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SHORT COMMUNICATION
BDNF attenuates hippocampal LTD via activation of phospholipase C: implications for a vertical shift in the frequency–response curve of synaptic plasticity

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Abstract

Recent evidence shows that neurotrophins are not only involved in neuronal survival and differentiation during development but also in modulating synaptic strength in the mature brain. To understand how neurotrophins alter this synaptic modification, we have investigated the effect of brain-derived neurotrophic factor (BDNF) on long-term depression (LTD) at Schaffer collateral–CA1 synapses in rat hippocampal slices. The slices treated with BDNF for 5 min showed significantly less LTD in response to a 1-Hz tetanus compared with controls but displayed normal LTD when the afferents were tetanized at 10 Hz. Because BDNF enhanced long-term potentiation (LTP) induced by a 30-Hz tetanus, the synaptic modification threshold (θ_m) as defined in the 'BCM' theory of Bienenstock Cooper & Monroe [Bienenstock *et al.* (1982), *J. Neurosci.*, 2, 32–48] was not shifted. BNDF is likely to alter the capability of the plastic changes in synaptic efficacy, i.e. to produce an upward shift in the BCM curve. The suppressive effect of BDNF on LTD was prevented by either the tyrosine kinase (Trk) receptor inhibitor K252a or the phospholipase C inhibitor U73122. Thus, TrkB activation may attenuate LTD through phospholipase C signalling pathway.

Introduction

One of the fundamental features of the central nervous system is the malleability of its synaptic connections. The activity-dependent modification of the strength of specific synapses is a principal way in which experiences and their consequences are etched into neural circuits. The activity history has also been suggested to influence future responses to synaptic input in one prominent model of experience-dependent synaptic plasticity (the 'BCM' theory of Bienenstock Cooper & Monroe), often termed metaplasticity (Bienenstock *et al*., 1982; Martin *et al*., 2000). Experimentally, afferent stimulation over a range of frequencies produces a frequency–response curve of synaptic plasticity, i.e. long-term potentiation (LTP) and long-term depression (LTD), as proposed by the BCM theory. Thus, a change in the LTD/LTP crossover point θ_m, i.e. a horizontal shift in the LTD/LTP curve, is believed to represent an experience-dependent mechanism capable of modifying the synaptic plasticity phenomena involved in developmental and learning/memory processes in the brain (Bienenstock *et al*., 1982).

Accumulating evidence shows that brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays an essential role in activity-dependent plastic changes in synaptic strength (Thoenen, 1995). So far the most extensive studies regarding the role of BDNF have been carried out in the hippocampus. For example, studies from the CA1 area have demonstrated that exogenous application of BDNF rapidly potentiates basal synaptic transmission (Kang & Schuman, 1995; but see Figurov *et al*., 1996; Patterson *et al*., 1996; Tanaka *et al*., 1997) and facilitates LTP induction (Figurov *et al*., 1996). It has also been shown that stimulation evoking LTP of pyramidal neurons causes an increase in BDNF mRNA in the regions stimulated (Patterson *et al*., 1992). On the other hand, tetanus-induced LTP is prevented after application of the BDNF scavenger protein TrkB-IgG (Figurov *et al*., 1996), function-blocking TrkB antiserum (Kang *et al*., 1997), or function-blocking anti-BDNF antibody (Kossel *et al*., 2001). These studies suggest that endogenous BDNF contributes to hippocampal LTP; this is further supported by works showing that two independent lines of mutant mice lacking BDNF exhibit a severe impairment in hippocampal LTP (Korte *et al*., 1995; Patterson *et al*., 1996). Although the effects of BDNF on LTD have been studied in the visual cortex (see Akaneya *et al*., 1996; Huber *et al*., 1998), surprisingly, there has been no study of the effect of BDNF on hippocampal LTD, and it remains to be determined how BDNF alters the synaptic modification threshold θ_m in the hippocampus. Using hippocampal slices, the present work shows that brief treatment with BDNF attenuates the magnitude of CA1 LTD in response to low-frequency stimulation. Further pharmacological investigation shows that this BDNF effect is mediated by activation of the receptor tyrosine kinase TrkB and phospholipase C (PLC).

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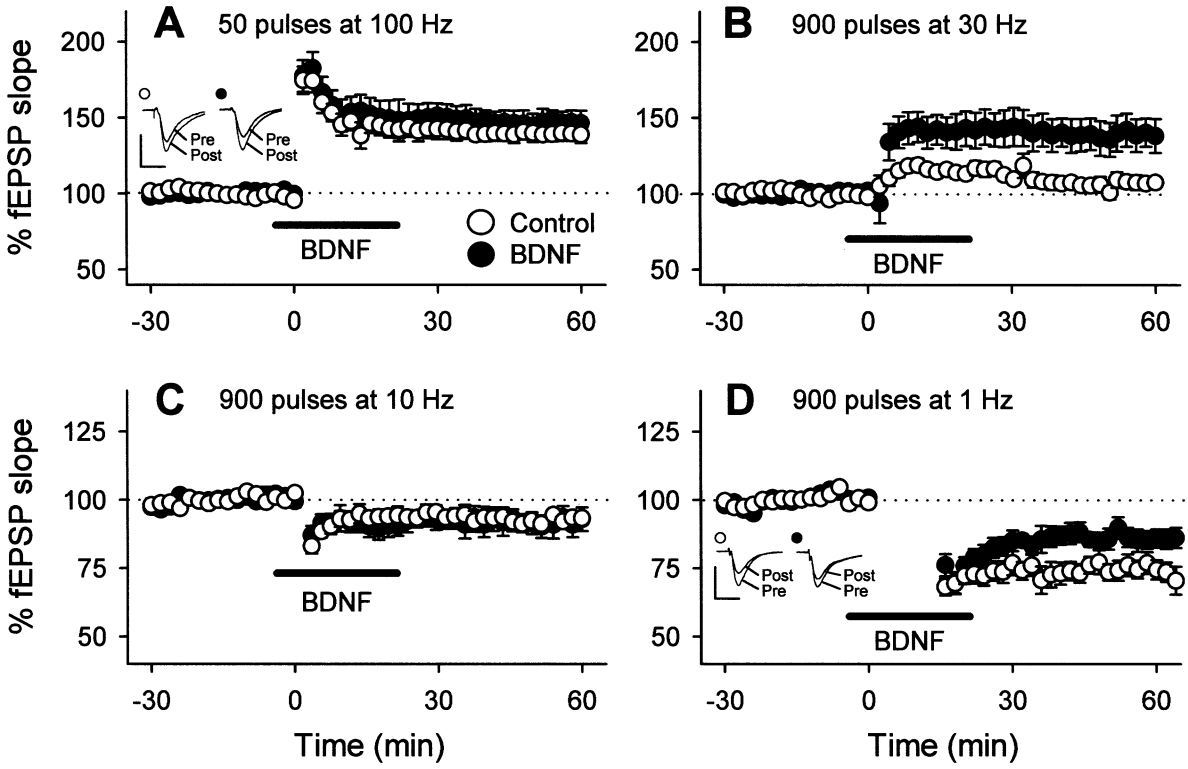

FIG. 1. BDNF treatment facilitates LTP and suppresses LTD at hippocampal CA1 synapses. Five minutes after application of 100 ng/mL BDNF, slices were tetanized with 50 pulses at 100 Hz (A), 900 pulses at 30 Hz (B), 900 pulses at 10 Hz (C) or 900 pulses at 1 Hz (D). BDNF was washed out at time 20 (min). Representative fEPSP recordings at time –5 and 50 min are shown in the inset. Calibration bars: vertical 0.5 mV; horizontal 10 ms. BDNF enhanced 30-Hz tetanus-induced LTP and attenuated 1-Hz tetanus-induced LTD. Data represent means ± SEM of each 7–9 slices.

Materials and methods

Recombinant human BDNF was a gift from Sumitomo pharmaceuticals (Osaka, Japan). Bicuculline was obtained from Sigma (St Louis, MO, USA). K252a was purchased from Kyowa Medex (Tokyo, Japan). U73122 and U73343 were purchased from Wako (Osaka, Japan).

Hippocampal slices were prepared from male Wistar/ST rats (19–25 days old) in accordance with the Japanese Pharmacological Society guide for the care and use of laboratory animals. After decapitation, the brain was removed, and cut into four to six slices (400 μm each) in ice-cold media containing (in mM): NaCl, 127.0; KCl, 1.6; KH_2PO_4, 1.24; $CaCl_2$, 2.4; $MgSO_4$, 1.3; $NaHCO_3$, 26.0; and D-glucose, 10. The slices were equilibrated on nets positioned over wells at the interface of humidified O_2 (95%) and CO_2 (5%), and maintained at 32 °C for at least 1 h before physiological recordings. Bipolar stimulating electrodes (200 μm pole separation) were positioned about 500 μm apart, and a single extracellular recording electrode (glass micropipette filled with 0.12 M NaCl) was placed in the middle of CA1 stratum radiatum. Test stimuli (50 μs duration) were delivered at a rate of one per 30 s. The half-maximal responses of the initial slopes of field excitatory postsynaptic potentials (fEPSPs) were monitored for 30 min before the induction of LTP or LTD. The fEPSP slope was defined as the maximal slope in a rise phase of the negative field potential via a computational analysis (Wave-kun Ver. 1.0, developed by Y. Ikegaya), in which two cursors, separated by 1 ms, were placed on the analogue-to-digital converted signals (20 kHz). Agents were delivered by superperfusion with the media containing the desired concentration of drugs. Bovine serum albumin (1 μg/mL) was used as a carrier protein to prevent the loss of BDNF by nonspecific binding to the equipment (e.g. tubing, slice chamber). The albumin alone had no effect on synaptic transmission, or the magnitude ofLTP or LTD (data not shown).

Results

Bath application of 100 ng/mL BDNF did not affect basal CA1 synaptic transmission; the average percentage of fEPSP slopes after 25-min perfusion was 96.1 ± 4.0% of the control average (mean ± SEM of 5 slices, $P < 0.1$, Student's *t*-test).

The initial set of experiments was designed to examine the effect of BDNF on the induction of LTP. Five minutes after application of 100 ng/mL BDNF, slices were tetanized at 100 Hz (50 pulses) or 30 Hz (900 pulses). BDNF was washed out 20 min after the tetani. BDNF-treated slices showed no difference in the magnitude of LTP from control slices when a 'strong' tetanus (100 Hz) was used to elicit a maximal (saturated) level of LTP (Fig. 1A) but displayed significantly greater synaptic potentiation in response to a 'mild' (30 Hz) tetanus (Fig. 1B). These data suggest that BDNF increases the probability of potentiation in response to a suboptimal tetanus but not the maximum attainable level of LTP.

We next investigated the effect of BDNF on the induction of LTD. The magnitude of LTD was greatly reduced when low-frequency stimulation (900 pulses at 1 Hz) was applied in the presence of 100 ng/mL BDNF (Fig. 1D) whereas synaptic depression in response to a 'weak' tetanus (900 pulses at 5 or 10 Hz) was virtually unaffected (see e.g. Fig. 1C). Thus, BDNF can modulate both activity-dependent increases and decreases in synaptic efficacy. Figure 2 A summarizes the BDNF effects on the plastic changes in

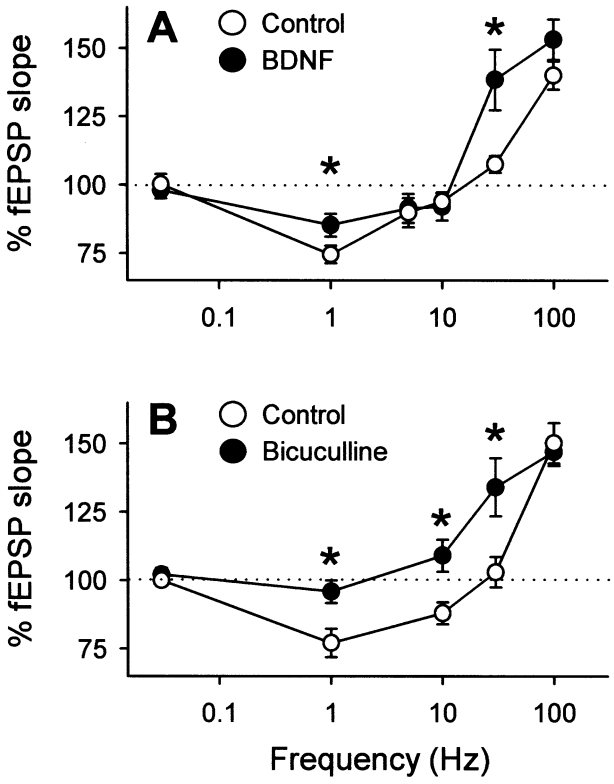

FIG. 2. BDNF does not shift the function θ_m relating stimulation frequency and synaptic plasticity in the hippocampus. (A) Summary data for the effect of 100 ng/mL BDNF on synaptic plasticity at different frequencies (0.33, 1, 5, 10, 30 and 100 Hz). (B) A similar experiment was performed with 3 μM bicuculline. Bicuculline caused a left shift of θ_m. *$P < 0.05$ vs. control, Tukey's test following two-way analysis of variance. Each data point is an average (± SEM of 7–9 slices but of 3 slices for 5 Hz) fEPSP slope 50–60 min after tetanus onset at the indicated frequency.

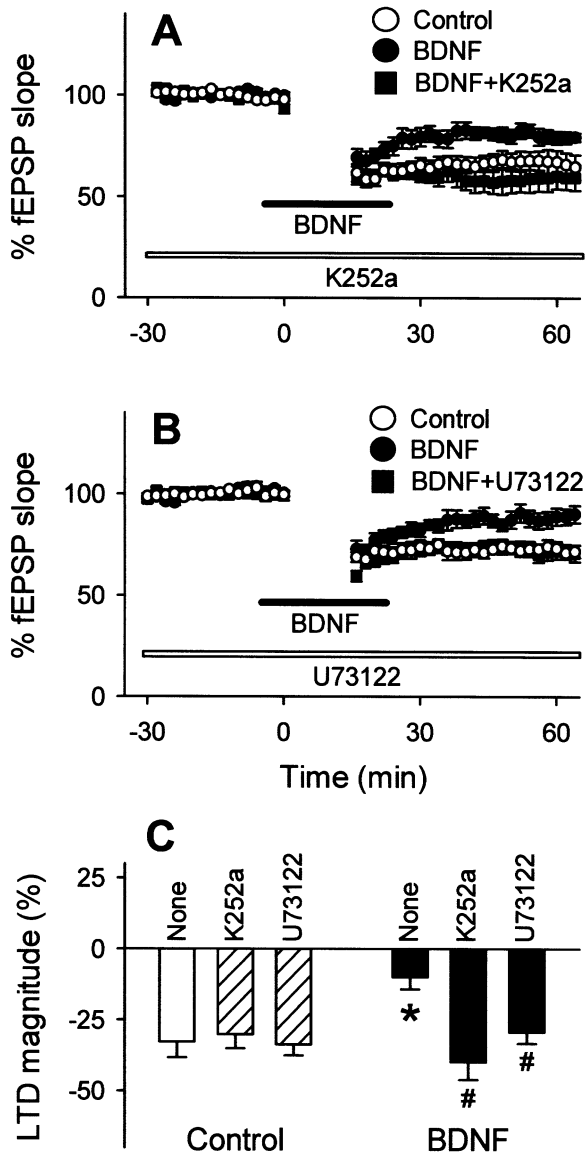

FIG. 3. The suppressive effect of BDNF on LTD depends on activation of Trk receptor and phospholipase C. (A) Low-frequency stimulation (900 pulses at 1 Hz) was applied in the presence and absence of 100 ng/mL BDNF and 200 nM K252a. (B) Low-frequency stimulation was applied in the presence and absence of 100 ng/mL BDNF and 5 μM U73122. (C) Summary data for the effects of K252a and U73122. The ordinate shows average changes in fEPSP slopes 50–60 min after tetanus onset. *$P < 0.05$ vs. none in control slices, $^{\#}P < 0.05$ vs. none in BDNF-treated slices, Tukey's test following one-way analysis of variance. Data represent means ± SEM of each 8–9 slices.

synaptic strength induced by various repetitive stimuli in the 0.33–100 Hz frequency range. BDNF caused no horizontal shift in the synaptic modification threshold θ_m but seems more likely to alter the sensitivity to each tetanus, resulting in an apparent vertical shift in the curve.

We and others have previously shown that BDNF depresses GABAergic transmission in the CA1 region of the hippocampus (Tanaka *et al*., 1997; Frerking *et al*., 1998). To determine whether the BDNF effect is mediated by inhibition of GABAergic transmission, we examined the effect of 3 μM bicuculline, a $GABA_A$ receptor antagonist. In contrast with BDNF, bicuculline treatment produced an obvious left shift in the function relating stimulation frequency and synaptic plasticity (Fig. 2B). Thus, we suggest that GABAergic disinhibition alone cannot account for the BDNF effect.

The suppressive effect of BDNF on LTD was completely prevented by K252a, a general inhibitor of tyrosine kinases, including the Trk receptors (Figs 3A and C), which suggests that TrkB activation mediates the LTD-blocking action of BDNF. Although Trk receptors are known to stimulate diverse signalling pathways including those mediated by PLC, mitogen-associated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) (Kaplan & Stephens, 1994; Segal & Greenberg, 1996), it remains to be seen which signalling pathway is involved in the BDNF modulation of LTD, even in the visual cortex. In this study we found that the blockade of hippocampal LTD was efficiently attenuated by the PLC inhibitor U73122 (Figs 3B and C) but not by its inactive analogue U73343 (data not shown). Thus, it is likely that TrkB activates PLC, thereby attenuating LTD. Neither K252a nor U73122 *per se* affected LTD induction (Fig. 3C), which suggests that endogenous BDNF did not work under our experimental conditions.

Discussion

Although the BDNF modulation of hippocampal synaptic transmission and plasticity has attracted much attention, it remains unclear how BDNF alters the frequency–response profile, mainly because no previous study has elucidated the effect of BDNF on hippocampal

LTD. We have shown for the first time that BDNF prevents hippocampal LTD and that this LTD-blocking action is abolished by pharmacological blockade of PLC activity.

We found no evidence that BDNF causes a left shift in the frequency–response curve. Rather, our data suggest that BDNF modulates LTP and LTD independently and thereby produces an apparent upward shift in the curve. This pseudo-vertical shift was previously suggested by Beggs (2001), who performed computational analyses of a 'bin' model in which long-term synaptic changes arise from a Hebbian rule when statistically unlikely conjunctions of pre- and postsynaptic activity occur. Our study provides the first evidence that a vertical shift actually occurs at hippocampal synapses and that it can be readily induced by neurotrophins. The 'bin' model predicts that an upward shift is produced when a presynaptic spike rarely encounters a postsynaptic spike and therefore conveys more information per spike (Beggs, 2001). Such conditions may also be accompanied by a decrease in postsynaptic activity (Beggs, 2001). It has been recently demonstrated that BDNF excites postsynaptic dendrites directly at perforant path–dentate granule cell synapses (Kovalchuk *et al*., 2002). However, it is possible that BDNF-induced enhancement of the firing activity of postsynaptic neurons does not occur at Schaffer collateral–CA1 synapses (Kanhema *et al*., 2001; Ying *et al*., 2002). Thus, to confirm the hypothesis proposed by the bin model, i.e. whether or not BDNF reduces the synchrony of timing of pre- and postsynaptic firings at CA1 synapses, needs to be determined by further investigation.

It is intriguing to find that PLC activity is required for the LTD-blocking action of BDNF because Gottschalk *et al*. (1999) reported that inhibition of MAPK and PI3K, but not PLC, prevented the BDNF modulation of high-frequency synaptic transmission at hippocampal CA1 synapses. It may be that BDNF only activates the PLC signalling pathway when the afferents are activated at relatively low frequencies; at higher frequencies it may trigger MAPK and PI3K signalling cascades selectively. Although the mechanism by which BDNF-stimulated PLC prevents LTD cannot be deduced from these data alone, our previous study indicated that BDNF-induced PLC activation causes a rapid depression of GABAergic transmission (Tanaka *et al*., 1997). One possibility is therefore that disinhibition of GABAergic influences is involved in the reduction of LTD magnitude. Consistent with this theory, the $GABA_A$ receptor antagonist bicuculline mimicked the BDNF effect on LTD induced by a 1-Hz tetanus. For tetani with higher frequencies, however, the antagonist did not always imitate the BDNF modulation of synaptic plasticity. Therefore, the effects of BDNF cannot all be explained merely by GABAergic disinhibition. These results may also support the proposal that BDNF activates distinct intracellular signalling pathways in a frequency-dependent manner.

In conclusion, we have shown that BDNF/TrkB activation causes a novel type of alteration, i.e. a vertical upward shift, in the frequency-LTP/LTD profile at hippocampal CA1 synapses. The horizontal left shift in the frequency–response curve is adequate to broadly augment the plastic ability of the entire neural network but may be inappropriate to extract a specific synaptic connection from a given network because it would force a synapse that should be depressed, i.e. a synapse with the activity just below θ_m, to be oppositely potentiated, probably resulting in a widespread, relatively nonspecific, reinforcement of neural connections. BDNF can therefore serve as a contrast enhancer by potentiating synaptic strengthening and reducing synaptic weakening processes without affecting θ_m; this, in turn, would precisely extract a specific synaptic connection from active neural networks.

Abbreviations

BDNF, brain-derived neurotrophic factor; fEPSP, field excitatory postsynaptic potential; GABA, γ-aminobutyric acid; LTD, long-term depression; LTP, long-term potentiation; MAPK, mitogen-associated protein kinase; PI3K, phosphatidylinositol 3-kinase; PLC, phospholipase C; Trk, tyrosine eceptor kinase.

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