Protein Synthesis-dependent and -independent Regulation of Hippocampal Synapses by Brain-derived Neurotrophic Factor*

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A fundamental difference between short-term and long-term forms of synaptic plasticity is the dependence on transcription and translation of new genes. Using organotypic cultures of hippocampal slices, we have investigated whether the modulation of synapses by brain-derived neurotrophic factor (BDNF) also requires protein synthesis. Long-term treatment of hippocampal slice cultures with BDNF increased the number of docked vesicles, but not that of reserve pool vesicles, at CA1 excitatory synapses. BDNF also increased the levels of the vesicle proteins synaptophysin, synaptobrevin, and synaptotagmin, without affecting the presynaptic membrane proteins syntaxin and SNAP-25, or the vesicle-binding protein synapsin-I. The increase in synaptophysin and synaptobrevin expression was moderate (2-fold) and occurred within 6 h after BDNF application. In contrast, synaptotagmin expression took 24 h to reach maximum levels (5-fold). The delayed increase in synaptotagmin was blocked by protein synthesis inhibitors, while the early increase in synaptophysin and synaptobrevin was not. Moreover, the BDNF-induced increase of synaptotagmin was blocked by inhibiting the cAMP/protein kinase A (PKA) pathway. However, BDNF did not activate PKA, and application of a PKA activator did not mimic the BDNF effect. Taken together, these results suggest a novel, protein synthesis-dependent form of BDNF modulation that requires cAMP gating.

As the cellular basis for learning and memory, two forms of synaptic plasticity have been subjects of intensive study: short-term changes in synaptic strength within minutes and hours, and long-term modulation of the structure and function of synapses over the course of days. In the mammalian hippocampus, a single high-frequency stimulation of afferent fibers elicits an early phase of long-term potentiation (E-LTP) lasting for 2–3 h, while repeated (3–4 times) high-frequency stimulation results in long-lasting, late phase LTP (L-LTP) that lasts for 2–3 h, while repeated (3–4 times) high-frequency stimulation results in long-lasting, late phase LTP (L-LTP) that lasts for 2–3 h, while repeated (3–4 times) high-frequency stimulation results in long-lasting, late phase LTP (L-LTP) that lasts for 2–3 h, while repeated (3–4 times) high-frequency stimulation results in long-lasting, late phase LTP (L-LTP) that lasts as long as the recordings can be maintained (1). Similar short- and long-term forms of synaptic plasticity have been observed in the sea slug Aplysia (2) and the fruit fly Drosophila (3, 4). A single application of the neuromodulator serotonin facilitates synaptic transmission at these synapses for a few hours. In contrast, 4–5 repeated applications of serotonin elicit a long-term facilitation that lasts for days. These forms of short- and long-term facilitation of synaptic transmission are thought to underlie short- and long-term sensitization, a simple form of learning and memory (2). Similarly, brief training of the fruit fly Drosophila results in short-term memory, while repeated, spaced training leads to long-term memory formation (3, 4). A unique feature sets long-term modulation apart from short-term plasticity: its dependence on protein synthesis (1–3). It has been proposed that repeated stimulation leads to a cAMP-dependent, sustained phosphorylation of the transcription factor CREB (cAMP response element binding protein), which in turn triggers the expression of several genes responsible for long-term structural and functional changes at synapses (2, 3, 5). Serotonin has been identified as the neuromodulator that mediates both short- and long-term synaptic facilitation in Aplysia (2). Recent studies suggest that neurotrophins, originally defined as a family of trophic factors for neuronal survival and differentiation, may serve as a new class of neuromodulators that regulate synaptic transmission, synapse development, and plasticity in vertebrates (6). Two major effects have been described at the neuromuscular synapse: (i) acute potentiation of transmitter release; (ii) long-term regulation of synaptic transmission. Acute application of BDNF or neurotrophin-3 (NT-3) rapidly enhances synaptic transmission at the neuromuscular junction (7). The acute effect of neurotrophins is due strictly to an enhancement of transmitter release probability at presynaptic sites (7, 8). Moreover, the protein synthesis inhibitor anisomycin or cycloheximide does not prevent the effects of neurotrophins, suggesting that acute potentiation of synaptic transmission by neurotrophins is completely independent of protein synthesis (8, 9). In the long-term mode, treatment with BDNF or NT-3 (2–3 days) results in a sustained increase in quantal size as well as a more reliable impulse-evoked synaptic transmission (10). Moreover, BDNF or NT-3 enhances the expression of synaptic vesicle proteins such as synaptophysin and synapsin-I, and increases the number of synaptic varicosities in the presynaptic site (10).

Acute neurotrophic modulation of synaptic transmission and plasticity has also been observed in the central nervous system (CNS). For example, application of BDNF or NT-3 to cultured hippocampal or cortical neurons rapidly enhances neuronal activity and synaptic transmission (11–13). Moreover, substantial evidence indicates that BDNF acutely modulates E-LTP in the hippocampus (14–16). Application of exogenous BDNF fa-
cilliates tetanus-induced LTP at the CA1 synapses in neonatal hippocampal slices, in which the endogenous BDNF levels are low (15). In contrast, inhibition of endogenous BDNF activity, either by the BDNF scavenger Trk-B-IgG, or by BDNF gene knockdown, reduces the magnitude of tetanus-induced LTP in adult hippocampus, in which the endogenous BDNF levels are high (14–17). Acute modulation of hippocampal LTP by BDNF may result from the BDNF-induced increase in the synaptic responses to high-frequency stimulation (15, 18). This effect requires activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase, and is insensitive to inhibitors of new protein synthesis (19). Analysis of the hippocampal synapses in BDNF knockout mice reveals three major deficits: (i) a pronounced impairment in the synaptic responses to high frequency stimulation that correlates with a reduction in LTP; (ii) a selective decrease in synaptotagmin and synaptophysin in synaptosomes; and (iii) a marked reduction in the number of docked synaptic vesicles, without affecting the total number of vesicles (20). The defects in LTP and synaptic responses to high frequency stimulation, as well as the reduction in the two vesicle proteins, can be rescued by treatment of the knockout slices with BDNF for a few hours (16, 20). Moreover, application of BDNF to cortical synaptosomes elicits a mitogen-activated protein kinase-dependent phosphorylation of synapsin-I, leading to an increase in availability of synaptic vesicles for release (21). These results suggest that acute modulation of CNS synapses by BDNF is achieved by protein synthesis-independent modifications of existing presynaptic proteins.

Neurotrophins are also involved in long-term modulation of CNS synapses. Substantial evidence suggests their role in the development of ocular dominance columns in cat and rodents in vivo (22, 23). Long-term treatment of slices derived from the visual cortex with neurotrophins elicits profound effects on dendritic growth (24). In dissociated cultures of hippocampal or cortical neurons, chronic application of BDNF results in complex effects on synaptic transmission (25–29). However, the molecular mechanisms underlying long-term modulation of synaptic transmission in the CNS by BDNF are essentially unexplored. Using organotypic slice cultures as a model system, the present study shows that long-term BDNF treatment increases the number of synaptic vesicles docked at active zones of excitatory CA1 synapses. The expression of specific synaptic vesicle proteins synaptophysin, synaptobrevin, and synaptotagmin, hippocampal slices is also enhanced by long-term treatment with BDNF. The delayed increase in synaptotagmin levels is prevented by inhibiting either protein synthesis, while the early increases in synaptophysin and synaptobrevin are not. Moreover, the effect of BDNF on synaptotagmin could be blocked by the inhibitor of cAMP-signaling cascade, but the activation of the cascade does not mimic the BDNF effect. These results suggest that BDNF exerts its actions on hippocampal synapses by two different mechanisms, a rapid protein synthesis-independent pathway, and a delayed cAMP- and protein synthesis-dependent cascade reminiscent of the late phase of LTP.

**EXPERIMENTAL PROCEDURES**

**Hippocampal Slice Cultures—**Hippocampal slices (400 μm thick) were prepared from postnatal day 7 (P7) rats. The slices were cultured on Unlaminated Hydrophilic Fluoropore membranes (Millipore) placed on the inserts of 6-well plates (Fisher) in a 37 °C, 5% CO2, 95% humidity incubator. The slices were maintained for 5 days in horse serum-containing medium (50% minimal essential medium with Earle’s salts, 25% HBSS, 10 mM HEPES, 0.5% GlutaMax II, glucose, pH 7.2, 25% horse serum), then for 3 days in Dulbecco’s modified Eagle’s medium N1 biont-containing medium plus 3% serum, and finally for 1 day in serum-free Dulbecco’s modified Eagle’s medium N1 biont-containing medium. The medium was then replaced with the serum-free medium containing 6 nM BDNF (kindly provided by Regeneron Pharmacuti-

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2 W. J. Tyler and L. Pozzo-Miller, unpublished observations.
(1:10,000), SNAP-25 (1:500), neuron-specific enolase (1:1,000), or the following antibodies from Dr. M. Takahashi synaptotagmin (monoclonal, 1:10,000), or antibody for synaptobrevin (polyclonal, 1:20,000). In most cases, two membranes were used. One membrane was cut to three pieces and reacted with synaptotagmin (65 kDa), synaptophysin (38 kDa), and SNAP-25 (25 kDa) antibodies, while the other membrane was cut to three pieces and reacted with synapsin I (85 kDa), actin (43 kDa), and synaptobrevin (18 kDa) antibodies. The membranes were rinsed with washing buffer (0.1% Tween 20, 0.2 mM Tris, 137 mM NaCl) and incubated with secondary antibody (1:10,000, monoclonal, 1:10,000), or antibody for synaptobrevin (monoclonal from Dr. Takahashi). After incubation 5-μl aliquots were spotted onto P81 Whatman filter papers and the filters were washed 5 × 20 min in 75 mM phosphoric acid. The samples were air-dried and the radioactivity counted. A standard curve using different amounts of recombinant PKA was constructed and the PKA activities in slices treated with or without forskolin or BDNF were calculated using the standard curve.

RESULTS

BDNF Increases the Number of Docked Synaptic Vesicles—Organotypic hippocampal slice cultures prepared from P7 rats were used to determine the structural changes at the hippocampal synapses induced by long-term treatment with BDNF. After 9 days in vitro, the hippocampal slice cultures were treated with 6 nM BDNF for 48 h, before harvesting for quantitative electron microscopy (EM) analysis. The slices were fixed, stained, and embedded. The Epon blocks were trimmed, and the CA1 region identified. Excitatory synapses on CA1 dendritic spines were identified by the following criteria: (i) presynaptic profiles containing small (~50 nm diameter), round and clear synaptic vesicles; (ii) prominent electron-dense postsynaptic densities, characteristic of type-I asymmetric synaptic junctions; and (iii) postsynaptic dendritic spines, identified by their lack of microtubules and mitochondria, continuity with a dendritic shaft, and overall size and shape. Synaptic junctions fulfilling the above criteria are well established to represent glutamatergic excitatory synapses (32). To avoid the bias of selecting the larger synapses during sampling in single thin sections, only synapses having approximately equal area of presynaptic terminals and length of active zones were chosen for analysis. From three independent pairs of slices, we analyzed a total of 84 synapses (control n = 43; BDNF n = 41) that fitted the above criteria. On average, the terminal areas were 0.229 ± 0.009 μm² in control and 0.233 ± 0.009 μm² in BDNF-treated slices, and the active zone lengths (L) were 0.251 ± 0.006 μm in control and 0.264 ± 0.006 μm in BDNF-treated slices.

Small, round and clear synaptic vesicles were clearly ob-
served in the CA1 spine synapses. The distribution of synaptic vesicles was measured in two distinct and mutually exclusive pools within presynaptic terminals at CA1 excitatory synapses. Docked vesicles are defined as those in close apposition to, or within 50 nm from the presynaptic active zone (33). Reserve pool vesicles are identified as those synaptic vesicles within the presynaptic terminals, but outside of the docked vesicle region (20). Long-term BDNF treatment significantly increased the number of docked vesicles per unit length of active zone at these excitatory spine synapses in CA1 stratum radiatum (Fig. 1A). The number of docked vesicles per micron active zone (DV/μm) increased from 14.7 ± 0.7 in control, to 17.3 ± 0.8 after BDNF treatment (p = 0.0139; Fig. 1B). In contrast, there were no differences in the number of reserve pool vesicles per unit terminal area between control (304.4 ± 7.3 reserve vesicle/μm² of presynaptic terminal area) and BDNF-treated slices (311.9 ± 9.2 reverse vesicle/μm²; p = 0.5269; Fig. 1B). From the length of the active zone (L) and the number of docked vesicles counted in the two-dimensional electron micrographs, we estimated the total number of docked vesicles per active zone (TDV = 0.907 × (DV/μm²) × L² (20). TDV was 14.1 in control and 21.0 in BDNF-treated slices, a 50% increase. These results suggest that BDNF selectively increases the number of docked vesicles without affecting the reserve pool vesicles at excitatory CA3-CA1 synapses in hippocampal slices.

**BDNF Differentially Regulates the Expression Levels of Specific Synaptic Vesicle Proteins**—To determine whether long-term treatment with BDNF also elicits biochemical changes in the hippocampal synapses, we performed Western blot analysis. After 9 days in vitro, the hippocampal slice cultures were treated with 6 nM BDNF for 48 h. In each experiment, samples from the same experimental conditions were run in triplicate (n = 3), and multiple proteins were simultaneously measured on the same blots. The same experiment was repeated 5–10 times (N = 5–10), using samples from independent culture preparations. As shown in Fig. 2, long-term (48 h) treatment with BDNF elicited a significant increase in the levels of several synaptic vesicle proteins. When all data from BDNF-treated group were normalized to those from control group, we found that levels of synaptophysin and synaptobrevin, two integral membrane proteins of the synaptic vesicle membrane, increased by 2-fold in slices treated with BDNF (Fig. 2B). A 5-fold increase was observed in the level of synaptotagmin, a vesicle membrane protein proposed to be the Ca²⁺ sensor for vesicle fusion (Fig. 2B) (34). The levels of the cytoskeletal protein actin and of the neuron-specific marker neuron-specific enolase did not show any significant changes after BDNF treatment (Fig. 2B), suggesting that there was no general increase in cellular proteins.

The increase in synaptotagmin, synaptobrevin, and synaptophysin were relatively specific. The levels of syntaxin and SNAP-25, two proteins located on presynaptic membrane, did not change after BDNF treatment (Fig. 2B), suggesting that the total number of synapses was not significantly altered. Synapsin-I, a non-integral membrane protein tightly associated with synaptic vesicles, remained unchanged in BDNF-treated slices (Fig. 2B). Furthermore, the increase in synaptotagmin was far more pronounced (5-fold) than that of synaptobrevin and synaptophysin (2-fold). These results are consistent with the idea that BDNF differentially increases the amount of certain proteins per vesicle rather than elevating the total number of synaptic vesicles per synapse (see below).

The effects of BDNF on synaptic protein expression were further confirmed by immunocytochemistry, using antibodies against aforementioned synaptic proteins. Control and BDNF-treated slices from sister cultures were processed side by side for immunofluorescence staining, and images were acquired by a confocal microscope using exactly the same conditions. As shown in Fig. 3, A and B, the BDNF-treated slices exhibited brighter immunofluorescence for synaptobrevin and synaptophysin, as compared with untreated controls. The increase in the immunofluorescence appears to be widespread in the hippocampus, rather than limited to the CA1 area. Although the immunofluorescence studies were not quantitative, it was quite clear that the BDNF-induced increase in synaptotagmin expression was much more pronounced (Fig. 4), as compared with that in synaptobrevin and synaptophysin expression (Fig. 3). The increase in synaptotagmin was observed throughout hippocampus, but most obviously in CA1 and dentate areas (Fig. 4).

To reveal the kinetics of the long-term BDNF effects, we examined the time course of the changes in various synaptic proteins by treating the slice cultures with or without BDNF at different time points (6, 12, 24, 48, and 72 h) before harvesting. This procedure allowed all slices to be cultured for the same length of time while being exposed to BDNF for different durations. The expression levels of synaptophysin and synaptobrevin increased within 6 h of BDNF treatment, an effect that lasted for at least 72 h (Fig. 5). In contrast, the increase in...
synaptotagmin occurred more slowly, reaching its maximum levels by 48 h (Fig. 5). The difference in the kinetics of these protein changes suggests that the mechanism by which BDNF regulates synaptotagmin may be different from that used to modulate synaptobrevin and synaptophysin.

**BDNF Modulation of Synaptotagmin Requires Protein Synthesis and cAMP**—Several forms of long-term synaptic plasticity require new protein synthesis (1–3). To determine whether the increase in synaptic protein levels after long-term BDNF treatment also depends on new protein synthesis, we investigated the effect of the protein synthesis inhibitor anisomycin on the BDNF-induced increase in synaptic proteins. Cultures were treated with BDNF, anisomycin, or a combination of BDNF and anisomycin for 12 h, since the increase in synaptotagmin was evident during this period (Fig. 5). Anisomycin (20/μM) was added 30 min prior to BDNF treatment. Pretreatment with anisomycin blocked the BDNF-induced increase in synaptotagmin expression (Fig. 6A). Similar results were obtained when another protein synthesis inhibitor cycloheximide was used (data not shown). We also examined the effect of anisomycin on BDNF modulation of synaptophysin and synaptobrevin. In most cases, we determined the levels of synaptophysin and synaptobrevin after 6–12 h of BDNF/anisomycin treatment because the maximal effect of BDNF was already achieved during this short period (Fig. 5). Surprisingly, the effects of BDNF on the levels of synaptophysin or synaptobrevin were not altered by the pretreatment with anisomycin (Fig. 6B). Treatment with anisomycin alone for the same duration of time did not decrease the levels of any of three synaptic proteins measured, implying a slow turnover of these proteins (Fig. 6, A and B). Furthermore, the levels of actin and neuron-specific enolase were not significantly affected by the anisomycin treatment, suggesting that anisomycin treatment for 6–12 h was not overtly toxic (data not shown).

Protein synthesis-dependent long-term synaptic plasticity often involves a cAMP/PKA-dependent signaling pathway (2, 3, 5). Therefore, we tested whether the BDNF-induced increase in synaptic proteins requires activation of this pathway, using a potent inhibitor for the cAMP/PKA pathway, Rp-cAMPs (100 μM). Cultures were treated with BDNF, Rp-cAMPs alone, or a combination of BDNF and Rp-cAMPs for 12 h. Treatment with Rp-cAMPs alone had no effect on any of the proteins examined (Fig. 7, A and B). Treatment with BDNF markedly increased the levels of synaptotagmin, but this effect was specifically blocked by Rp-cAMPs (Fig. 6B). In contrast, the increase of synaptophysin (Fig. 7B) or synaptobrevin (data not shown) was not affected by Rp-cAMPs. Neither BDNF, Rp-cAMPs, nor the two together had any effect on the levels of Syntaxin (Fig. 7B) or actin (data not shown). Thus, the effect of BDNF on synaptotagmin involves activation of a cAMP/PKA pathway, making it distinct from the effects of BDNF on synaptophysin and synaptobrevin.

The BDNF-induced increase in synaptotagmin expression could be mediated by the cAMP/PKA pathway. Alternatively, cAMP could serve as a "gate" that allows BDNF to achieve this effect (35–37). If the effect of BDNF were mediated by cAMP, one would predict that: 1) cAMP/PKA pathway should be activated by BDNF; and 2) cAMP analogues should mimic the BDNF effect. However, BDNF was unable to activate PKA in the cultured hippocampal slices. PKA activity did not increase,
but slightly decreased, upon application of BDNF (Fig. 7C). Moreover, enhancement of endogenous PKA activity by Sp-cAMPs (100 μM), a potent activator of PKA, did not alter the expression of synaptotagmin, synaptophysin, or synaptobrevin in the hippocampal slices. The levels of these proteins in slice cultures treated with Sp-cAMPs for 12 (not shown) or 24 (Fig. 7D) h were the same as those in control cultures. These results, together with the finding that the BDNF-induced increase in synaptotagmin expression could be blocked by Rp-cAMPs, strongly suggest that cAMP is not a downstream effector in the signaling cascade activated by BDNF, but instead acts in a permissive capacity for the BDNF effect.

DISCUSSION

While significant progress has been made in studying the acute effects of neurotrophins, the molecular mechanisms underlying long-term modulation of synaptic transmission in the CNS by neurotrophins are much less well understood. In dissociated cultures, long-term treatment with BDNF increases the AMPA-receptor-mediated synaptic currents in hippocampal neurons (29), but inhibits the increase of AMPA currents induced by activity blockade in cortical neurons (27). Moreover, chronic application of BDNF or NT-3 may or may not increase the number of synaptic connections depending on the age of embryonic brain used for cultures (26, 28). These discrepancies may be due largely to the heterogeneity of the culture systems, which lack the appropriate afferent and efferent synaptic connections observed in vivo. To avoid problems associated with dissociated cultures, we investigated the molecular mechanism(s) mediating long-term BDNF modulation of hippocampal synapses using organotypic slice cultures, which maintain a pattern of synaptic connectivity quite similar to that of the hippocampal formation in vivo. We have made two interesting observations. First, we found that BDNF exerts a long-term modulatory effect on the expression levels of several proteins on synaptic vesicles, as well as changes in the distribution of synaptic vesicles within presynaptic terminals at excitatory spine synapses. Second, we showed that the BDNF-induced increase in synaptotagmin is mediated through a protein synthesis and cAMP-dependent mechanism, an effect reminiscent of long-term facilitation in Aplysia sensory-motor synapses, and of L-LTP. Thus, long-term modulation by BDNF may share mechanisms similar to other types of long-term synaptic modifications.

In the brain of awake, living animals, acute and long-term actions of BDNF on synapses may have different physiological consequences. Acute application of neurotrophins to neuromuscular or central synapses has been shown to cause an instant but transient modulation of the efficacy of synaptic transmission (7, 8, 11–13). These short-term effects are mediated by rapid changes in intracellular Ca2+ and protein phosphorylation, rather than new protein synthesis (8, 9, 38, 39). It is conceivable that activity dependent secretion of neurotrophins may play an important role in this type of modulation. On the other hand, BDNF mRNA and protein are highly expressed in the brain, particularly in the hippocampus (40, 41), suggesting that CNS synapses are constantly exposed to BDNF under physiological conditions in vivo. Thus, BDNF may play a long-
term modulatory role in the development and/or function of hippocampal synapses under physiological conditions, in addition to its acute effects on synaptic transmission and plasticity. It has been shown that repeated high-frequency afferent stimulation elicits a marked increase in BDNF mRNA in the hippocampus (42). Time course studies indicate that the activity-dependent increase in BDNF gene expression occurs within 3–5 h, correlating very well with the occurrence of protein synthesis and cAMP-dependent L-LTP. BDNF knockout mice exhibit impairments in L-LTP, in addition to defects in E-LTP (43). Application of TrkB-IgG, a scavenger for BDNF and NT-4, 30 min after tetanic stimulation to hippocampal slice cultures reverses previously established LTP (17). All these studies raise the possibility that activity-dependent expression of BDNF plays a role in L-LTP. The mechanisms by which BDNF modulates hippocampal L-LTP are unknown. Long-term treatment of cortical slices with BDNF regulates dendritic growth of cortical pyramidal neurons (24). We demonstrated here that chronic exposure of hippocampal slices to BDNF increases the number of vesicles docked at active zones of CA1 synapses. Furthermore, BDNF increases the expression of synaptotagmin in a protein synthesis and cAMP-dependent manner. It is tempting to speculate that these changes contribute to the BDNF modulation of L-LTP.

Takei et al. (25) reported that treatment of newly dissociated cultures of cortical neurons with BDNF in serum-containing conditions for 5 days appears to increase all synaptic proteins, suggesting an increase in synapse number. The present paper has made a number of conceptual and technical advances. First, we studied the long-term effects of BDNF on hippocampal synapses in slices, which resemble more closely the hippocampus in vivo in terms of synaptic circuits. Second, our experiments were done in better controlled conditions. We examined the effects of BDNF in serum-free conditions, avoiding the potential interactions between BDNF and other unknown serum factors. Our slices were derived from postnatal day 7 (P7) hippocampus, right around the time of synapse formation. We also treated slices for much shorter periods (maximum 2 days). These measures made it less likely that we were looking at the survival effect of BDNF. Third, we showed that BDNF selectively enhances the expression of synaptophysin, synaptobrevin, and synaptotagmin, without affecting other synaptic proteins. Thus, we demonstrated specific changes in the properties of synapses, rather than general increase in synapse number. Finally and most importantly, our study revealed mechanistic differences between the short-term and long-term synaptic effects of BDNF: dependence on protein synthesis and cAMP. We believe that this represents a novel and significant conceptual advance.

We have previously shown a selective reduction in the amount of synaptophysin and synaptobrevin in hippocampal synaptosomes prepared from BDNF knockout mice (20). Moreover, the reduction in the levels of synaptobrevin and synaptophysin was reversed after incubation with BDNF for a few hours, suggesting an acute, rather than a long-term effect of BDNF. Using hippocampal slice cultures, we now studied acute as well as long-term effects of BDNF on the expression of synaptic proteins in the hippocampus. We found that the levels of synaptobrevin and synaptophysin increase within a few hours after BDNF application, and this increase is sustained as long as BDNF is present in the cultures. Interestingly, the increase in synaptobrevin and synaptophysin cannot be blocked by the protein synthesis inhibitor anisomycin. We do not know how the increase in synaptobrevin and synaptophysin could occur without protein synthesis, but it is possible that BDNF could alter the processing, post-translational modification, or redistribution between pools of proteins, leading to a better detection by the antibodies (44). Further experiments are required to distinguish these possibilities. The most interesting finding in the present study is the BDNF-induced increase in synaptotagmin, a multifunctional protein on the synaptic vesicles (34). We demonstrated here that long-term treatment of cultured hippocampal slices with BDNF elicits an increase in synaptotagmin in ways quite distinct from the BDNF-induced increase in synaptobrevin and synaptophysin. First, the increase in synaptotagmin requires a considerably longer time of BDNF exposure (12–24 h), and is much more pronounced. Second, this increase is dependent on protein synthesis, and on a cAMP-signaling pathway. The present results, together with other studies discussed above, suggest that BDNF has two modes of actions on hippocampal synapses: an acute effect due at least in part to rapid protein phosphorylation, and a long-term effect mediated by a protein synthesis and cAMP-dependent mechanism.

There are two potential mechanisms that cAMP pathway could be involved in neurotrophin signaling. One is that a particular neurotrophin activates the cAMP/PKA pathway, which in turn mediates its neurotrophic effects. BDNF does not seem to activate the cAMP/PKA pathway in a number of systems tested (45–47). In the present study, we found that BDNF does not activate PKA in hippocampal neurons in slice cultures. The second mechanism is the “cAMP gating.” This mechanism was first proposed to explain the regulatory role of cAMP...
showing that the effect of BDNF on synaptotagmin, but not synaptophysin, is blocked by 
standard curved generated using the recombinant catalytic subunit of PKA.

logically docked vesicles are reduced in the synaptotagmin
and recycling of synaptic vesicles (56, 57). Moreover, morpho-
vesicle fusion (34). It has also been implicated in endocytosis
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aptobrevin and synaptotagmin.

Whether and how changes in synaptic vesicle proteins con-
tribute to the BDNF modulation of vesicle docking are inter-
esting questions for further investigation. Synaptophysin is
tightly associated with synaptobrevin (49, 50), but there is
little evidence for its involvement in vesicle docking. Rather,
recent studies in the squid giant synapse have implicated synap-
tophysin in rapid clathrin-independent vesicle endocytosis at
the active zone (51). The role of synaptobrevin in vesicle docking
has been controversial. Biochemical experiments indicate
that the v-SNARE synaptobrevin binds to the t-SNARE pro-
teins syntaxin and SNAP25 to form the SNARE core complex
(20 S), suggesting that synaptobrevin is a required component
for vesicle docking (52, 53). However, cleavage of synaptobrevin
in squid giant synapses in Drosophila neuromuscular junction
by tetanus toxin caused a slight increase, rather than a de-
crease, in the numbers of docked vesicles due to an accumu-
lation of vesicles after fusion is blocked by the toxin (54, 55).
Synaptotagmin is a Ca^2+ sensor known to play a key role in
vesicle docking (34). It has also been implicated in endocytosis
and recycling of synaptic vesicles (56, 57). Moreover, morpho-
logically docked vesicles are reduced in the synaptotagmin
mutant of Drosophila (58). A recent study suggests the involve-
ment of C terminus of synaptotagmin in vesicle docking (59). In
the present study, we demonstrated that long-term exposure of
hippocampal slices to BDNF increases the number of vesicles
docked at the active zone, as well as the level of synaptotagmin.
These experiments support the notion that synaptotagmin
plays a role in vesicle docking. It is also possible that both
synaptobrevin and synaptotagmin participate in vesicle dock-
ing, but the former is involved in the initiation of docking
complex, while the later may be required for maintenance or
stabilization of vesicles docked at the active zone.

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