BDNF Evokes Release of Endogenous Cannabinoids at Layer 2/3 Inhibitory Synapses in the Neocortex

Fouad Lemtiri-Chlieh and Eric S. Levine

Department of Neuroscience, University of Connecticut Health Center, Farmington, Connecticut

Received 26 May 2010; accepted in final form 12 August 2010

Lemtiri-Chlieh F, Levine ES. BDNF evokes release of endogenous cannabinoids at layer 2/3 inhibitory synapses in the neocortex. J Neurophysiol 104: 1923–1932, 2010. First published August 18, 2010; doi:10.1152/jn.00472.2010. The neurotrophin brain-derived neurotrophic factor (BDNF) is a potent regulator of inhibitory synaptic transmission, although the locus of this effect and the underlying mechanisms are controversial. We explored a potential interaction between BDNF and endogenous cannabinoid (endocannabinoid) signaling because activation of type 1 cannabinoid (CB1) receptors potently regulates γ-aminobutyric acid (GABA) release and both trkB tyrosine kinase receptors and CB1 receptors are highly expressed at synapses in neocortical layer 2/3. Here, we found that the effects of BDNF at inhibitory cortical synapses are mediated by the release of endocannabinoids acting retrogradely at presynaptic CB1 receptors. Specifically, acute application of BDNF rapidly reduced the amplitude of inhibitory postsynaptic currents (IPSCs) via postsynaptic trkB receptor activation because intracellular delivery of the tyrosine kinase inhibitor K252a completely blocked the BDNF effect. Although triggered by postsynaptic trkB activation, BDNF exposure decreased presynaptic release probability, as evidenced by increases in the paired-pulse ratio and coefficient of variation of evoked responses. In addition, BDNF decreased the frequency but not the amplitude of action potential–independent miniature IPSCs and BDNF did not alter the postsynaptic response to locally applied GABA. These results suggest that BDNF induces the release of a retrograde messenger from the postsynaptic cell that regulates presynaptic neurotransmitter release. Consistent with a role for endocannabinoids as the retrograde signal, the effect of BDNF on IPSCs was blocked by CB1 receptor antagonists and was occluded by a cannabinoid receptor agonist. Furthermore, inhibiting endocannabinoid synthesis or transport also disrupted the BDNF effect, implicating postsynaptic endocannabinoid release triggered by BDNF.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin gene family that has well-characterized effects on neuronal survival and phenotypic differentiation during development (Lu et al. 2005; McAllister et al. 1999; Thoenen et al. 1987). Neurotrophins have attracted much interest for their therapeutic potential in a range of neurologic disorders (Schulte-Herbruggen et al. 2007). BDNF has also emerged as a potent synaptic modulator involved in many forms of activity-dependent synaptic plasticity (Lu 2003) and may play an important role in learning and memory (Tyler et al. 2002). BDNF and its receptors, the tropomyosin receptor kinase B (trkB) receptor and the pan-neurotrophin (p75) receptor, are expressed throughout the nervous system, with the highest levels in the neocortex and hippocampus (Masana et al. 1993). Within the neocortex, trkB is predominantly localized to layers 2/3 and 5 (Cabelli et al. 1996; Fryer et al. 1996; Miller and Pitts 2000). At the subcellular level, trkB and p75 are expressed in postsynaptic dendrites and axon terminals (Aoki et al. 2000; Gomes et al. 2006), in glutamatergic neurons (Cabelli et al. 1996), and in GABAergic neurons (Drake et al. 1999).

BDNF is a potent modulator of GABAergic transmission, but the nature of this effect and its site of action are controversial. In the cortex, hippocampus, and cerebellum, trkB receptors are expressed not only in postsynaptic dendrites but also in GABAergic axon terminals (Drake et al. 1999; Fryer et al. 1996; Rico et al. 2002). In the CA1 region of the hippocampus, BDNF reduces inhibitory transmission via both presynaptic (Frerking et al. 1998) and postsynaptic mechanisms (Brunig et al. 2001; Hewitt and Bains 2006; Mizoguchi et al. 2003; Tanaka et al. 1997). In cerebellar granule cells, BDNF decreases the amplitude and frequency of spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs, respectively), suggesting both pre- and postsynaptic effects (Cheng and Yeh 2003). The effect of BDNF on IPSCs, however, was blocked by inclusion of the trkB inhibitor K252a to the postsynaptic cell (Cheng and Yeh 2003; Hewitt and Bains 2006; Tanaka et al. 1997). Taken together, these results suggest that postsynaptic trkB receptors contribute to the initiation of many of the effects of BDNF at GABAergic synapses.

Interestingly, the highest levels of CB1 cannabinoid receptors in the neocortex are also found in layer 2/3 (Egertova et al. 2003; Marsicano and Lutz 1999; Matsuda et al. 1993; Tsou et al. 1998), similar to trkB, and there is evidence of endogenous cannabinoid (endocannabinoid)–BDNF interactions in layer 2/3 of visual cortex (Huang et al. 2008), hippocampus (Khaspekov et al. 2004; Roloff et al. 2010), and cerebellum (Maison et al. 2009). In neocortical layers 2/3 and 5, endocannabinoid signaling modulates γ-aminobutyric acid (GABA) release by activating type 1 cannabinoid (CB1) receptors expressed in presynaptic terminals (Bodor et al. 2005; Fortin et al. 2004; Lemtiri-Chlieh and Levine 2007; Trettel and Levine 2002; Trettel et al. 2004), suggesting possible interactions with BDNF-trkB signaling. These two modulatory systems have been extensively studied in isolation to characterize their molecular and cellular effects, although little is known of their potential interdependence.

METHODS

Slice preparation

All animal procedures were conducted according to the protocols approved by the University of Connecticut Health Center Animal...
Care Committee. Briefly, Swiss CD-1 mice (P13 to P21) were decapitated under isoflurane anesthesia and the brains were harvested quickly and placed in ice-cold “cutting and incubating” (CI) solution composed of (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 0.5 CaCl2, 4 MgCl2, 4 MgSO4, 4 lactic acid, 2 pyruvic acid, 20 glucose, and 0.4 acetic acid, carbogemixed with 95% O2-5% CO2 (pH 7.3, 310 ± 5 mmol·kg⁻¹). Transverse slices (350 μm) containing somatosensory and auditory cortices were cut using a vibromate (Microslicer; Dosaka EM, Kyoto, Japan). The slices were placed in a large incubating chamber containing CI solution at a temperature of 34–35°C for 30 min before being transferred to room temperature for ≥30 min prior to recording. Slices were then individually transferred to the recording chamber (room temperature) fixed to the stage of an Olympus BX51WI upright microscope fitted with a ×40 water-immersion objective lens (0.8 NA). During record-

ings, slices were continuously perfused at 2 ml/min with artificial cerebrospinal fluid (aCSF) consisting of (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 2 CaCl2, 2 MgCl2, and 15 glucose (pH 7.3, 310 ± 5 mmol·kg⁻¹); pH was equilibrated by continuous bubbling with 95% O2-5% CO2.

Electrophysiology

Whole cell voltage-clamp recordings (Vhold = −70 mV) were obtained from layer 2/3 pyramidal neurons (PNs) in the neocortex and were visually identified by their morphology and position using an infrared–differential interference contrast video microscope. Patch electrodes (3–5 MΩ) were pulled from borosilicate glass capillaries using a Flaming/Brown P-97 micropipette puller (Sutter Instrument, Novato, CA). The pipette solution contained (in mM): 120 CsCl, 10 HEPES, 1 EGTA, 0.1 CaCl2, 1.5 MgCl2, 4 Na-ATP, 0.3 Na-GTP, 10 phosphocreatine, and 0.5 QX-314 (pH 7.2, 290 mOsmol/kg). Series resistance was compensated 70% at 10–100 μs lag. On breaking into whole cell configuration, a brief series of voltage ramps (50 ms, 2 mV/ms) was applied to promote the activity-dependent block of the sodium conductance by QX-314.

All electrical events were filtered at 2.9 kHz and digitized at 70 ms sampling rate. Paired-pulse responses were obtained by stimulating with two square pulses (3 psi) delivered every 30 s. Stimulus consisted of a single square-wave current pulse (duration: 50–150 μs; amplitude: 50–200 μA at a frequency of 0.067 Hz), designed to evoke half-maximal responses. In some experiments, paired stimuli were delivered with an interstimulus interval of 75 ms to obtain the paired-pulse ratio (PPR). The PPR of evoked postsynaptic currents was calculated by dividing the mean peak amplitude of the second evoked response (A2) by the mean peak amplitude of the first response (A1; PPR = mean of A2/mean of A1). Responses to locally applied GABA puffs were measured using a pipette positioned 40–50 μm from the cell soma. The pipette contained 50 μM GABA, which was applied with 10-ms pulses (3 psi) delivered every 30 s.

Neurons were discarded from analyses if 1) Rf was >25 MΩ at the time of break-in or >10.5 MΩ after compensation, 2) if Rf changed by >15% during the course of an experiment, or 3) if Rf fell to <100 MΩ. Off-line analysis was carried out using Clampfit (Axon Instruments). The effects of the test solutions were determined by comparing the currents evoked during a 2-min baseline period (BL, eight sweeps) to the last 2 min of the 10-min drug exposure (eight sweeps). Time of onset for drug effects was quantified by calculating the mean and SD of the baseline period and locating the first response that was >2SDs above or below baseline. Tests of statistical significance were based on Student’s paired t-test or ANOVA. Group data are reported as means ± SE.

Chemicals

Unless otherwise stated, all drugs were from Sigma-Aldrich (St. Louis, MO) and were delivered by bath perfusion or by intracellular loading through the patch pipette, as indicated. The stock solution of BDNF (10 μg/ml; PeproTech, Rocky Hill, NJ) was made in aCSF and stored at −80°C. Stock solutions of K252a (Calbiochem, La Jolla, CA), WIN55,212-2, SR141716A, AM251, capsazepine, RHC-80267, and AM404 (Tocris Bioscience, Elllisville, MO) were dissolved in 100% DMSO at 5 × 10⁵ times their final concentration and stored at −20°C. This gave a final DMSO concentration of 0.02%, which by itself had no effect on synaptic transmission (Trettel and Levine 2002).

RESULTS

BDNF depresses evoked IPSCs via activation of postsynaptic trkB receptors

The first set of experiments examined the effect of BDNF on evoked IPSCs (eIPSCs) recorded from layer 2/3 PNs. As shown in the example in Fig. 1A, BDNF (20 ng/ml; 0.8 nM) rapidly reduced peak eIPSC amplitude and this effect lasted for the duration of BDNF exposure. On average, as seen in the group data in Fig. 1B, the time of onset of the BDNF effect was 2.7 ± 0.3 min and eIPSC amplitude was reduced to 61.2 ± 5.2% of baseline (BL) by the end of a 10-min exposure period (P < 0.01, n = 9, range: 74–35% of BL). The effect of BDNF did not reverse immediately on termination of BDNF exposure, but eIPSC amplitude generally returned to baseline levels 15–20 min following wash-out of BDNF (see examples in Supplemental Figs. S1 and S2). In contrast, application of the aCSF vehicle solution had no significant effect on eIPSC amplitude, although a small rundown of the evoked response was seen over time (Supplemental Fig. S1).

We determined whether the effect of BDNF on eIPSCs was mediated by trkB receptors by using K252a (200 nM), a relatively specific inhibitor of autophosphorylation of trk tyrosine kinase receptors (Berg et al. 1992; Hashimoto 1988; Kusuel and Hefti 1992; Nye et al. 1992). Layer 2/3 PNs were pretreated for 10 to 15 min with bath-applied K252a prior to recording. As shown in the example in Fig. 1C and group data in Fig. 1D, application of BDNF had no significant effect on eIPSC amplitude in the presence of K252a (95 ± 2.8% of BL; n = 4), indicating a requirement for trk signaling. K252a applied alone did not have any significant effect on eIPSC amplitude (93 ± 9.7%; n = 4). As an additional control, we found that bath application of the K252a analog K252b, which has a lower membrane permeability, did not block the effect of BDNF (Supplemental Fig. S2). Because trkB receptors are expressed at both pre- and postsynaptic sites in layer 2/3 (Aoki et al. 2000; Gomes et al. 2006), the effect of BDNF could be mediated by trkB receptors at either side of the synapse. We addressed the question of which subset of trkB receptors are involved in the BDNF-induced suppression of eIPSCs by

1 The online version of this article contains supplemental data.
including K252a (200 nM) in the internal patch pipette solution. We allowed 5 to 10 min for the internal solution to equilibrate before evoking IPSCs. As shown in Fig. 2A (individual example) and Fig. 2B (group data), postsynaptic delivery of K252a prevented the effect of BDNF. The average decrease in the presence of BDNF reached only 90 ± 3.3% of BL (n = 4, range: 92 to 85%) compared with 57.9 ± 3% when including the vehicle alone (0.02% DMSO; Fig. 2C, open symbols, n = 3). These results suggest that activation of postsynaptic trkB receptors is required for the effect of BDNF.

Another factor often implicated in BDNF–trkB signaling pathways is a rise in intracellular free Ca^{2+} (Amaral and Pozzo-Miller 2007a; Tanaka et al. 1997). To address the role of postsynaptic calcium in the effect of BDNF on eIPSCs, layer 2/3 PNs were loaded intracellularly with the Ca^{2+} chelator BAPTA (20 mM). Under these conditions, BDNF had no effect on eIPSCs (see example in Fig. 2C). The group data (Fig. 2D) show a nonsignificant decrease to 88.5 ± 10.2% of BL (n = 6; P > 0.3), suggesting a key role for postsynaptic calcium in mediating the effect of BDNF. The small residual decrease that may be present after blocking the BDNF effect with K252a or BAPTA probably reflects simple rundown, given that a similar effect was seen in vehicle control experiments (Supplemental Fig. S1).

**BDNF suppresses spontaneous IPSCs via postsynaptic trkB receptors**

We next examined the effect of BDNF on spontaneous inhibitory activity. Under basal conditions, bath application of BDNF (20 ng/ml) had no significant effect on either the mean frequency of spontaneous IPSCs (n = 6; 115 ± 6.9% of BL) or their amplitude, with 200 nM K252a intracellularly loaded through the patch pipette. B: group data showing the effect of BDNF (20 ng/ml) on eIPSC amplitude in the presence of K252a (200 nM; bath-applied). C: individual example of the lack of effect of BDNF (20 ng/ml) on eIPSCs in the presence of bath-applied K252a (n = 4). Note that K252a was applied ≥10 min prior to BDNF application. BL, baseline.

**FIG. 1.** Brain-derived neurotrophic factor (BDNF) depresses evoked inhibitory postsynaptic current (eIPSC) amplitude in cortical layer 2/3 pyramidal neurons (PNs) via activation of trkB receptors. A: example time course of the effect of BDNF (20 ng/ml) on eIPSC peak amplitude. Inset: example sweeps of 2 superimposed eIPSCs recorded before (a) and after (b) bath application of BDNF (scale bars: 0.2 nA, 0.05 s). B: group data (n = 9) showing the effect of BDNF on normalized peak amplitude of eIPSCs as a function of time. C: individual example of the time course of the effect of BDNF (20 ng/ml) on eIPSC peak amplitude in the presence of the trk tyrosine kinase inhibitor K252a (200 nM; bath-applied). D: group data showing the effect of BDNF on normalized peak amplitude of eIPSCs in the presence of bath-applied K252a (n = 4). Note that K252a was applied ≥10 min prior to BDNF application. BL, baseline.

**FIG. 2.** BDNF-induced suppression of eIPSCs is dependent on both postsynaptic trkB receptors and postsynaptic calcium elevation. A: individual example of the lack of effect of BDNF (20 ng/ml) on eIPSC amplitude, with 200 nM K252a intracellularly loaded through the patch pipette. B: group data showing the time course of the experiments using K252a in the patch pipette (n = 4; filled symbols). Note that K252a was dialyzed into the cell ≥10 min prior to BDNF application. Also shown are the group data using the vehicle alone (0.02% DMSO; n = 3; open symbols). C: individual example of the lack of effect of BDNF (20 ng/ml) on eIPSC amplitude, with the calcium chelator BAPTA (20 mM) in the patch pipette. D: group data showing the time course for the BAPTA experiments (n = 6).
amplitude (87.5 ± 6.4% of BL; see example in Fig. 3A). To enhance spontaneous inhibitory activity, slices were treated with the muscarinic agonist carbachol (CCh; 5 μM) (Kawaguchi 1997; Lemtiri-Chlieh and Levine 2007; Trettel et al. 2004). In the presence of CCh, mean IPSC amplitude increased more than twofold: from 52 ± 6.4 to 114 ± 21.4 pA (n = 8, P < 0.01) and the frequency increased from 2.4 ± 0.5 to 6.4 ± 0.6 Hz (P < 0.01). In contrast to the lack of effect under basal conditions, adding BDNF in the presence of CCh caused a dramatic decrease in the amplitude of CCh-induced IPSCs, as shown in the example in Fig. 3B (also see time course of BDNF effect on CCh-induced IPSCs in Fig. 7A). On average, spontaneous IPSC amplitude decreased significantly to 66.5 ± 9.5 pA in the presence of BDNF (n = 8; P < 0.05), but there was no significant change in IPSC frequency with BDNF (5.9 ± 0.6 Hz). The time of onset for this effect was 2.8 ± 0.3 min (n = 6), not significantly different from the onset time for the BDNF effect on evoked responses (2.7 ± 0.3 min).

Similar to the results obtained with eIPSCs, K252a (200 nM) either in the bath (Fig. 3C) or in the patch pipette (Fig. 3D) prevented the BDNF effect on CCh-induced IPSCs. Figure 3E compiles the group data for the effect of BDNF on spontaneous IPSC amplitude and frequency in layer 2/3 PNs. First, BDNF had no significant effect on amplitude or frequency of spontaneous IPSCs recorded under basal conditions. In the presence of CCh, however, BDNF decreased spontaneous IPSC amplitude to 63.5 ± 8.2% of BL (n = 8; P < 0.05) and had a small but nonsignificant effect on IPSC frequency. The effect of BDNF on IPSC amplitude was completely prevented in the presence of K252a in the bath (106.8 ± 5.7% of CCh BL, n = 4) or when K252a was loaded intracellularly through the patch pipette (105.7 ± 10.0% of CCh BL, n = 8).

**BDNF reduces presynaptic release probability**

The results with intracellular K252a and with BAPTA suggest that postsynaptic trkB receptor signaling is required for initiating the effect of BDNF on IPSCs. The resultant BDNF-induced suppression of IPSC amplitude, however, could result from decreased postsynaptic GABA receptor responsiveness, decreased presynaptic transmitter release, or both. To determine whether BDNF had presynaptic effects, we used three different approaches: paired-pulse ratio (PPR), coefficient of variation (CV), and action potential (AP)–independent mIPSCs. First, we found that the PPR was increased in all cells tested with BDNF. An individual example is represented in Fig. 4A, showing two consecutive eIPSCs separated by a 75-ms interstimulus interval. The PPR for this cell increased from 1.57 during BL (black trace) to 2.15 during BDNF (gray trace). The group data shown in Fig. 4A demonstrate an average increase in PPR to 121.4 ± 7.3% of BL (n = 5, P < 0.05, range: 6–47%). Second, 1/CV2 decreased in all cells tested with BDNF. Figure 4B shows the individual experiments with an average decrease to 34 ± 5.2% of BL (n = 9, P < 0.05, range: 62–10%). Finally, as shown in Fig. 4, C and D, as well as the cumulative distribution in Supplemental Fig. S3, BDNF significantly decreased the frequency of mIPSCs (1.6 ± 0.3 vs. 0.7 ± 0.06, n = 5, P < 0.05). Taken together, these results suggest that the BDNF-induced suppression of IPSCs was at least in part due to presynaptic effects.

Two different approaches were used to determine whether BDNF also had direct postsynaptic effects. First, we examined the amplitudes of AP-independent mIPSCs. BDNF had no significant effect on average mIPSC amplitude (32 ± 3.3 vs. 29 ± 1.8 pA, n = 5; Fig. 4E) or on the cumulative amplitude distribution, as shown in Supplemental Fig. S3. We also explored this issue by monitoring the effects of BDNF on local puff application of GABA. As shown in Fig. 5, BDNF had no effect on postsynaptic responses to locally applied GABA. Taken together, these results suggest that BDNF decreased presynaptic release probability without affecting postsynaptic GABA receptor responsiveness.

**Effect of BDNF on IPSCs is mediated by endocannabinoids**

The above-cited evidence for postsynaptic induction and presynaptic expression suggests that the BDNF effect may be...
mediated by a retrograde messenger released from the postsynaptic PN. We explored the role of endocannabinoids as the retrograde signal because these lipid messengers can be released by layer 2/3 PNs and have been shown to suppress GABA release via activation of CB1 cannabinoid receptors expressed at presynaptic inhibitory terminals in layer 2/3 (Kan- tona et al. 1999; Kreitzer and Regehr 2001; Ohno-Shosaku et al. 2001; Wilson and Nicoll 2001). As shown in Fig. 6, A and B, pretreatment with either of the CB1 receptor antagonists, SR141716A (SR; 5 μM) or AM251 (5 μM), prevented the effect of BDNF on eIPSCs (SR: 97.9 ± 2.6% of baseline, n = 7; AM251: 89.2 ± 10.2% of baseline, n = 5). In addition, we examined the potential role of vanilloid VR1 receptors because SR has been shown to also antagonize these receptors (Gibson et al. 2008). We found, however, that the VR1 antagonist capsazepine did not block the effect of BDNF (Fig. 6C). In the presence of capsazepine, BDNF caused an average decrease of eIPSC amplitude to 68 ± 5.3% of BL (n = 6, P < 0.01), with an onset time of 2.5 ± 0.6 min, not significantly different from the effect of BDNF alone. Application of a CB1 receptor antagonist alone did not alter eIPSC amplitude (105 ± 7.8%; n = 7), indicating a lack of endocannabinoid tone under these conditions.

If the effect of BDNF is mediated by the release of endocannabinoids, this effect should be mimicked and occluded by prior treatment with a CB1 receptor agonist. Consistent with our previous results (Trettel and Levine 2002, 2003), we found that acute application of the cannabinoid receptor agonist WIN55,212-2 (WIN; 5 μM) led to a quick and stable inhibition of eIPSC amplitude, as shown in Fig. 7A. The group data in Fig. 7B show that the average time of onset for the WIN effect was 2.9 ± 0.3 min and eIPSC amplitude reached 62.7 ± 5.6% of BL by the end of WIN exposure (n = 4, P < 0.05, range: 84–55%). Figure 7B also illustrates the striking similarity in time course and magnitude for the BDNF and WIN effects. Furthermore, pretreatment with WIN (5 μM) significantly blocked the effect of BDNF. A typical example is shown in Fig. 7C and Fig. 7D summarizes the group data. The application of WIN alone led to a decrease to 61.7 ± 4.7% of BL (n = 4, P < 0.05, range: 72–52%) and subsequent addition of BDNF had no significant further effect on eIPSC amplitude (88.2 ± 7.5% of WIN baseline).

In addition to blocking the effect of BDNF on eIPSCs, we found that the CB1 receptor antagonists SR (5 μM) and AM251 (5 μM) also prevented the effect of BDNF on CCh-induced sIPSCs. An example of such an effect for SR
is represented in Fig. 8B, compared with the effect of BDNF in the absence of SR (Fig. 8A). Time courses of the amplitude of the IPSCs in the absence and presence of SR from both traces are also shown (the data are represented as 1-min bins of the mean IPSC amplitude over time). BDNF rapidly decreased the amplitude of the CCh-induced IPSCs in BL conditions but failed to do so in the presence of SR. Application of the CB1 antagonist AM251 produced similar results. As shown in Fig. 8C, BDNF significantly decreased CCh-induced IPSCs (63.5 ± 8.2%, n = 8, P < 0.05) and either SR or AM251 prevented the BDNF effect (SR: 111.1 ± 8.2% of BL, n = 4; AM251: 106.8 ± 5.6% of BL, n = 4), paralleling the results obtained with eIPSCs. Finally and for the sake of comparison, this figure also includes data obtained using the cannabinoid agonist WIN either in control conditions or in the presence of SR. Note the similarity between the effect of WIN (56.3 ± 3.5%, n = 5, P < 0.01) and the effect of BDNF on CCh-induced IPSCs under control conditions and that the addition of SR prevents the WIN effect (99.7 ± 11.1%, n = 5).

We further explored BDNF-induced release of endocannabinoids by blocking endocannabinoid transport and synthesis. It has been shown that inhibition of membrane transport can disrupt endocannabinoid release. In particular, the cannabinoid transport inhibitor AM404 loaded intracellularly via the patch pipette has been shown to block endocannabinoid-mediated long-term depression in striatal medium spiny neurons (Ronesi et al. 2004). As shown in the individual example in Fig. 9A and group data in Fig. 9B, intracellular AM404 (5 μM) significantly disrupted the effect of BDNF on eIPSCs (82 ± 3.7% of BL; n = 6; range: 95–68%) compared with vehicle alone (0.02% DMSO; 57.9 ± 3%; n = 3; Fig. 2B). The small residual effect of BDNF in the presence of AM404 may reflect alternate pathways for endocannabinoid release from the postsynaptic PN. We also used the diacylglycerol (DAG) lipase inhibitor RHC 80267 to prevent synthesis of the endocannabinoid 2-arachidonylglycerol (2-AG). As shown in Fig. 9, C and D, DAG lipase inhibition completely prevented the effect of BDNF. These results strongly suggest a role for BDNF-induced release of endocannabinoids from the postsynaptic pyramidal neuron.

**DISCUSSION**

This report establishes that BDNF inhibits synaptic transmission at GABAergic synapses received by cortical layer 2/3 PNs in juvenile mice through an interaction with the endocannabinoid system. We found that the effects of BDNF were triggered by postsynaptic trkB activation, but resulted in a presynaptic change in transmitter release probability. Our results further suggest that endocannabinoids are the retrograde messenger induced by postsynaptic trkB activation because the effect of BDNF was blocked by two different CB1 antagonists, was mimicked and occluded by a cannabinoid agonist, and was prevented by postsynaptic loading with the endocannabinoid transport blocker AM404 and the DAG lipase inhibitor RHC 80267.

The effect of BDNF on both evoked and CCh-induced spontaneous IPSCs was triggered by activation of trkB receptors because bath application of K252a, a relatively specific inhibitor of trk receptor autophosphorylation, resulted in a total loss of BDNF effectiveness. Loading the postsynaptic PN with K252a was also sufficient to prevent the BDNF effect, suggesting that most, if not all, of the BDNF effect depends on trkB receptors located on the postsynaptic neuron. It is important to note that K252a is membrane permeable and therefore could diffuse outside the cell to also block trkB receptors located on the presynaptic side. However, we previously demonstrated that at glutamatergic synapses, intracellular K252a prevented the postsynaptic effect of BDNF on mEPSC decay time but did not prevent the presynaptic increase in mEPSC frequency, indicating specificity in its site of action (Madara and Levine 2008). It should be noted that K252a can inhibit other protein kinases as well, although at the concentration used it is relatively specific for trk receptors (Berg et al. 1992; Nye et al. 1992). Importantly, however, blockade of the BDNF effect after direct postsynaptic application of K-252a indicates a critical role for postsynaptic signaling in the initiation of the BDNF effect. This conclusion is further supported by the
findings that the effect of BDNF was also blocked by intracellular application of either the calcium chelator BAPTA or the endocannabinoid transport inhibitor AM404. Blockade of the BDNF effect by intracellular loading of a single postsynaptic cell also indicates that BDNF-induced endocannabinoids that may have been generated in neighboring neurons did not affect synaptic inputs to the cell under study. This limited diffusion of lipophilic endocannabinoids has also been observed during depolarization-induced suppression of inhibition (DSI) (Wilson and Nicoll 2001). Although triggered by postsynaptic trkB activation,

**FIG. 7.** Cannabinoid agonist WIN55,212-2 (WIN) depresses eIPSCs and occludes the effect of BDNF. A: example time course of the effect of WIN (5 μM) on eIPSC amplitude. Inset shows example eIPSCs recorded before (a) and during (b) bath application of WIN (scale bars: 0.5 nA, 0.05 s). B: group data (n = 4) showing the effect of WIN on normalized eIPSC amplitude as a function of time. Also shown for comparison are the control BDNF group data (taken from Fig. 1B). C: time course of a representative example of the lack of effect of BDNF (20 ng/ml) on eIPSCs following pretreatment with WIN (5 μM). D: group data (n = 4) showing the occlusion of BDNF effect by WIN pretreatment.

**FIG. 8.** CB1 receptor antagonists block the effect of BDNF on CCh-induced IPSCs. A and B: individual time courses and representative sweeps for the effect of BDNF (20 ng/ml) on the mean amplitude of CCh-induced IPSCs in the absence (A) or presence (B) of the CB1 antagonist SR141716A (SR; 5 μM). Scale bars: 0.4 nA, 10 s. C, left: group data showing the effects of BDNF alone on CCh-induced IPSC amplitude (n = 8) or in the presence of the CB1 receptor antagonists SR or AM251 (5 μM; n = 4 for each condition). Right: group data for the effect of WIN alone (5 μM; n = 6) or in the presence of SR (n = 5). *P < 0.05.

*J Neurophysiol • VOL 104 • OCTOBER 2010 • www.jn.org*
receptors, BDNF caused a decrease in presynaptic release probability, as indicated by changes in the paired-pulse ratio, the coefficient of variation, and the frequency of mIPSCs.

Several previous studies identified acute postsynaptic effects of BDNF that were also triggered by postsynaptic trkB activation. For example, BDNF rapidly down-regulates GABA receptor surface expression in cultured neurons from the hippocampus (Cheng and Yeh 2003) and cerebellum (Brunig et al. 2001) and in hypothalamic slices (Hewitt and Bains 2006). BDNF has also been shown to decrease postsynaptic responses to applied GABA agonists. These effects were prevented by direct postsynaptic application of K252a (Cheng and Yeh 2003; Hewitt and Bains 2006; Tanaka et al. 1997), suggesting a postsynaptic trkB signaling requirement for these effects. In the present studies, we found no evidence for direct postsynaptic effects in layer 2/3 cortical pyramidal neurons, evidenced by the lack of effect of BDNF on mIPSC amplitude and the lack of effect on responses to locally applied GABA. These results mirror the lack of effect of exogenous cannabinoids on both mIPSC amplitude and postsynaptic GABA responses in layer 2/3 PNs that we previously reported (Trettel et al. 2004), although it should be noted that endocannabinoids may have direct postsynaptic effects on a subset of cortical interneurons and principal neurons (Bacci et al. 2004; Marinelli et al. 2009).

BDNF has been shown to have acute postsynaptic effects in various brain regions. In CA1 pyramidal neurons in hippocampal slices, for example, BDNF caused a rapid decrease in evoked responses that was accompanied by an increase in both the paired-pulse ratio and the coefficient of variation, suggesting a presynaptic locus (Frerking et al. 1998). In cultured cerebellar granule cells, in addition to its postsynaptic effects, BDNF caused a decrease in mIPSC frequency that may reflect presynaptic effects (Cheng and Yeh 2003). In the neocortex, we found in the present studies that BDNF had clear presynaptic effects, although these effects were triggered by postsynaptic trkB activation, suggesting that a retrograde messenger induced by postsynaptic trkB activation mediated the presynaptic effect of BDNF at these synapses. An endocannabinoid, specifically 2-AG, is likely retrograde messenger released by the postsynaptic PN because the effect of BDNF was blocked by two different CB1 receptor antagonists and was mimicked and occluded by a cannabinoid agonist. In addition, the effect of BDNF was prevented by inhibiting DAG lipase, the enzyme responsible for 2-AG synthesis, and was also prevented by postsynaptic loading with the endocannabinoid transport blocker AM404.

Other lines of evidence also support mutual interactions between BDNF and the endocannabinoid system. In addition to the evidence presented here that BDNF can induce release of endocannabinoids, BDNF may also increase sensitivity to endocannabinoids via modulation of CB1 expression (Maison et al. 2009). In contrast to the suppressive effect of BDNF at GABAergic synapses, we have shown that BDNF enhances transmission at glutamatergic synapses via a combination of pre- and postsynaptic effects (Madara and Levine 2008). It is not known as yet whether BDNF-induced endocannabinoids also play a mitigating role at excitatory synapses. Furthermore, endocannabinoids themselves have been implicated in the regulation of BDNF synthesis. For example, BDNF levels can be up-regulated by cannabinoid receptor activation (Butovsky et al. 2005), kainate-induced seizures have been shown to induce BDNF expression in a CB1-dependent manner (Marsicano et al. 2003), and BDNF levels are decreased in CB1 knockout mice (Aso et al. 2008). This interaction may have physiological significance because BDNF has been reported to mediate CB1 receptor-dependent protection against excitotoxicity (Khaspekov et al. 2004; Marsicano et al. 2003).

Although the underlying signaling mechanisms for BDNF-induced endocannabinoid mobilization are not yet known, a potential role for postsynaptic PLCγ signaling is consistent with involvement of endocannabinoids. TrkB receptor activation and subsequent PLCγ signaling can generate DAG, a
calcium chelator BAPTA. Interestingly, BDNF–trkB activation may lead to an intracellular Ca++ increase via routes that are independent of membrane depolarization or N-methyl-D-aspartate receptor activation. These include activation of the canonical transient receptor potential channel 3 (TRPC3; Amaral and Pozzo-Miller 2007a,b) or release from internal stores mediated by PLCγ1-IP3 signaling (Amaral and Pozzo-Miller 2007a; Li et al. 1998; Tanaka et al. 1997). In the present studies, where neurons were voltage-clamped and there were no signs of inward currents in the postsynaptic cell, the source of the calcium required for the BDNF response is most likely release from intracellular stores.

In conclusion, our data support a new model for the acute effect of BDNF on layer 2/3 neocortical GABAergic synapses. BDNF–trkB signaling in the postsynaptic dendrite results in a rapid mobilization of endocannabinoids into the synaptic cleft, leading to a decrease in the probability of presynaptic GABA release. Further experimentation is needed to elucidate the intracellular pathways by which BDNF–trkB signaling induces the synthesis and release of endocannabinoids from neocortical PNs and the interactions between BDNF and other regulators of endocannabinoid mobilization.

GRANTS

This work was supported by National Institute on Drug Abuse Grant DA-16791 to E. S. Levine.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


