF-induced Ca^{2+} signals require functional Trk and IP_3 receptors. *A*: BDNF-evoked Ca^{2+} signals and I_{BDNF} are sensitive to the Trk inhibitor k-252a (200 nM). *B*: The IP_3R inhibitor xestospongine-C (1 μM intracellular) blocked BDNF-evoked Ca^{2+} signals and I_{BDNF} .

FIG. 3.

BDNF-induced Ca²⁺ signals require full intracellular Ca²⁺ stores, Ca²⁺ influx from the extracellular space, and activation of TRPC channels. **A:** depletion of intracellular Ca²⁺ stores with thapsigargin (1 μM) abolished BDNF Ca²⁺ responses and I_{BDNF} . **B:** applying BDNF in the absence of extracellular Ca²⁺ failed to evoke Ca²⁺ elevations and significantly reduced I_{BDNF} . **C:** The TRPC inhibitor SKF-96365 (30 μM) prevented Ca²⁺ signals and I_{BDNF} .

TABLE 1

BDNF-Induced Dendritic Ca^{2+} Signals in CA1 Pyramidal Neurons

ACSF Composition	Baseline Pre-BDNF 360/380 Ratio	Initial Signal (Before I_{BDNF}) (100 s From BDNF Puff) 360/380 Ratio	Sustained Signal (at I_{BDNF} Peak) (440 s From BDNF Puff) 360/380 Ratio
TTX ($n = 7$)	0.89 ± 0.03 (0.08)	1.26 ± 0.14 (0.19)	1.71 ± 0.09 (0.14)
TTX, APV, Cd^{2+} ($n = 4$)	1.15 ± 0.11 (0.18)	1.31 ± 0.26 (0.28)	2.12 ± 0.35 (0.33)
Pooled data ($n = 11$)	0.98 ± 0.05 (0.18)	1.28 ± 0.11 (0.2)	1.86 ± 0.14 (0.25)

Maximum values (means $\pm$ SE) of brain-derived neurotrophic factor (BDNF)-induced Ca^{2+} elevations in voltage-clamped CA1 pyramidal neurons in the absence of action potential firing (TTX). Blocking *N*-methyl-D-aspartate receptors (NMDARs; APV) and voltage-gated Ca^{2+} channels (Cd^{2+}) yielded similar responses. Initial Ca^{2+} signals always occurred in the targeted dendritic locations, whereas the sustained signals were widespread throughout the somato-dendritic compartment. Background-subtracted fluorescence intensity measurements were obtained within regions of interest (ROIs) defined over apical primary and secondary dendrites as well as somata. The average ratio of 360 and 380 nm fluorescence within each ROI was used as an estimate of intracellular Ca^{2+} concentration as these two parameters are directly proportional to each other (see METHODS for more details). The coefficient of variance (in parentheses, $\text{CV} = \text{SD}/\text{mean}$) is provided as a measure of consistency.