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

Michelle D. Amaral and Lucas Pozzo-Miller
Department of Neurobiology, Civitan International Research Center and McKnight Brain Institute, University of Alabama at Birmingham, Birmingham, Alabama

Submitted 16 July 2007; accepted in final form 9 August 2007

Amaral MD, Pozzo-Miller L. BDNF induces calcium elevations associated with $I_{BDNF}$, a nonselective cationic current mediated by TRPC channels. J Neurophysiol 98: 2476–2482, 2007. First published August 15, 2007; doi:10.1152/jn.00797.2007. 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 bisphosphate (PIP$_2$) by phospholipase C gamma (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 (Berminger 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 Nakabeura 2003; Mizoguchi et al. 2002; Yang and Gu 2005; Zirgriege et al. 1995). In contrast, BDNF failed to affect $Ca^{2+}$ levels in cultured cerebellar granule cells (Gaidon 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 (Boulangier 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 Na$_A$1.9 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 intracellular $Ca^{2+}$ 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.

Address for reprint requests and other correspondence: L. Pozzo-Miller, Dept. of Neurobiology, SHEL-1002, University of Alabama at Birmingham, 1825 University Blvd., Birmingham, AL 35294-2182 (E-mail: lucaspm@uab.edu). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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% CO2, 98% relative humidity incubators (Thermo-Forma; Waltham, MA). Slices were maintained in culture media (Neurobasal-A plus B27, InVitrogen; Carlsbad, CA) containing (in mM) 124 NaCl, 2 KCl, 1.24 KH2PO4, 1.3 MgSO4, 17.6 NaHCO3, 2.5 CaCl2, 10 glucose, and 29.2 sucrose (310–320 mosM); ACSF was bubbled with 95% O2-5% CO2 (pH 7.4). 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 m/min) with artificial cerebrospinal fluid (ACSF) at room temperature (24°C), containing (in mM) 124 NaCl, 2 KCl, 1.24 KH2PO4, 1.3 MgSO4, 17.6 NaHCO3, 2.5 CaCl2, 10 glucose, and 29.2 sucrose (310–320 mosM); ACSF was bubbled with 95% O2-5% CO2 (pH 7.4). Superficial CA1 neurons were visualized with a water-immersion 40× objective (0.9 NA) using IR-DIC microscopy. Simultaneous whole cell recording and microfluorometric Ca2+ 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 10 Na2 creatine phosphate; 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 10 Na2 creatine phosphate. 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 (Ri) was measured with hyperpolarizing voltage pulses (50 ms, −20 mV), and cells were discarded if any of those cell parameters (Cm, Rm, Ri) 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.

BDNF-EVOKED Ca2+ ELEVATIONS

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 m/min) with artificial cerebrospinal fluid (ACSF) at room temperature (24°C), containing (in mM) 124 NaCl, 2 KCl, 1.24 KH2PO4, 1.3 MgSO4, 17.6 NaHCO3, 2.5 CaCl2, 10 glucose, and 29.2 sucrose (310–320 mosM); ACSF was bubbled with 95% O2-5% CO2 (pH 7.4). Superficial CA1 neurons were visualized with a water-immersion 40× objective (0.9 NA) using IR-DIC microscopy. Simultaneous whole cell recording and microfluorometric Ca2+ 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). Nominal 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 (Ri) was measured with hyperpolarizing voltage pulses (50 ms, −20 mV), and cells were discarded if any of those cell parameters (Cm, Rm, Ri) 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 (Gryniewicz 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 (5–7 μM) 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 using 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 (Frdfelder et al. 1996). These power analyses yielded values of statistical Power (1-β; where β is the Type-II error) larger than 0.95 (i.e., 95% confidence of accepting the null hypothesis when it is true).

The application of BDNF to CA1 pyramidial neurons evoked transient fura-2 ratio elevations that preceded IBDNF 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 IBDNF 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 ~100 μm above hippocampal slice cultures to avoid pressure and mechanical artifacts, and over s. radiatum dendrites ~200 μm 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 IBDNF, 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'-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 \(\mu\)M fura-2 or 500 \(\mu\)M 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 \((0.5 \mu\)M\), 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 \(\sim 100 \mu\)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\(^{2+}\) \((200 \mu\)M\) and D,L-APV \((50 \mu\)M\) 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\(^{2+}\) \((\text{Amaral and Pozzo-Miller 2007})\) 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 \((\text{Fig. 1A})\). 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 \((n = 7 \text{ of 7 cells})\; CV = 0.14\). 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\(^{2+}\) \((200 \mu\)M\) and D,L-APV \((100 \mu\)M\); peak amplitude 2.12 ± 0.35, \(n = 4 \text{ of 4 cells}\), \(P = 0.03 \text{ vs. pre-BDNF baseline}\), 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 \((200 \text{nM})\) (Knusel and Hefti 1992) completely prevented BDNF-induced Ca\(^{2+}\) signals \((\text{peak} \; 0.86 \pm 0.03, \; n = 6, \; P > 0.05 \text{ vs.})\).

### TABLE 1. BDNF-Induced Dendritic Ca\(^{2+}\) Signals in CA1 Pyramidal Neurons

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

Maximum values \((\text{means} \pm \text{SE})\) of brain-derived neurotrophic factor \((\text{BDNF})\)-induced Ca\(^{2+}\) elevations in voltage-clamped CA1 pyramidal neurons in the absence of action potential firing \((\text{TTX})\). Blocking N-methyl-d-aspartate receptors \((\text{NMDARs}; \text{APV})\) and voltage-gated Ca\(^{2+}\) channels \((\text{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 \((\text{ROIs})\) 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 \((\text{see METHODS for more details})\). The coefficient of variance \((\text{in parentheses}, CV = \text{SD/mean})\) is provided as a measure of consistency.
pre-BDNF baseline; Fig. 2A) as well as \( I_{\text{BDNF}} \) recorded in the same cells (5.57 \pm 7.67 pA, \( n = 6 \), \( P < 0.05 \)). Second, the IP\(_3\)R inhibitor xestospongin-C (1 \( \mu \)M intracellular) (Gafni et al. 1997) also completely blocked BDNF-induced \( \text{Ca}^{2+} \) elevations (peak: 0.89 \pm 0.01, \( n = 3 \), \( P > 0.05 \) vs. baseline; Fig. 2B) and \( I_{\text{BDNF}} \) in the same cells (14.17 \pm 16.45 pA, \( n = 6 \), \( P < 0.05 \)). Consistent with a requirement of IP\(_3\)R-dependent \( \text{Ca}^{2+} \) mobilization, pretreatment (30 min) with 1 \( \mu \)M thapsigargin (in 0.01% DMSO), which depletes intracellular \( \text{Ca}^{2+} \) stores by inhibiting SERCA pumps (Thastrup et al. 1990), also prevented BDNF-induced \( \text{Ca}^{2+} \) signals (peak: 0.9 \pm 0.03, \( n = 6 \), \( P < 0.05 \) vs. baseline; Fig. 3A) as well as \( I_{\text{BDNF}} \) in the same cells (0.24 \pm 3.23 pA, \( n = 6 \), \( P < 0.05 \)). Intriguingly, removal of extracellular \( \text{Ca}^{2+} \) also prevented BDNF-induced fura-2 ratio elevations (peak: 0.79 \pm 0.03, \( n = 6 \), \( P > 0.05 \) vs. baseline; Fig. 3B) and \( I_{\text{BDNF}} \) (9.97 \pm 9.14 pA, \( n = 6 \), \( P < 0.05 \)). Taken together, these observations demonstrate that Trk receptors, IP\(_3\)Rs, full intracellular \( \text{Ca}^{2+} \) stores and \( \text{Ca}^{2+} \) influx are all required for BDNF-induced \( \text{Ca}^{2+} \) elevations and membrane currents.

Collectively, the features of BDNF-induced \( \text{Ca}^{2+} \) signals in voltage-clamped CA1 pyramidal neurons resemble capacitative \( \text{Ca}^{2+} \) entry, a process thought to be mediated by \( \text{Ca}^{2+} \)-permeable TRPC channels (Clapham 2003; Mikoshiba 1997; Parekh and Putney 2005; Putney 2003). The imidazole SKF-96365, an inhibitor of store-operated \( \text{Ca}^{2+} \) 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 \( I_{\text{BDNF}} \) in CA1 pyramidal neurons (Amaral and Pozzo-Miller 2007). Consistent with these observations, peak amplitudes of BDNF-induced \( \text{Ca}^{2+} \) signals after application of SKF-96365 (30 \( \mu \)M in 0.01% DMSO) were indistinguishable from baseline levels (0.88 \pm 0.03, \( n = 4 \), \( P > 0.05 \) vs. pre-BDNF baseline, Fig. 3C); SKF-96365 also prevented \( I_{\text{BDNF}} \) recorded in these same cells (0.53 \pm 2.55 pA, \( n = 4 \), \( P < 0.05 \)). Taken together, these results indicate that BDNF induces intracellular \( \text{Ca}^{2+} \) elevations through the activation of the IP\(_3\) signaling cascade leading to TRPC channel activation.
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 IP\(_3\) 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\(_{\gamma}\) pathway. PLC\(_{\gamma}\) 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 mechanisms 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 homologue of TRPC1, plays a similar role in BDNF-induced growth signaling 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 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).

ACKNOWLEDGMENTS

We are indebted to Dr. Takaumi Inoue (Waseda University) for continuous support of the acquisition and analysis software (TIWorkBench). We thank AMGEN for the supply of BDNF. We also thank the technical assistance of the UAB Neuroscience Cores (P30-NS-47466, P30-HD-38985, P30-NS-57098).

REFERENCES


