BDNF pro-peptide actions facilitate hippocampal LTD and are altered by the common BDNF polymorphism Val66Met

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Most growth factors are initially synthesized as precursor proteins and subsequently processed into their mature form by proteolytic cleavage, resulting in simultaneous removal of a pro-peptide. However, compared with that of mature form, the biological role of the pro-peptide is poorly understood. Here, we investigated the biological role of the pro-peptide of brain-derived neurotrophic factor (BDNF) and first showed that the pro-peptide is expressed and secreted in hippocampal tissues and cultures, respectively. Interestingly, we found that the BDNF pro-peptide directly facilitates hippocampal long-term depression (LTD), requiring the activation of GluN2B-containing NMDA receptors and the pan-neurotrophin receptor p75NTR. The BDNF pro-peptide also enhances NMDA-induced α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor endocytosis, a mechanism crucial for LTD expression. Thus, the BDNF pro-peptide is involved in synaptic plasticity that regulates a mechanism responsible for promoting LTD. The well-known BDNF polymorphism valine for methionine at codon 66 in the prodomain of human BDNF (11). In that study, we show that Val66Met mutation affects human memory retention and the activity-dependent secretion of BDNF, indicating that both the prodomain of proBDNF and the BDNF pro-peptide play functional roles. The primary sequence of the BDNF prodomain is conserved among species and differs significantly from that of other neurotrophins, indicating that the BDNF proregion, the pro-peptide, or both may have unique functions. Thus, as is the case for neurotrophines such as β-endorphin (12), the BDNF pro-peptide may serve as a bioactive molecule in neurons.

Recent accumulated evidence demonstrates that BDNF regulates synaptic plasticity and neuronal morphology in the adult brain (5–7).

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that elicits biological effects on synaptic plasticity. BDNF is initially synthesized as precursor proBDNF, and then the pro-peptide is simultaneously produced from the precursor protein. However, the physiological functions of the pro-peptide are largely unknown. Here, we demonstrate that the BDNF pro-peptide is a facilitator of hippocampal long-term depression (LTD), requiring the activation of GluN2B-containing NMDA-type receptors and the pan-neurotrophin receptor p75NTR. Second, a common BDNF polymorphism substitutes valine for methionine at amino acid position 66 (Val66Met) in the pro-peptide of BDNF and impairs memory function. Unexpectedly, the pro-peptide with Met mutation completely inhibits hippocampal LTD. These findings provide insights into the physiological role of the BDNF pro-peptide in the brain.

Significance

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that elicits biological effects on synaptic plasticity. BDNF is initially synthesized as precursor proBDNF, and then the pro-peptide is simultaneously produced from the precursor protein. However, the physiological functions of the pro-peptide are largely unknown. Here, we demonstrate that the BDNF pro-peptide is a facilitator of hippocampal long-term depression (LTD), requiring the activation of GluN2B-containing NMDA-type receptors and the pan-neurotrophin receptor p75NTR. Second, a common BDNF polymorphism substitutes valine for methionine at amino acid position 66 (Val66Met) in the pro-peptide of BDNF and impairs memory function. Unexpectedly, the pro-peptide with Met mutation completely inhibits hippocampal LTD. These findings provide insights into the physiological role of the BDNF pro-peptide in the brain.

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The BDNF pro-peptide is a novel facilitator of hippocampal LTD. The BDNF pro-peptide is a portion of proBDNF (Fig. 1A). Recently, Dieni et al. demonstrated that the BDNF pro-peptide is endogenously detectable in hippocampal tissues and is located at presynaptic sites (8). More recently, Anastasia et al. reported that the expression of the BDNF pro-peptide increases during postnatal development and plateau in adult mice and that it is secreted from neuronal cells in an activity-dependent manner (9). They also showed that the enzymatic removal of N-linked glycan moieties resulted in a reduction of the molecular mass of the endogenous BDNF pro-peptide from 15 to 12 kDa (9). In the present study, we detected the BDNF pro-peptide in hippocampal tissues of 3-wk-old WT mice (Fig. 1B) and in the supernatants of hippocampal neurons cultured for 14 d (Fig. 1C). Consistent with the report of Anastasia et al., the band of the endogenous BDNF pro-peptide was ∼15 kDa (Fig. 1B and C), whereas that of the nonglycosylated recombinant BDNF pro-peptide (rec BDNF pro-peptide) was 12 kDa. Moreover, the concentration of the BDNF pro-peptide was determined using the method described by Anastasia et al. (9). The estimated concentrations of the BDNF pro-peptide in the hippocampal lysate and culture medium were 1.63 ± 0.13 pg/μg total protein (n = 4 mice) and 320.52 ± 44.49 pg/mL (n = 8 independent culture dishes), respectively. These results together suggest that the BDNF pro-peptide functions in postnatal stages and raise the possibility that the BDNF pro-peptide modulates synaptic plasticity in the brain.

To test this hypothesis, we chose a paradigm of LTD, which is modulated by BDNF and proBDNF (15, 20). In practice, we applied a sequence of low-frequency stimulation (LFS; 1 Hz, 900 pulses, 15 min) to Schaffer collaterals of hippocampal slices from juvenile mice and then measured field excitatory postsynaptic potential (fEPSP) slopes in the CA1, as previously described (15). The application of LFS led to robust LTD at the CA3–CA1 synapses (Fig. 1D, Control, and Fig. S1, Control). As reported previously (15, 20), BDNF treatment (10 ng/mL, 30 min) attenuated LTD (Fig. 1D, BDNF, and Fig. S1, BDNF). A statistical analysis revealed that the fEPSP slope values recorded 60 min after LFS stimulation were 80.9 ± 2.0% in the control group and 89.2 ± 2.5% in the BDNF-treated group (Fig. 1G; P < 0.05 relative to the control).

Next, we purified recombinant BDNF pro-peptide (Fig. S2, Val) and examined the effect of the pro-peptide on LTD. A 30-min treatment with the BDNF pro-peptide at a subnanomolar concentration (10 ng/mL; approximately 0.8 nM) significantly enhanced LTD in hippocampal slices (Fig. 1D, BDNF pro-peptide, and Fig. S1, BDNF pro-peptide). The mean fEPSP slope values recorded 60 min after the application of this protocol were 80.9 ± 2.0% in the control group and 70.4 ± 1.4% in BDNF pro-peptide–treated group (Fig. 1G; P < 0.01 relative to the control). Importantly, the application of BDNF pro-peptide (10 ng/mL, 30 min) enhanced LTD in Bdnf−/− hippocampal slices, which lack...
endogenous expression of BDNF (Fig. 1 E and H; control, 80.3 ± 3.0%; BDNF pro-peptide, 68.2 ± 4.0%; P < 0.05 relative to the control), demonstrating that the BDNF pro-peptide is functional in vivo.

We next investigated whether the BDNF pro-peptide affected basal synaptic transmission in the absence of LFS. First, application of the BDNF pro-peptide had no effect on basal synaptic transmission elicited by test-pulse stimulation throughout the recording period, compared with nonapplied control (Fig. 1 F and I; control, 95.5 ± 1.8%; BDNF pro-peptide, 93.1 ± 3.6%; P = 0.9, between control and BDNF pro-peptide–treated groups).

Second, we analyzed input-output (I/O) relationship of the Schaffer collaterals–CA1 pyramidal synapses and did not find significant difference in I/O relationship between before and after a treatment of the BDNF pro-peptide (Fig. S3A, Val-BDNF pro-peptide). Third, we tested a presynaptic form of short-term plasticity, paired-pulse facilitation (PPF). The analysis of PPF indicated that there was no significant change in the PPF ratio between before and after treatment of the BDNF pro-peptide (Fig. S3B, Val-BDNF pro-peptide).

Thus, these electrophysiological data together suggest that the BDNF pro-peptide is a novel facilitator of hippocampal LTD. The Role of p75NTR in the BDNF Pro-Peptide–Enhanced LTD. We next sought to explore the mechanisms underlying BDNF pro-peptide–enhanced LTD. Because KO of the pan-neutrophin receptor p75NTR results in impaired hippocampal LTD (15, 21), we questioned whether p75NTR plays a role in the facilitation of LTD by the BDNF pro-peptide. To address this question, we first investigated whether the BDNF receptor, a reagent that blocks the function of p75NTR, affects pro-peptide–enhanced hippocampal LTD. Similar to previous reports (15, 18), REX (100 μg/mL) inhibited LFS-induced LTD (Fig. 2 A and G; control, 80.9 ± 2.0%; REX, 91.6 ± 2.8%; P < 0.05 relative to the control). We also found that the BDNF pro-peptide failed to facilitate LTD in the presence of REX (Fig. 2 A and G; BDNF pro-peptide, 70.4 ± 1.4%; BDNF pro-peptide+ REX, 93.2 ± 5.6%; P < 0.01 between BDNF pro-peptide and BDNF pro-peptide + REX). This result was supported by another experiment using p75NTR−/− (Ngfr+/−) animals, which lack the gene encoding p75NTR. The treatment of Ngfr+/− hippocampal slices with BDNF pro-peptide did not facilitate LTD (Fig. 2 B and H; control, 83.6 ± 3.0%; BDNF pro-peptide, 84.4 ± 2.0%; P = 0.8 relative to the control). We also examined whether the BDNF pro-peptide required the BDNF receptor TrkB to facilitate hippocampal LTD. To address this, we used a pan-TrkB inhibitor K252a according to a previous report (20) and found that, in the presence of K252a (200 nM), the BDNF pro-peptide–enhanced hippocampal LTD (Fig. 2 C and I; control, 80.9 ± 2.0%; BDNF pro-peptide, 70.4 ± 1.4%; K252a, 87.8 ± 3.1%; BDNF pro-peptide + K252a, 65.9 ± 3.0%; P = 0.48 between BDNF pro-peptide and BDNF pro-peptide + K252a).

We further tested the effect of the BDNF pro-peptide on the activity of TrkB. To this end, we applied BDNF or its pro-peptide (10 ng/mL) to 3-wk cultured hippocampal neurons for 5 min and investigated the TrkB phosphorylation levels by Western blotting with an anti-phospho-TrkB antibody (pY490) as described previously (22). We showed that the BDNF pro-peptide had no apparent effect on TrkB phosphorylation (Fig. S4A; BDNF pro-peptide, p-TrkB and TrkB). However, as a positive control, BDNF treatment increased the phosphorylation levels of TrkB (Fig. S4A; BDNF, p-TrkB and TrkB). Moreover, to investigate the interaction of the BDNF pro-peptide with TrkB receptor, we carried out a BiAcore binding assay on the chip immobilized with recombinant TrkB-IgG fusion protein, corresponding to extracellular domain of TrkB receptor. The resultant data indicated that BDNF bound the extracellular domain of TrkB receptor, whereas the BDNF pro-peptide did not significantly bind (Fig. S4B). Thus, these data together show that the BDNF pro-peptide–enhanced hippocampal LTD is independent of TrkB receptor.

NMDAR Activation Is Required for BDNF Pro-Peptide–Dependent Facilitation of Hippocampal LTD. Ngfr−/− mice exhibit a selective deficit in the NMDAR-dependent form of LTD (15), demonstrating that p75NTR plays a unique role in this form of hippocampal synaptic plasticity. In the present study, we investigated whether the effect of the BDNF pro-peptide depended on the activation of NMDARs, i.e., we explored the mechanistic roles of the NMDAR and GluN2B subunit in BDNF pro-peptide–induced facilitation of hippocampal LTD. To this end, we treated slices by bath application of either a general NMDAR antagonist, δ-2-amino-5-phosphonovaleric acid (APV, 25 μM), or the noncompetitive, selective GluN2B-NMDAR antagonist ifenprodil
BDNF Pro-Peptide Promotes NMDA Stimulation-Induced Internalization of AMPARs, and This Pro-Peptide Effect Is Implicated in the Activation of the p75NTR Receptor. Endocytosis of AMPARs is a crucial mechanism underlying activity-induced synaptic plasticity and LTD (30, 31). In the light of that finding, we next investigated whether the BDNF pro-peptide affects trafficking of a distinct AMPAR subunit, GluA1, in cultured hippocampal neurons (27). Using a procedure that allowed labeling of both surface and internalized GluA1 with different secondary antibodies (29), we found that a 5-min treatment with NMDA (100 μM) led to the internalization of GluA1, i.e., a significant increase was observed for the internalized GluA1 in the dendrites (Fig. 3 D and E; 100 μM NMDA; internalized); the mean ratio of the internalized to total GluA1 in the NMDA-treated group was 1.39 ± 0.02-fold higher than that in controls (Fig. 3 D and E; NMDA; n = 30 cells; P < 0.01 compared with the control). A 30-min pretreatment with the BDNF pro-peptide (10 ng/mL) further increased the NMDA-triggered endocytosis of GluA1 (Fig. 3 D and E; 10 ng/mL BDNF pro-peptide + 100 μM NMDA, arrows in the bottom row); under these conditions, the mean ratio of the internalized to total GluA1 was 1.74 ± 0.02-fold higher in the cells treated with BDNF pro-peptide + NMDA than in that in control cells (Fig. 3 D and E; BDNF pro-peptide + NMDA; n = 30 cells; P < 0.01 compared with the control). Moreover, treatment with the BDNF pro-peptide alone (10 ng/mL, 30 min) promoted endocytosis of GluA1 (Fig. 3 D and E; BDNF pro-peptide; 1.39 ± 0.02; n = 30 cells; P < 0.01 relative to the control), and there was a significant difference in the internalized GluA1 between the group treated with the BDNF pro-peptide alone and the group treated with the pro-peptide + NMDA (P < 0.01).

Regarding the internalization of GluA2, an AMPAR subunit essential for LTD (24), a 5-min treatment with NMDA promoted internalization of GluA2 (Fig. 3 F and G; NMDA; 1.55 ± 0.03; n = 30 cells; P < 0.01 compared with the control). Pretreatment with the BDNF pro-peptide (10 ng/mL, 30 min) significantly increased the NMDA-triggered endocytosis of GluA2 (Fig. 3 F and G; 10 ng/mL BDNF pro-peptide + 100 μM NMDA, arrows in the bottom row). Thus, the mean ratio of the internalized to total GluA2 following the pretreatment was 1.70 ± 0.03-fold higher than that in control cells (Fig. 3 F and G; BDNF pro-peptide + NMDA; n = 30 cells; P < 0.01 compared with the control). In addition, as was observed for GluA1, GluA2 was internalized on stimulation with the pro-peptide alone (Fig. 3 F and G; BDNF pro-peptide, 1.36 ± 0.03; n = 30 cells; P < 0.01 relative to the control), and we observed a significant difference in the internalized levels of GluA2 between cells treated with the BDNF pro-peptide alone and those treated with the pro-peptide plus NMDA (P < 0.01). Thus, the BDNF pro-peptide decreases the surface expression of GluA2 and increases the NMDA-triggered internalization of GluA1 and GluA2, providing a plausible mechanistic explanation for the BDNF pro-peptide–induced facilitation of hippocampal LTD. Moreover, the trafficking of AMPARs in the chemical LTD is controlled additively by the BDNF pro-peptide and NMDA (Fig. 3 B, E, and G).

Because the p75NTR functional blocker REX attenuated BDNF pro-peptide–dependent LTD in hippocampal slices (Fig. 2 A), we next examined the influence of REX on the pro-peptide-induced endocytosis of GluA2. Treatment with the BDNF pro-peptide alone (10 ng/mL, 30 min) promoted endocytosis of GluA2 (Fig. 3 H; BDNF pro-peptide; 1.21 ± 0.03; n = 30 cells; P < 0.01 relative to the control). However, in the presence of REX, treatment with the BDNF pro-peptide did not affect GluA2 endocytosis (Fig. 3 H; REX, 0.99 ± 0.01; BDNF pro-peptide + REX, 0.98 ± 0.02; P = 0.29 relative to the control), demonstrating that the p75NTR receptor is required for the BDNF pro-peptide–dependent dynamics of AMPARs.

The Common BDNF Polymorphism Val66Met Alters the Biological Activity of BDNF Pro-Peptide. To understand the physiological role of the BDNF pro-peptide, we investigated the effect of a
human BDNF polymorphism, Val66Met, on the biological action of the BDNF pro-peptide. To this end, we generated a recombinant pro-peptide containing a Met mutation (Fig. S2, Met) and investigated the effects of the mutant pro-peptide on hippocampal LTD. Notably, unlike the Val-BDNF pro-peptide, pretreatment with the Met-BDNF pro-peptide (10 ng/mL, 30 min) inhibited the LFS-induced hippocampal LTD (Fig. 4B, Met-pro-peptide, Fig. 4B, 60 min after LFS; control, 80.9 ± 2%; Met-BDNF pro-peptide, 97.1 ± 5.9%; P < 0.01 relative to the control; Met-BDNF pro-peptide). We next investigated whether the Met-BDNF pro-peptide affected basal synaptic transmission in the absence of LFS. Similarly to our earlier results (Fig. S3A and B; Val-BDNF pro-peptide), neither the I/O relationship nor PPF ratio showed significant difference between before and after treatment of the Met-BDNF pro-peptide (Fig. S3A and B, Met-BDNF pro-peptide).

Because the Val-BDNF pro-peptide enhanced NMDA-triggered endocytosis of GluA2 (Fig. 3A), we next investigated the effect of the Met-BDNF pro-peptide on this endocytosis. Unexpectedly, however, pretreatment with this mutant pro-peptide did not enhance the NMDA-induced decrease in the surface levels of GluA2. Moreover, the effect of the Met-BDNF pro-peptide was markedly less than that of the NMDA treatment alone (Fig. 4C; NMDA + Met-BDNF pro-peptide; 90.9 ± 0.2%; P < 0.01 relative to the NMDA stimulation), and the effect of the Met-BDNF pro-peptide was diminished by APV (Fig. 4D; ANOVA, P = 0.52). These results suggest that differently from the Val-BDNF pro-peptide, the Met-BDNF pro-peptide does not enhance the NMDA-induced endocytosis of GluA2.

Fig. 3. Role of the BDNF pro-peptide on endocytosis and cell surface expression of AMPA receptors. (A and B) Effect of the BDNF pro-peptide on the NMDA-induced reduction of GluA2 on the cell surface. (A) Representative fluorescence images of surface GluA2. All boxed regions throughout this figure are magnified and shown in the rows below those with the boxes. BDNF pro-peptide promotes NMDA-triggered endocytosis of GluA2. (B) Quantitation of the intensity of surface GluA2. (C) Effect of APV on the NMDA and BDNF pro-peptide-induced reduction of cell surface GluA2. The reduction in surface GluA2 mediated by NMDA and the pro-peptide is completely blocked by APV. ANOVA, P = 0.77. (D) Representative fluorescence images of surface (green) and internalized (red) GluA1. Pretreatment with BDNF pro-peptide increases NMDA-triggered internalization of GluA1 signals (arrows in the bottom row). (E) Quantitation of GluA1 endocytosis. (Scale bars, 10 μm) BDNF pro-peptide induces GluA1 endocytosis as efficiently as NMDA. (F) Representative fluorescence images of surface (green) and internalized (red) GluA2. BDNF pro-peptide enhances NMDA-triggered endocytosis of GluA2 (arrows in bottom row). (G) Quantitative data of GluA1 endocytosis. (Scale bars, 10 μm) BDNF pro-peptide induces endocytosis of GluA2 as efficiently as that of GluA1. **P < 0.01 relative to the NMDA stimulation). (H) Prevention of BDNF pro-peptide-induced endocytosis of GluA2 by REX, a functional inhibitor of p75NTR. To examine the role of p75NTR, cells were treated with or without BDNF pro-peptide (10 ng/mL) in the presence or absence of REX (100 μg/mL) for 30 min and fixed for labeling of GluA2. For B, E, G, and H, **P < 0.01; ANOVA with post hoc tests; n = 30 independent cells from six coverslips, except n = 33 in B.
In our pharmacological study, we showed that the BDNF pro-peptide–induced hippocampal LTD required the activation of GluN2B (Fig. 2 E and K). Previously, it was demonstrated that proBDNF enhances the GluN2B–mediated LTD induction and synaptic currents (15). We thus examined the effects of the Val- and Met-BDNF pro-peptides on the expression levels of surface GluN2B in cultured hippocampal neurons. As a positive control, the Val-BDNF pro-peptide significantly increased the density of GluN2B clusters on the cell surface in a concentration-dependent manner (Fig. 4 E and F; Val-BDNF pro-peptide, 10 ng/mL Val-BDNF pro-peptide, \( P < 0.05; 100 \text{ ng/mL Val-BDNF pro-peptide, } P < 0.01 \text{ relative to the control} \). However, applying the same concentrations of the Met-pro-peptide did not affect the density of GluN2B clusters (Fig. 4 E and F; Met-BDNF pro-peptide). These results provide a mechanistic explanation for the impairment of hippocampal LTD induced by the Met-BDNF pro-peptide.

Discussion

The present report demonstrates novel biological roles of the BDNF pro-peptide beyond that of assisting in the folding of BDNF (10). We found that the pro-peptide was a bioactive molecule that facilitated synaptic plasticity (LTD) by promoting the surface expression of GluN2B and the endocytosis of AMPAR, two crucial mechanisms for LTD expression. We also provided evidence demonstrating that these biological activities were specific to the Val-BDNF pro-peptide.

Within the present study, several lines of evidence indicate that the BDNF pro-peptide is a newly discovered facilitator of hippocampal LTD. Treatment with the BDNF pro-peptide for 30 min facilitated LTD in the hippocampus without affecting basal synaptic transmission. A subnanomolar concentration of the BDNF pro-peptide was sufficient to facilitate hippocampal LTD. Consistent with a previous report (32), LTD occurred normally in Bdnf−/− hippocampal slices, whereas application of the BDNF pro-peptide to the mutant slices facilitated LTD. These results demonstrated that the biological activity of the BDNF pro-peptide itself promoted LTD and that this effect of the pro-peptide was not mediated via an interaction with endogenous BDNF. Moreover, the pro-peptide of NGF, which has low sequence similarity to the BDNF pro-peptide (Fig. S5), did not facilitate LTD. Lastly, we found that the BDNF polymorphism Val66Met, which likely resulted in structural changes to the pro-peptide, led to the inhibition of hippocampal LTD. These results suggest that the biological action of the BDNF pro-peptide is based on its intrinsic structural information.

An important mechanism underlying LTD is the endocytosis and exocytosis of AMPARs at postsynaptic sites (33). Although the role of AMPAR trafficking in synaptic plasticity has been extensively studied (30, 31), the mechanisms underlying the modulation of AMPAR trafficking are not fully understood. In mature hippocampal neurons, AMPARs exist predominantly as complexes containing GluA1/2 or GluA2/3 (26), and the trafficking of GluA2 receptors is crucial for LTD (24, 25). In the present study, we explored the modulatory role of the BDNF pro-peptide during this trafficking by quantitating the AMPAR immunoreactivity in dissociated hippocampal neurons. Our investigation of AMPAR trafficking clarified several roles of the BDNF pro-peptide in this mechanism. First, in line with the electrophysiological data, a 30-min pretreatment with the Val-BDNF pro-peptide decreased the NMDA-triggered surface expression of GluA2 but increased the NMDA-triggered internalization of GluA1 and GluA2. These results provide a mechanistic explanation for the facilitation of hippocampal LTD mediated by the BDNF pro-peptide. Second, as with NMDA, the BDNF pro-peptide activated AMPAR trafficking. Thus, the effects of NMDA and the pro-peptide on AMPAR trafficking may be additive. Similarly, insulin and NMDA induce internalization of AMPARs using independent mechanisms (24). Therefore, both NMDA and growth factor signaling may be molecular mechanisms responsible for the induction/facilitation of LTD. Substantial evidence supports the idea that BDNF inhibits LTD (6) and that BDNF controls the surface expression of AMPARs on the plasma membrane by exocytosis (34). Thus, BDNF and its pro-peptide may exert opposing roles on the synaptic mechanisms underlying LTD and the trafficking of AMPARs.

Because a growing body of clinical evidence indicates that the BDNF Val66Met polymorphism increases susceptibility to a variety of brain disorders (35), we explored the role of this genetic variation in the actions of the BDNF pro-peptide. The Val-BDNF pro-peptide facilitated hippocampal LTD, whereas the Met-BDNF pro-peptide markedly inhibited LFS-induced hippocampal LTD, demonstrating that this genetic variation altered the biological activity of the mutant pro-peptide.

We next examined the molecular mechanisms underlying the Met-BDNF pro-peptide–induced LTD inhibition. Notably, although...
the Val-BDNF pro-peptide enhanced NMDA-induced endocytosis of GluA2, the Met-BDNF pro-peptide attenuated this endocytosis (Fig. 4C). This attenuation was diminished in the presence of APV, an antagonist of the NMDA receptor (Fig. 4D). Thus, the Met-BDNF pro-peptide may inhibit LFS-induced hippocampal LTD by attenuating the neuronal activity-dependent activation of NMDARs. Consequently, the Met-BDNF pro-peptide inhibited NMDA-induced endocytosis of GluA2, and this attenuation was diminished in the presence of the NMDA receptor antagonist APV (Fig. 4D), suggesting that the Met-BDNF pro-peptide modulates the neuronal activity-dependent endocytosis of GluA2. These results offer a plausible explanation for the Met-BDNF pro-peptide–induced impairment of hippocampal LTD.

Our previous study showed that BDNF Val66Met polymorphism affects human memory retention as well as the activity-dependent secretion of BDNF (11). A growing body of clinical evidence indicates that the BDNF Val66Met polymorphism increases susceptibility to a variety of brain disorders (35). Recently, it was reported that mice with the Val66Met mutation are defective in NMDAR-dependent plasticity in the hippocampus (36). Moreover, we showed that APV diminished the surface expression of GluA2 by the Val- and Met-BDNF pro-peptide at similar levels, suggesting that the Val- and Met-BDNF pro-peptide induced GluA2 endocytosis via NMDAR activation (Figs. 3C and 4D). Because this additive effect of NMDA and the Val-BDNF pro-peptide on the endocytosis of GluA2—a crucial mechanism for the expression of LTD—was reversed by this genetic variation, such mechanistic data may help explain how LTD is facilitated and inhibited by the Val- and Met-BDNF pro-peptide, respectively. These results together indicate that the Val66Met genetic variation may affect the regulation of NMDAR functions to ultimately impair NMDAR-dependent synaptic plasticity and provide new insights into the role of this BDNF polymorphism in synaptic plasticity, human brain function, and brain disorders.

Many previous reports have demonstrated the role of p75(−NTR) in synaptic plasticity. First, deletion of the gene encoding p75(−NTR) alters the expression of GluA2 and GluA3 (21). Second, the expression levels of GluN2B decrease in hippocampal tissues of p75(−NTR) KO mice (15). Third, expression levels of p75 and GluN2B, which involve the BDNF pro-peptide (48). In the present study, the concentration of the BDNF pro-peptide was found to be comparable to that reported by Anastasia et al. (48). Moreover, it was previously reported that proBDNF enhanced hippocampal LTD (15), and, in the present study, we showed that the BDNF pro-peptide facilitated LTD. Thus, these findings all together suggest that proBDNF and the pro-peptide both enhance synaptic depression in the postnatal stage of brain.

A recent report indicates that many eukaryotic proteins exist in a disordered form under physiological conditions and fold into ordered structures only upon binding to their cellular targets (42). Experimental evidence for this theory was provided by a recent extensive report that investigated how intrinsically disordered proteins fold on binding to their targets (43). According to the structure prediction software, the BDNF prodomain is disordered (38). Nevertheless, we showed that the BDNF pro-peptide promoted hippocampal LTD, and Anastasia et al. showed that the application of Met-BDNF prodomain induced acute growth cone retraction (9). Thus, the BDNF pro-peptide may exert its biological activity in a manner as reported by Sugase et al. (43). Our findings and recent reports (8, 9) together suggest that the BDNF pro-peptide plays important biological roles beyond its traditional role in assisting the folding of BDNF (10), and, in light of this possibility, we propose a multiligand model in which neurotrophins, via their pro-peptides, exert numerous biological functions in the nervous system.

The present study suggests that the BDNF pro-peptide and BDNF have antagonistic functions on LTD, i.e., facilitation and blockade of LTD induction. Given the antagonistic actions of BDNF and its pro-peptide in hippocampal LTD, how these antagonistic peptides operate in physiological condition is a fundamental question. There were recent noteworthy reports to provide the mechanistic possibility. First, Guo et al. demonstrated the role of neuronal activity on the levels of BDNF-induced TrkB activation: whereas field stimulation with TBS (θ-burst stimulation) converted BDNF-induced TrkB phosphorylation from a transient to a sustained mode, another stimulation protocol used to induce the LTD paradigm did not (44). The understanding of such mechanism of p75(−NTR), which involves the BDNF pro-peptide–dependent facilitation of hippocampal LTD, would be important to solve the question of how two antagonistic peptides (BDNF and its pro-peptide) operate as a whole. Solodyn, activity-dependent secretion of BDNF is an important mechanism of BDNF-dependent synaptic plasticity (5), and it was reported that the BDNF pro-peptide is stored in presynaptic dense-core vesicles in brain neurons (8) and releasable in an activity-dependent manner (9). Given the pro-peptide modulates LTD, the mechanism for the pro-peptide secretion should be clarified in future studies.

The present study may provide new insights in the field of neuroscience, as well as cell biology, for understanding the general physiological roles and modes of action for the pro-peptides of growth factors.

Materials and Methods

Rats and mice were maintained according to the guidelines of the National Institute of Advanced Industrial Science and Technology and the Nara Institute of Science and Technology. All experiments were approved by the Institutional Animal Care and Use Committees of these two organizations. Full methods, including reagents, production of recombinant proteins, SDS/PAGE and immunoblotting analysis, hippocampal slice preparation, electrophysiology, hippocampal cell cultures, quantitative analysis of AMPAR
trafficking and GluN2B on the cell surface, fluorescence microscopy, and statistics are described in SI Materials and Methods.

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