Brain-Derived Neurotrophic Factor Silences GABA Synapses Onto Hypothalamic Neuroendocrine Cells Through a Postsynaptic Dynamin-Mediated Mechanism

Sarah A. Hewitt and Jaideep S. Bains

JN 95:2193-2198, 2006. First published Jan 11, 2006; doi:10.1152/jn.01135.2005

You might find this additional information useful...

This article cites 34 articles, 15 of which you can access free at:
http://jn.physiology.org/cgi/content/full/95/4/2193#BIBL

Updated information and services including high-resolution figures, can be found at:
http://jn.physiology.org/cgi/content/full/95/4/2193

Additional material and information about ***Journal of Neurophysiology*** can be found at:
http://www.the-aps.org/publications/jn

This information is current as of March 21, 2006 .

Journal of Neurophysiology publishes original articles on the function of the nervous system. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2005 by the American Physiological Society. ISSN: 0022-3077, ESSN: 1522-1598. Visit our website at http://www.the-aps.org/.

Downloaded from jn.physiology.org on March 21, 2006

J Neurophysiol 95: 2193–2198, 2006.
First published January 11, 2006; doi:10.1152/jn.01135.2005.

Brain-Derived Neurotrophic Factor Silences GABA Synapses Onto Hypothalamic Neuroendocrine Cells Through a Postsynaptic Dynamin-Mediated Mechanism

Sarah A. Hewitt and Jaideep S. Bains
Hotchkiss Brain Institute and Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada

Submitted 27 October 2005; accepted in final form 4 January 2006

Hewitt, Sarah A. and Jaideep S. Bains. Brain-derived neurotrophic factor silences GABA synapses onto hypothalamic neuroendocrine cells through a postsynaptic dynamin-mediated mechanism. *J Neurophysiol* 95: 2193–2198, 2006. First published January 11, 2006; doi:10.1152/jn.01135.2005. In the paraventricular nucleus of the hypothalamus (PVN), experimental stress paradigms that suppress γ-aminobutyric acid (GABA) inputs to parvocellular neuroendocrine cells (PNCs) also increase the expression of brain-derived neurotrophic factor (BDNF). In the adult CNS, BDNF regulates the efficacy of GABAergic transmission, but its contributions to functional changes at inhibitory synapses in the PVN have not been investigated. Analysis of quantal transmission revealed a decrease in the frequency of miniature inhibitory postsynaptic currents (mIPSCs) in response to BDNF with no accompanying changes in their amplitude. These effects were completely blocked by prior inclusion of the TrKB receptor antagonist K252a in the patch pipette. Inclusion of a dynamin inhibitory peptide in the patch pipette also blocked the effects of BDNF, consistent with an all-or-none removal of clusters of postsynaptic $GABA_A$ receptors. Finally, to confirm a decrease in the availability of postsynaptic $GABA_A$ receptors, we tested the effects of BDNF on focal application of the $GABA_A$ agonist muscimol. Postsynaptic responses to muscimol were reduced after BDNF. Collectively, these data indicate that BDNF remodels functional synaptic contacts putatively by reducing the surface expression of postsynaptic $GABA_A$ receptors.

INTRODUCTION

The neurotrophins constitute a family of molecules critical for normal development of the CNS (Tapia-Arancibia et al. 2004). They play a vital role in facilitating the formation and maturation of the precise neural circuitry during the developmental phase through the strengthening of some synapses and pruning of others (Lu 2003; Tapia-Arancibia et al. 2004). Our knowledge of their scope of action has been expanded recently by the demonstrations that neurotrophins—in particular, brain-derived neurotrophic factor (BDNF)—can modify synaptic efficacy in neural circuitry derived from adult brain (Lu 2003). Although the focus, in the adult, has been on the ability of BDNF to alter synaptic strength, it may also play an important role in changing the relative weightings of individual synapses onto target neurons.

In response to stress paradigms, the expression of BDNF mRNA and protein increases in the adult hypothalamus (Givalois et al. 2004; Rage et al. 2002; Smith et al. 1995). This is especially evident in the neuroendocrine neurons in the paraventricular nucleus (PVN) of the hypothalamus and is paralleled by decreases in inhibitory synaptic drive to these cells (Verkuyl et al. 2004, 2005). BDNF can alter synaptic inhibition by decreasing the surface stability and expression of γ-aminobutyric acid type A ($GABA_A$) receptors through activation of postsynaptic TrkB receptors (Brunig et al. 2001; Jovanovic et al. 2004; Tanaka et al. 1997). An alteration in the surface stability of the receptor may underlie the demonstration that BDNF can promote loss of receptors at some sites and increased clustering at other sites (Elmariah et al. 2004), resulting in a remodeling of inhibitory synapses through changes in the localization of $GABA_A$ receptors.

To test the effects of BDNF on stress-relevant inhibitory circuitry, we obtained whole cell patch-clamp recordings from postnatal day 21 (P21) to P28 rat parvocellular PVN neurons in acute coronal slices. We recorded from parvocellular cells in the medial region of the nucleus and examined the effects of BDNF on miniature inhibitory postsynaptic currents (mIPSCs). Our findings indicate that BDNF selectively inhibits a subpopulation of GABA synapses through a dynamin-mediated endocytosis of postsynaptic $GABA_A$ receptors. These limited, yet precise, actions of BDNF unveil a mechanism by which neurons meet physiological demands by selectively altering the balance of inhibitory drive to fine-tune outputs.

METHODS

All experiments were performed according to protocols approved by the University of Calgary animal care and use committee in accordance with guidelines established by the Canadian Council on Animal Care.

Slice preparation

Hypothalamic slices containing the PVN were prepared from P21 to P28 male Sprague–Dawley rats. Animals were anesthetized with sodium pentobarbital (30–50 mg/kg) and decapitated, and the brains were rapidly removed into ice-cold high-sucrose slicing solution (saturated with 95% O_2-5% CO_2) and allowed to cool for about 3 min. The slicing solution contained the following (in mM): 87 NaCl, 2.5 KCl, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 1.25 NaH_2PO_4, 25 glucose, and 75 sucrose. The brain was then blocked, mounted on a vibrating slicer (Leica, Nussloch, Germany), and submerged in slicing solution that was constantly bubbled with 95% O_2-5% CO_2. The brain was cut in the coronal plane, and hemisected slices of 300 μM thickness, containing the hypothalamus, were incubated at 32.5°C in a sub-

Address for reprint requests and other correspondence: J. S. Bains, Hotchkiss Brain Institute and Department of Physiology and Biophysics, University of Calgary, Calgary, AB, Canada T2N 4N1 (E-mail: jsbains@ucalgary.ca).

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.

0022-3077/06 $8.00 Copyright © 2006 The American Physiological Society

Downloaded from jn.physiology.org on March 21, 2006

merged chamber of oxygenated artificial cerebrospinal fluid (aCSF) for a minimum of 90 min before recording. The aCSF contained the following (in mM): 126 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose.

Electrophysiological recordings

Whole cell recordings were obtained from medial parvocellular cells visually identified using an upright microscope (BX51W, Olympus Optical, Tokyo, Japan) fitted with infrared differential interference contrast optics. All recordings were obtained at 32.5°C using borosilicate glass microelectrodes (tip resistance of 3–7 MΩ) filled with intracellular solution containing the following (in mM): 150 CsCl, 1 EGTA, 10 HEPES, 0.1 $CaCl_2$, 4.6 $MgCl_2$, 2 Mg-ATP, and 0.3 NA-GTP. The internal solution was filtered before use. In preliminary experiments, recordings were obtained with a potassium gluconate–based internal solution to ascertain the phenotype of these cells based on their electrical fingerprint. Previous work has demonstrated that a lack of inward rectification and low-threshold spikes are consistent with a neuroendocrine phenotype (Luther et al. 2002). Once we were confident of our ability to faithfully record from neuroendocrine cells, the remaining recordings were performed with a Cs-based internal solution to increase the driving force for Cl^- ions and inhibit postsynaptic voltage-gated K^+ currents. All experiments were performed in voltage-clamp mode, with cells held at −80 mV (to block any *N*-methyl-D-aspartate currents) and recordings were accepted when access resistance changes were limited to <15%.

For all experiments, the perfusate solution contained 10 μM DNQX (Tocris Cookson, Ellisville, MO) to block excitatory α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–mediated synaptic currents and tetrodotoxin (TTX) 1 μM, to ensure only spontaneous mIPSCs through $GABA_A$ receptors were recorded. Signals were amplified with the Multiclamp 700A amplifier (Axon Instruments, Foster City, CA), low-pass filtered at 1 kHz, and digitized at 5–10 kHz using the Digidata 1322 (Axon Instruments).

Data analysis

Data were digitized (pClamp 9, Axon Instruments) and stored on computer for off-line analysis. Events were detected when they crossed a threshold set at three times the baseline noise. The detected events were confirmed as synaptic events by eye. mIPSCs in individual cells were compared between groups using a Kolmogorov–Smirnov statistic. All other values shown are means ± SE. Data points were compared using ANOVA with a post hoc Newman–Keuls test (multiple groups) or Student's *t*-test (two groups).

Drugs

TTX and K252B were purchased from Alamone Labs; bicuculine methiodide and basic lab salts from Sigma-Aldrich; BDNF from R&D Systems; K252A, DIP, DIP myristoylated, muscimol, and DNQX from Tocris Cookson.

RESULTS

Recordings were obtained from parvocellular neurons in medial PVN, identified by their location and their clearly distinct morphology from magnocellular cells (Cowley et al. 1999; Pronchuk et al. 2002; Verkuyl et al. 2005). All experiments were conducted in voltage-clamp mode at a holding potential of −80 mV.

BDNF decreases frequency of mISPCs

In addition to the action potential–driven release of GABA, PNCs in PVN are bombarded by a high tonic level of quantal, non-action potential–driven mIPSCs. The mean mIPSC frequency recorded from these neurons was 3.99 ± 0.22 Hz, n = 40 (data not shown), whereas the mean amplitude was 76.36 ± 1.11 pA, n = 40 (data not shown). The mIPSCs were reversibly blocked by the $GABA_A$ receptor antagonist, bicuculine methiodide, 10 μM (data not shown), thereby confirming that these events were mediated by the activation of postsynaptic $GABA_A$ receptors.

To test the effects of BDNF on GABAergic inputs, we bath applied 2 nM BDNF for 2 min and assessed changes in the frequency and amplitude of mISPCs. Previous investigators have reported multifaceted effects of BDNF that were a function of the duration for which BDNF was applied (Jovanovic et al. 2004). We failed to see any evidence for an increase in quantal amplitude or frequency in response to BDNF (n = 20). In response to BDNF, we observed a slowly developing decrease in the frequency of mISPCs that reached a new, stable level approximately 6 min after the termination of the BDNF application. Consequently, we examined changes only in synaptic strength during this stable period (6–12 min after BDNF) and compared these values to the control time period before BDNF application. BDNF elicited a significant decrease in mIPSC frequency (74.09 ± 3.87% of control, $P < 0.01$, n = 20, Fig. 1, *A–C*) but had no effect on amplitude (101.40 ± 2.91% of control, $P > 0.05$, n = 20, Fig. 1, *A* and *C*). Measurements of mIPSC frequency and amplitude were taken from 6 to 12 min after termination of BDNF, with no statistical differences in frequency noted between these time points. No further decrease in frequency was observed beyond this time period and the effects were not reversible ≤35 min of wash.

BDNF acts through postsynaptic TrKB receptors

Through interactions with the postsynaptic TrkB receptor, BDNF can decrease the surface stability of the $GABA_A$ receptor within the membrane (Brunig et al. 2001; Jovanovic et al. 2004; Tanaka et al. 1997). Although a decrease in the frequency of mIPSCs is classically taken as an indicator of a change in vesicular release probability, manipulations that remove entire clusters of postsynaptic receptors can also cause a decrease in quantal frequency (Beattie et al. 2000; Oliet et al. 1996). Combined with the data that BDNF can alter the postsynaptic localization of $GABA_A$ receptors (Elmariah et al. 2004), we were compelled to examine the possibility that BDNF decreases inhibitory signaling through a postsynaptic effect on $GABA_A$ receptors.

To test this hypothesis directly, we included the TrkB receptor inhibitor K252a (200 nM) (Cheng and Yeh 2003, 2005; Patapoutian and Reichardt 2001; Tanaka et al. 1997) in the patch pipette and repeated the experiments with BDNF. During the 15 min of control to allow for infusion of K252a into the cell, we did not see any consistent changes in event frequency or amplitude (data not shown). In the presence of K252a, BDNF had no effect on either the frequency or the amplitude of mIPSCs (frequency: 102.10 ± 4.06% of control, $P > 0.05$, $n = 6$, Fig. 2*A*; amplitude: 102.20 ± 4.32% of control, $P > 0.05$, Fig. 2*B*). To confirm that the effects of BDNF were the result of specific TrkB receptor activation, we repeated these experiments in the presence of K252B (200 nM), a compound that acts as a weak, nonspecific inhibitor of the Trk-type kinase and protein kinase C (PKC), commonly used as a control for K252a (Tanaka et al. 1997). No frequency or ampli-

Downloaded from jn.physiology.org on March 21, 2006

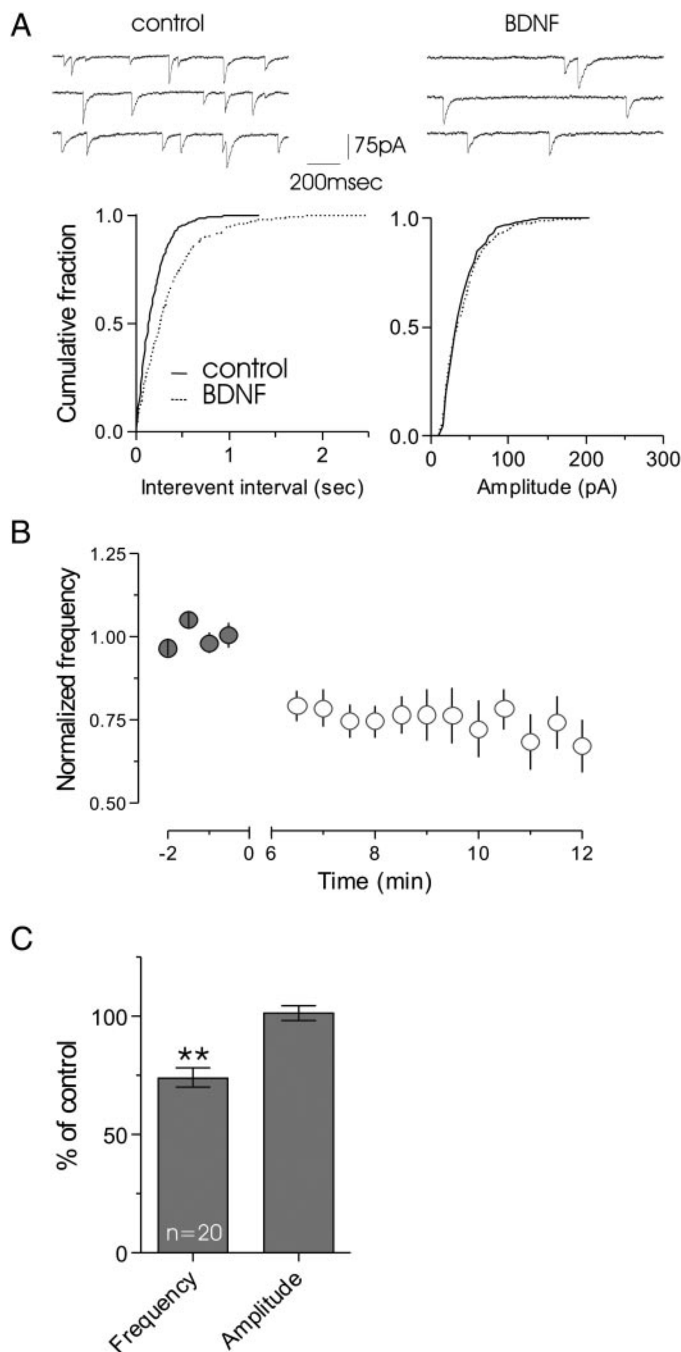

FIG. 1. Brain-derived neurotrophic factor (BDNF) decreases the frequency of miniature inhibitory postsynaptic currents (mIPSCs) with no effect on amplitude. *A*: representative traces (*top*) during control and after BDNF application (2 nM, 2 min). Decrease in frequency with no accompanying change in amplitude is shown in the cumulative fraction plots immediately *below*. *B*: event frequency of all cells tested with BDNF, normalized to control frequency ($n = 20$), shown as a fraction of the control values. Time points taken from 2 min control (dark circles) and from the 6–12 min after BDNF (clear circles). *C*: summary of the change in event frequency and amplitude after BDNF application. Data are shown as a percentage of control values.

tude effects were observed during the 15-min control period. In the presence of K252b, BDNF elicited a small but significant decrease in mIPSC frequency (85.30 ± 4.83% of control, $P < 0.05$, $n = 8$, Fig. 2*A*) but not event amplitude (101.10 ± 4.84% of control, $P > 0.05$, Fig. 2*B*). This decrease in mIPSC frequency was not as robust as that observed in BDNF alone ($P < 0.05$). To further assess the intracellular pathways mediating the effects of BDNF on mIPSCs, we conducted additional experiments with the calcium chelator EGTA (10 mM) in the patch pipette. Under these conditions, BDNF decreased mIPSC frequency (83.66 ± 3.97% of control, $P < 0.05$, $n = 6$, Fig. 2*A*) and had no effect on amplitude (95.23 ± 3.92% of control, $P > 0.05$, Fig. 2*B*). Like K252b, this change in frequency was also significantly different from that observed in BDNF alone ($P < 0.05$). From these observations, it appears that TrkB receptor activation is necessary for the actions of BDNF and that downstream effectors such as PKC and intracellular Ca^{2+} may be involved in an important, yet limited, fashion.

BDNF internalizes $GABA_A$ receptors through a dynamin-dependent mechanism

We next examined the potential cellular mechanism through which BDNF may exact a removal of $GABA_A$ receptor clus-

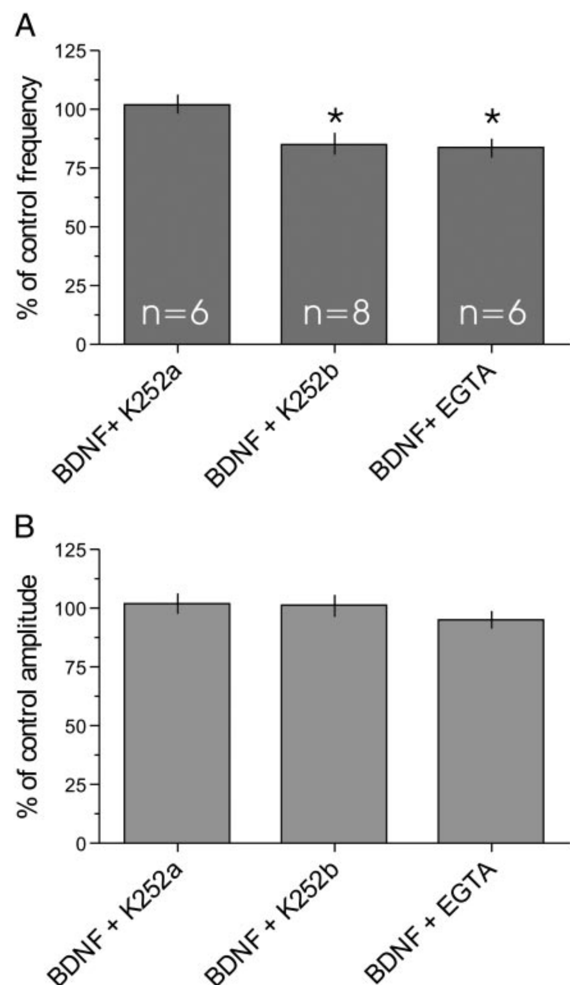

FIG. 2. BDNF effects are mediated through activation of the TrkB receptor. *A*: summary of the change in event frequency as a percentage of control values. Experiments were done with either K252a (200 nM), K252b (200 nM), or EGTA (10 mM) included in the patch pipette. BDNF had no effect on frequency in the presence of K252a ($P > 0.05$ vs. control, $n = 6$), but did decrease frequency in either K252b ($P < 0.05$ vs. control, $n = 8$) or EGTA ($P < 0.05$ vs. control, $n = 6$). *B*: summary of mIPSC amplitude in BDNF alone or BDNF + K252a, +K252b, +EGTA showing no change from control values.

Downloaded from jn.physiology.org on March 21, 2006

ters. One possibility is that BDNF decreases the surface expression of $GABA_A$ receptors through an internalization mechanism (Brunig et al. 2001; Cheng and Yeh 2003; Jovanovic et al. 2004). Endocytosis of membrane-bound receptors involves their association with adaptor proteins and the subsequent recruitment of clathrin-coated pits. With the receptor bound to an adaptor protein such as AP2, the complex is then able to interact with clathrin and the GTPase dynamin, both critical elements for the endocytotic process (Herring et al. 2003; Kittler et al. 2000). Because the endocytosis of $GABA_A$ receptors in the hippocampus is a clathrin-mediated event (Connolly et al. 1999; Herring et al. 2003; Kittler et al. 2000; van Rijnsoever et al. 2005) and dynamin is a crucial component to this process, we asked whether dynamin was involved in the postsynaptic actions of BDNF. To selectively block dynamin-mediated endocytosis, a 10 amino acid peptide, dynamin inhibitory peptide (DIP, Tocris Cookson), 50 μM, was added to the internal solution of the patch pipette. This molecule prevents endocytosis by interfering with the binding of amphiphysin with dynamin (Marks and McMahon 1998; Wigge and McMahon 1998). To permit sufficient time for the contents of the pipette to fully diffuse into the cell, we obtained control recordings for 15 min before application of BDNF. During this time, we did not observe any changes in the frequency or amplitude of mIPSCs. The subsequent application of BDNF did not replicate the changes in mIPSC frequency seen in BDNF alone. We observed no change in mIPSC frequency (95.55 ± 5.55% of control, $P > 0.05$, $n = 7$, Fig. 3, *A* and *B*) and no change in amplitude (98.95 ± 5.40% of control amplitude, $P > 0.05$, Fig. 3, *A* and *B*). To control for nonspecific actions of this inhibitor that may be independent of its actions on dynamin, we included a myristoylated scrambled version of the peptide (50 μM) in the patch pipette and tested the effects of BDNF on mIPSCs. Under these conditions, BDNF still robustly inhibited GABA transmission (mIPSC frequency: 82.11 ± 4.35% of control, $P < 0.05$, $n = 5$; mIPSC amplitude: 92.34 ± 6.42% of control, Fig. 3*B*). These findings indicate that BDNF decreases GABAergic signaling by the selective, dynamin-dependent internalization of $GABA_A$ receptors.

Agonist-induced inward currents are reduced after BDNF

To directly measure the consequences of postsynaptic receptor internalization, we examined the effects of BDNF on the inward current induced by focal application of the $GABA_A$ receptor agonist muscimol. If BDNF elicits the internalization of $GABA_A$ receptors, we would predict that fewer receptors would be available for activation after exposure to BNDF and the current induced by direct $GABA_A$ receptor activation will be reduced accordingly. Muscimol was pressure applied from the tip of a patch pipette in which the concentration of muscimol was 100 μM. The pipette tip was positioned about 20–30 microns away from the recording electrode in the cell body. Four epochs of muscimol application, spaced 1 min apart, were delivered. Each epoch consisted of three applications, 4 s apart. Average amplitudes from the control period and in the 6–12 min after BDNF application were used for analysis. As a result of the intercell variability in the amplitude of the responses to muscimol, data within each cell were normalized to the average control response (Fig. 4*B*). After BDNF, the amplitude of the inward current, in pA, was significantly smaller than that in

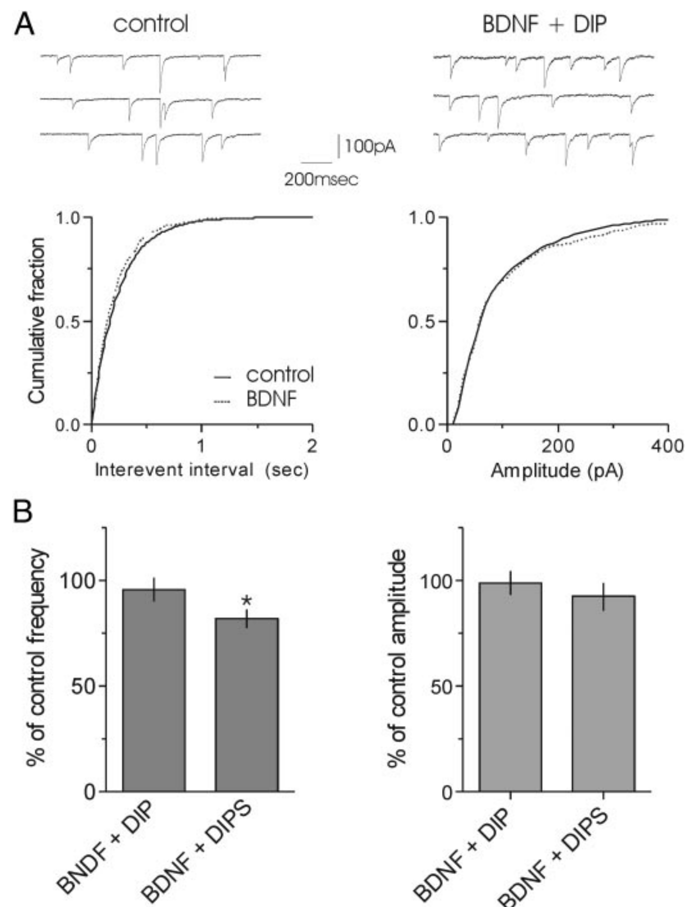

FIG. 3. BDNF effects require postsynaptic dynamin. *A*: sample traces (*top*) from control with dynamin inhibitory peptide (DIP) included in the patch pipette (50 μM) and after BDNF application. Effects on mIPSC frequency and amplitude in these cells are quantified immediately *below*. *B*: summary of BDNF effects on frequency (*left*) and amplitude (*right*) in the presence of DIP and the scrambled DIP, 50 μM (DIPS).

control (83.01 ± 6.11% of control, $P < 0.05$, $n = 8$, Fig. 4, *A* and *B*), indicative of a removal of available surface receptors.

DISCUSSION

Our data demonstrate that BDNF depresses transmission at inhibitory, GABAergic synapses onto parvocellular neuroendocrine neurons in PVN. This effect is mediated by the postsynaptic activation of TrkB receptors. Furthermore, we were able to block the actions of BDNF by inhibiting dynamin, a critical component in the clathrin-mediated endocytosis of $GABA_A$ receptors. This decrease in the surface expression of functional $GABA_A$ receptors provides a reasonable mechanism underlying the previously observed decreases in inhibitory signaling at the onset of the stress response when BDNF expression is increased in PVN.

Our findings demonstrating the TrkB-mediated inhibition of GABA transmission are consistent with earlier observations (Brunig et al. 2001; Cheng and Yeh 2003; Gottschalk et al. 1998). Although there is also evidence for a BDNF-induced potentiation of $GABA_A$ receptor function (Baldelli et al. 2002) we did not see any evidence of increases in GABAergic drive to parvocellular PVN neurons. These differential effects may be time dependent, with an initial potentiation giving way to a

Downloaded from jn.physiology.org on March 21, 2006

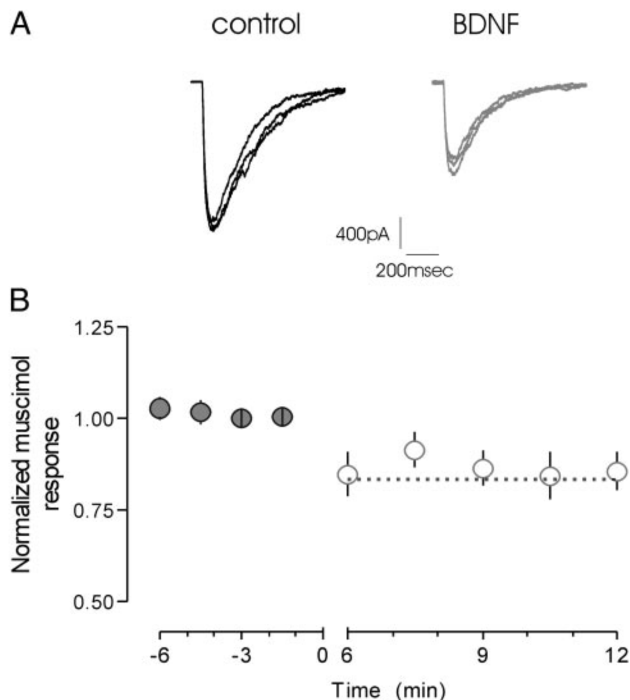

FIG. 4. Postsynaptic responses to focal γ-aminobutyric acid type A ($GABA_A$) agonist application are attenuated after BDNF. *A*: muscimol, a $GABA_A$ agonist, was focally applied to the soma of the neuron being recorded. Three pressure-applied puffs of muscimol were given every 4 s, spaced 1 min apart. Sample traces of the inward currents observed in response to 3 successive puffs (one epoch) are shown both in control (black) and after BDNF (gray) application. *B*: summary of the change in the amplitude of the muscimol-evoked inward current inward current from all cells, normalized as a ratio of control.

depression on prolonged exposure to BDNF (Jovanovic et al. 2004). To circumvent these biphasic effects, we assessed BDNF effects in a very narrow time window (6–12 min after BDNF application) during which synaptic depression is dominant (Jovanovic et al. 2004).

The most intriguing aspect of our observations is the fact that BDNF appears to selectively target a subset of synapses by removing clusters of $GABA_A$ receptors in an "all-or-none" fashion. Because we observed a decrease in mIPSC frequency, but did not observe any changes in mIPSC amplitude, we hypothesized that BDNF was selectively "switching off" clusters of postsynaptic $GABA_A$ receptors (Beattie et al. 2000; Oliet et al. 1996). Our experiments in which inclusion of the TrkB inhibitor K252a in the patch pipette blocked the effects of BDNF are consistent with a postsynaptic locus of action of BDNF. We further hypothesized that this may result from a TrkB-driven endocytosis of receptor clusters. $GABA_A$ receptors are recycled at the membrane surface, the rate of which is modulated by a number of factors (Kittler and Moss 2003). BDNF-induced $GABA_A$ receptor internalization was previously demonstrated and, consistent with our findings, does not occur until about 5 min after exposure to BDNF (Cheng and Yeh 2003). The phosphorylation state of the receptor plays a major role in determining the surface stability of the receptor at the membrane and thus the number of receptors present at any one time (Henneberger et al. 2002; Jovanovic et al. 2004; Kittler and Moss 2003; Krishek et al. 1994; Moss et al. 1995). There is extensive evidence that BDNF can alter the phosphorylation state and thus influence the rate of internalization of $GABA_A$ receptors (Brunig et al. 2001; Kittler and Moss 2001; Kittler et al. 2000; Wan et al. 1997). Although there is conflicting evidence for the internalization process for $GABA_A$ receptors, a clathrin-mediated, dynamin-dependent mechanism is most commonly attributed to this receptor (Connolly et al. 1999; Kittler et al. 2000). Although we did not explicitly rule out a role for alterations in the phosphorylation state of the $GABA_A$ receptor, our data are most consistent with a BDNF-mediated internalization of $GABA_A$ receptors through a dynamin-dependent mechanism.

The TrkB receptor was previously linked to the activation of both PKC and intracellular calcium (Henneberger et al. 2002; Tanaka et al. 1997). This prompted us to investigate a role for intracellular calcium using the calcium chelator EGTA (10 mM) in the patch pipette. With this manipulation, BDNF decreased frequency to a level that was different from that of control but also much attenuated from its effects when acting alone. Interestingly, we also noted a partial inhibition of the effects of BDNF when K252b was used. This compound is a derivative of K252a, commonly used as a control, and although it does not directly interfere with the TrkB receptor, it does, at the dose used here, negatively affect PKC. Because PKC has also been shown to reduce $GABA_A$ receptor immunoreactivity (Chapell et al. 1998) and BDNF activation of the TrkB receptor has been previously linked to the elevation of intracellular calcium and the activation of PLC (Tanaka et al. 1997), it is not surprising that inhibiting PKC, even partially, should mitigate some of the effects of BDNF observed initially. It appears therefore that TrkB receptor activation is crucial, whereas the role of these specific downstream effectors is more limited in mediating the BDNF response.

Our findings demonstrate a novel link between the BDNF activation of TrkB receptors and dynamin-mediated endocytosis of $GABA_A$ receptors in PVN parvocellular neurons. These data indicate that a decrease in frequency is likely attributable to a loss of postsynaptic sites, further supported by the decreased inward current elicited by direct activation of $GABA_A$ receptors after BDNF application. This reflects a condition whereby release at the presynaptic terminals is unchanged, although there are fewer receptors available to transduce the signal.

Physiological significance

In PVN parvocellular neurons, during the onset of the stress response, BDNF activity is increased in CRH cells and may be released in an autocrine fashion to act on GABA synapses. Based on the data presented here, we propose that this causes the internalization of receptors at postsynapic sites. Our data reinforce recent work using acute and chronic stress paradigms (Verkuyl et al. 2004, 2005) that attenuate the frequency of mIPSCs with no effect on amplitude or event kinetics (Verkuyl et al. 2004, 2005). Furthermore, after chronic stress this decrease in GABA mIPSC frequency was not accompanied by changes in presynaptic release probability (Verkuyl et al. 2004). The authors propose that this reflects a loss of synaptic contacts under the stressed condition. Our data are consistent with this interpretation of a reduction in the number of synaptic contacts and we would further postulate that this is the result of a BDNF-mediated internalization of receptors at specific synapses. This functional alteration may provide

Downloaded from jn.physiology.org on March 21, 2006

a means through which inputs can be selectively attenuated to increase the specificity of physiologically distinct signals and provide an additional level of control in fine-tuning neuroendocrine output.

REFERENCES

Baldelli P, Novara M, Carabelli V, Hernandez-Guijo JM, and Carbone E. BDNF up-regulates evoked GABAergic transmission in developing hippocampus by potentiating presynaptic N- and P/Q-type Ca^{2+} channels signalling. *Eur J Neurosci* 16: 2297–2310, 2002.

Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, and Malenka RC. Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat Neurosci* 3: 1291–1300, 2000.

Brunig I, Penschuck S, Berninger B, Benson J, and Fritschy JM. BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of GABA(A) receptor surface expression. *Eur J Neurosci* 13: 1320–1328, 2001.

Chapell R, Bueno OF, varez-Hernandez X, Robinson LC, and Leidenheimer NJ. Activation of protein kinase C induces gamma-aminobutyric acid type A receptor internalization in Xenopus oocytes. *J Biol Chem* 273: 32595–32601, 1998.

Cheng Q and Yeh HH. Brain-derived neurotrophic factor attenuates mouse cerebellar granule cell GABA(A) receptor-mediated responses via postsynaptic mechanisms. *J Physiol* 548: 711–721, 2003.

Cheng Q and Yeh HH. PLCgamma signaling underlies BDNF potentiation of Purkinje cell responses to GABA. *J Neurosci Res* 79: 616–627, 2005.

Connolly CN, Kittler JT, Thomas P, Uren JM, Brandon NJ, Smart TG, and Moss SJ. Cell surface stability of gamma-aminobutyric acid type A receptors. Dependence on protein kinase C activity and subunit composition. *J Biol Chem* 274: 36565–36572, 1999.

Cowley MA, Pronchuk N, Fan W, Dinulescu DM, Colmers WF, and Cone RD. Integration of NPY, AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. *Neuron* 24: 155–163, 1999.

Elmariah SB, Crumling MA, Parsons TD, and Balice-Gordon RJ. Postsynaptic TrkB-mediated signaling modulates excitatory and inhibitory neurotransmitter receptor clustering at hippocampal synapses. *J Neurosci* 24: 2380–2393, 2004.

Givalois L, Naert G, Rage F, Ixart G, Arancibia S, and Tapia-Arancibia L. A single brain-derived neurotrophic factor injection modifies hypothalamo-pituitary-adrenocortical axis activity in adult male rats. *Mol Cell Neurosci* 27: 280–295, 2004.

Gottschalk W, Pozzo-Miller LD, Figurov A, and Lu B. Presynaptic modulation of synaptic transmission and plasticity by brain-derived neurotrophic factor in the developing hippocampus. *J Neurosci* 18: 6830–6839, 1998.

Henneberger C, Juttner R, Rothe T, and Grantyn R. Postsynaptic action of BDNF on GABAergic synaptic transmission in the superficial layers of the mouse superior colliculus. *J Neurophysiol* 88: 595–603, 2002.

Herring D, Huang R, Singh M, Robinson LC, Dillon GH, and Leidenheimer NJ. Constitutive GABAA receptor endocytosis is dynamin-mediated and dependent on a dileucine AP2 adaptin-binding motif within the beta 2 subunit of the receptor. *J Biol Chem* 278: 24046–24052, 2003.

Jovanovic JN, Thomas P, Kittler JT, Smart TG, and Moss SJ. Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating GABA(A) receptor phosphorylation, activity, and cell-surface stability. *J Neurosci* 24: 522–530, 2004.

Kittler JT, Delmas P, Jovanovic JN, Brown DA, Smart TG, and Moss SJ. Constitutive endocytosis of GABAA receptors by an association with the adaptin AP2 complex modulates inhibitory synaptic currents in hippocampal neurons. *J Neurosci* 20: 7972–7977, 2000.

Kittler JT and Moss SJ. Neurotransmitter receptor trafficking and the regulation of synaptic strength. *Traffic* 2: 437–448, 2001.

Kittler JT and Moss SJ. Modulation of GABAA receptor activity by phosphorylation and receptor trafficking: implications for the efficacy of synaptic inhibition. *Curr Opin Neurobiol* 13: 341–347, 2003.

Krishek BJ, Xie X, Blackstone C, Huganir RL, Moss SJ, and Smart TG. Regulation of GABAA receptor function by protein kinase C phosphorylation. *Neuron* 12: 1081–1095, 1994.

Lu B. BDNF and activity-dependent synaptic modulation. *Learn Mem* 10: 86–98, 2003.

Luther JA, Daftary SS, Boudaba C, Gould GC, Halmos KC, and Tasker JG. Neurosecretory and non-neurosecretory parvocellular neurones of the hypothalamic paraventricular nucleus express distinct electrophysiological properties. *J Neuroendocrinol* 14: 929–932, 2002.

Marks B and McMahon HT. Calcium triggers calcineurin-dependent synaptic vesicle recycling in mammalian nerve terminals. *Curr Biol* 8: 740–749, 1998.

Moss SJ, Gorrie GH, Amato A, and Smart TG. Modulation of GABAA receptors by tyrosine phosphorylation. *Nature* 377: 344–348, 1995.

Oliet SH, Malenka RC, and Nicoll RA. Bidirectional control of quantal size by synaptic activity in the hippocampus. *Science* 271: 1294–1297, 1996.

Patapoutian A and Reichardt LF. Trk receptors: mediators of neurotrophin action. *Curr Opin Neurobiol* 11: 272–280, 2001.

Pronchuk N, Beck-Sickinger AG, and Colmers WF. Multiple NPY receptors inhibit GABA(A) synaptic responses of rat medial parvocellular effector neurons in the hypothalamic paraventricular nucleus. *Endocrinology* 143: 535–543, 2002.

Rage F, Givalois L, Marmigere F, Tapia-Arancibia L, and Arancibia S. Immobilization stress rapidly modulates BDNF mRNA expression in the hypothalamus of adult male rats. *Neuroscience* 112: 309–318, 2002.

Smith MA, Makino S, Kim SY, and Kvetnansky R. Stress increases brain-derived neurotrophic factor messenger ribonucleic acid in the hypothalamus and pituitary. *Endocrinology* 136: 3743–3750, 1995.

Tanaka T, Saito H, and Matsuki N. Inhibition of GABAA synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. *J Neurosci* 17: 2959–2966, 1997.

Tapia-Arancibia L, Rage F, Givalois L, and Arancibia S. Physiology of BDNF: focus on hypothalamic function. *Front Neuroendocrinol* 25: 77–107, 2004.

van Rijnsoever C, Sidler C, and Fritschy JM. Internalized GABA-receptor subunits are transferred to an intracellular pool associated with the postsynaptic density. *Eur J Neurosci* 21: 327–338, 2005.

Verkuyl JM, Hemby SE, and Joels M. Chronic stress attenuates GABAergic inhibition and alters gene expression of parvocellular neurons in rat hypothalamus. *Eur J Neurosci* 20: 1665–1673, 2004.

Verkuyl JM, Karst H, and Joels M. GABAergic transmission in the rat paraventricular nucleus of the hypothalamus is suppressed by corticosterone and stress. *Eur J Neurosci* 21: 113–121, 2005.

Wan Q, Man HY, Braunton J, Wang W, Salter MW, Becker L, and Wang YT. Modulation of GABAA receptor function by tyrosine phosphorylation of beta subunits. *J Neurosci* 17: 5062–5069, 1997.

Wigge P and McMahon HT. The amphiphysin family of proteins and their role in endocytosis at the synapse. *Trends Neurosci* 21: 339–344, 1998.

Downloaded from jn.physiology.org on March 21, 2006