Gating of BDNF-Induced Synaptic Potentiation by cAMP

Lisa Boulanger and Mu-ming Poo*

Neurotrophins have been implicated in activity-dependent synaptic plasticity, but the underlying intracellular mechanisms remain largely unknown. Synaptic potentiation induced by brain-derived neurotrophic factor (BDNF), but not neurotrophin 3, was prevented by blockers of adenosine 3′,5′-monophosphate (cAMP) signaling. Activators of cAMP signaling alone were ineffective in modifying synaptic efficacy but greatly enhanced the potentiation effect of BDNF. Blocking cAMP signaling abolished the facilitation of BDNF-induced potentiation by presynaptic activity. Thus synaptic actions of BDNF are gated by cAMP. Activity and other coincident signals that modulate cAMP concentrations may specify the action of secreted neurotrophins on developing nerve terminals.

Neurotrophins, a family of proteins related to nerve growth factor, are best known for their role in regulating the differentiation and survival of specific populations of developing neurons (*1*). Neurotrophins can also regulate the morphology of nerve processes (*2, 3*) and the efficacy of synaptic transmission (*4–6*). Electrical activity can regulate the synthesis of neurotrophins (*7*) and trigger their secretion (*8*). In the developing visual system, secreted neurotrophins play a critical role in the activity-dependent refinement of thalamocortical projections (*9*). Activity-induced long-term potentiation (LTP) in the hippocampus is reduced by depleted endogenous BDNF (*10*) and is impaired in BDNF knockout mice, a deficit that is rescued by exogenous BDNF (*11*). Thus, neurotrophins have been proposed to be mediators of activity-dependent synaptic plasticity (*12*). For a neurotrophin to mediate synaptic changes at active synapses, it might be secreted locally only at the active synapses. Alternatively, electrical activity itself may confer a synapse-specific restriction to neurotrophin action. Activity can modulate neurotrophin effects on neuronal survival (*13*), dendritic morphology (*14*), and synaptic efficacy (*15, 16*) in different systems, but the underlying cellular mechanisms are largely unknown. In this study, we found that BDNF-induced synaptic potentiation at developing neuromuscular synapses can be regulated by cAMP, and that such regulation is required for activity-dependent modulation of the synaptic action of BDNF.

Application of a high dose (50 ng/ml) of BDNF to developing *Xenopus* neuromuscular synapses in culture (*17, 18*) resulted in an increase in the amplitude of evoked excitatory postsynaptic currents (EPCs) and in the frequency of miniature excitatory postsynaptic currents (MEPCs) (Fig. 1) (*4, 5*). No change in the amplitude of MEPCs was observed (*4, 5*), which suggests a presynaptic action of BDNF on transmitter secretion. Exposure of the synapse to Rp-cAMPS (adenosine 3′,5′- monophosphothioate, 8-bromo-, rp-isomer; 20 μM), a nonhydrolyzable competitive antagonist of cAMP for binding to protein kinase A (PKA), had no significant effect on the basal level of spontaneous or evoked synaptic currents (Fig. 1, B and C). However, treatment with Rp-cAMPS abolished the potentiating effects of BDNF (50 ng/ml) on EPCs and MEPCs (Fig. 1). The BDNF-induced increase in MEPC frequency was similarly abolished by KT5720 (200 μM), a specific inhibitor of PKA, and by ACPD [(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (50 μM)], a specific metabotropic glutamate receptor agonist known to reduce cAMP concentrations in some neurons (Fig. 1C). Because inhibitors of cAMP signaling alone had no effect on the synaptic currents (Fig. 1, B and C), they did not act directly on presynaptic transmitter secretion or on postsynaptic sensitivity to transmitters.

Further studies showed that cAMP not only acts in a permissive capacity but also modulates the potency of BDNF action at the synapse. Treatments that enhance cAMP signaling caused an increase in spontaneous and evoked synaptic currents upon exposure to a low dose (10 ng/ml) of BDNF (Fig. 2), whereas exposure to such a low dose by itself had no effect on synaptic activity. In the presence of forskolin, an activator of adenyl-

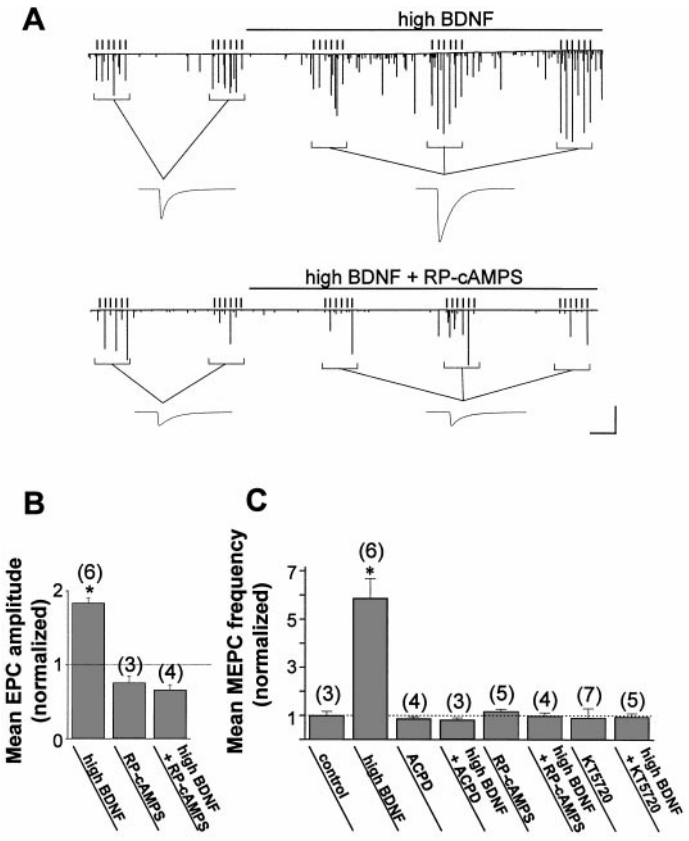


Fig. 1. Inhibition of endogenous cAMP-dependent activity blocks BDNF-induced synaptic potentiation. Synaptic currents at developing *Xenopus* neuromuscular junctions were recorded from the postsynaptic myocyte and BDNF was applied to the bath after a 10-min control recording. (**A**) Membrane currents recorded in voltage clamp ($V_c = -70$ mV). Downward deflections are inward currents representing either MEPCs or EPCs (EPCs evoked by extracellular stimulation of the presynaptic neuronal soma at times marked by vertical lines). (Top) Synapse exposed to BDNF (50 ng/ml) alone. (Bottom) Synapse exposed to BDNF (50 ng/ml) and 20 μM Rp-cAMPS. Averages of all EPCs before and after treatment are shown below at a higher time resolution. Scales: slow traces, 1 nA, 125 s; fast traces, 1 nA, 15 ms. (**B** and **C**) Effects of various treatments on EPCs and MEPCs. Bars represent normalized values (mean ± SEM) for 10 to 50 min after treatments, which include control medium; high BDNF (50 ng/ml); 50 μM ACPD, 20 μM Rp-cAMPs, or 200 nM KT5720 with or without BDNF (50 ng/ml). Significant difference from corresponding controls is indicated by an asterisk ($P < 0.05$; Student's t test). Number of synapses examined is shown in parentheses.

Department of Biology, University of California at San Diego, La Jolla, CA 92093–0357, USA.

*To whom correspondence should be addressed. E-mail: mpoo@ucsd.edu

ate cyclase, this low dose of BDNF produced a marked increase in the amplitude of EPCs and in the frequency of MEPCs (Fig. 2, A to D). Forskolin alone had no effect. Facilitating effects on the synaptic action of BDNF were also found for Sp-cAMPS (adenosine 3′,5′- monophosphorothioate, 8-Br-, sp-isomer; 20 μM), a membrane-permeable activator of PKA, and for okadaic acid (0.5 nM), a potent activator of cAMP-dependent signaling that acts by inhibiting protein phosphatase I, whereas okadaic acid or Sp-cAMPS alone had no effect (Fig. 2C). Taken together, these results indicate that cAMP is not a downstream effector in the signal transduction cascade activated by BDNF, but it appears to facilitate either BDNF signaling or the action of its effectors on the secretion machinery. Furthermore, cAMP-dependent processes themselves do not exert acute effects directly on the basal synaptic transmission in this system.

At these *Xenopus* synapses, treatment with neurotrophin 3 (NT-3) also results in potentiation of spontaneous and evoked transmitter release (*4*). The actions of BDNF and NT-3 are initiated by different membrane receptors (TrkB and TrkC, respectively), which are likely to trigger different cytoplasmic signal pathways (*19*). Potentiation of MEPC frequency by NT-3 was not affected by either inhibiting or activating the cAMP pathway (Fig. 2E), which suggests that effectors in the NT-3 transduction cascade were insensitive to the amount of cAMP. A similar situation has been found for the effect of cAMP on the growth cone turning responses induced by neurotrophins in *Xenopus* spinal neurons (*20*).

We have shown that presynaptic depolarization at these *Xenopus* neuromuscular synapses facilitates the synaptic potentiation induced by BDNF (*15*). A low dose of BDNF (10 ng/ml) or presynaptic depolarization (15 pulses, 2 Hz) by itself does not have any effect on synaptic efficacy. However, when presynaptic activity is induced in the presence of the same low dose of BDNF, marked potentiation of both spontaneous and evoked transmitter secretion is observed (*15*) (see also Fig. 3). Because neuronal activity can elevate cytoplasmic cAMP concentrations (*21, 22*) by increasing the activity of calmodulin-dependent and voltage-sensitive adenylate cyclases, the facilitating effect of presynaptic depolarization could be mediated by cAMP. We thus examined the consequence of manipulating cAMP concentrations on the facilitating effect of depolarization on BDNF-induced potentiation. As shown in Fig. 3, in the presence of Rp-cAMPS (20 μM), the facilitating effect of presynaptic stimulation was completely abolished. These results demonstrate that synaptic potentiation by BDNF is regulated by cAMP. Whether this mechanism also regulates neurotrophin-induced synaptic changes in other systems remains to be examined.

cAMP has been implicated in several forms of synaptic plasticity, including synaptic facilitation in *Aplysia* and LTP (*23*). In addition, quantities of available BDNF receptor (*24*) and the effects of chronic neurotrophin exposure on neuronal survival (*13*) can be modulated by changes in cAMP or by chronic K^+-induced depolarization. It has been shown that, during induction of LTP, cAMP acts in a purely regulatory role, a mechanism referred to as gating (*25*). Gating by cAMP was also found for transformation of fibroblasts by components of the growth factor signaling pathway (*26*) and for morphogenic patterning by sonic hedgehog (*27*).

The amount of cytosolic cAMP can be modulated by many cellular processes. Depolarization and synaptic activity are known to elevate cAMP (*21*), possibly through Ca^{2+}-dependent adenylate cyclases. In addition, a voltage-sensitive, Ca^{2+}-independent adenylate cyclase has recently been described (*22*). Depolarization can also trigger the secretion of neuromodulators that affect cAMP (*28*). In spinal motor neurons, acetylcholine secretion is accompanied by co-release of calcitonin-gene-related peptide, which can stimulate accumulation of cAMP in cultured muscle cells (*29*). BDNF itself can also cause rapid increases in cAMP in some systems (*30*), probably by Ca^{2+}-calmodulin–activated adenylate cyclases. These *Xenopus* spinal neurons may undergo developmental changes in cAMP, as suggested by altered growth responses to neurotrophins (*3, 31*). Thus developmental state as well as other transient cellular signaling events may lead to selective potentiation of synaptic function by BDNF.

The cAMP-dependent regulation of neurotrophin signaling described here is bidirectional: synaptic responses to neurotrophin can be either increased or decreased, depending on the direction of change in the cAMP. Such regulatory control by cAMP provides a flexible signaling pathway leading to synaptic potentiation. The precise step or steps in the BDNF-induced cascade gated by cAMP remains unclear. cAMP can induce a variety of cellular processes in different systems, including expression of mRNA for Trk receptors and neurotrophins (*32*), incorporation of

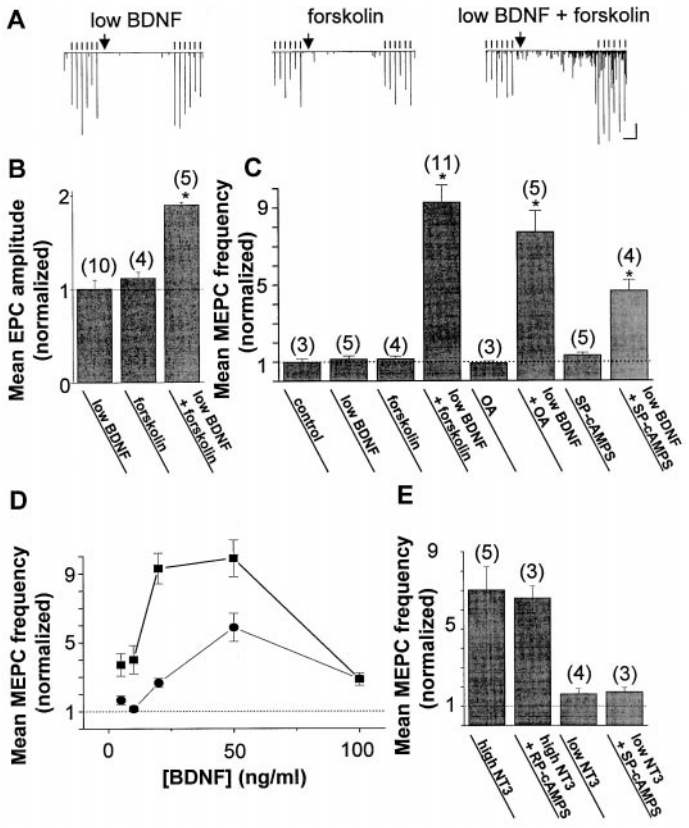


Fig. 2. Elevation of cAMP signaling facilitates the synaptic action of small amounts of BDNF. (**A**) EPCs and MEPCs before and after addition (arrow) of BDNF (10 ng/ml), 20 μM forskolin, or both. Scales: 1 nA, 100 s. (**B** and **C**) Data summarizing all experiments on changes in EPC amplitude (B) and MEPC frequency (C) after treatments, normalized against average control values at the same synapses before treatment (0.5 nM okadaic acid, 20 μM Sp-cAMPS, 20 μM forskolin). Number of synapses recorded is shown in parentheses. Significant difference from corresponding controls is indicated by an asterisk ($P < 0.05$; Student's t test). (**D**) Changes in MEPC frequency in response to different concentrations of BDNF in the absence (circles) or presence (squares) of 20 μM forskolin. Each point represents normalized MEPC frequency ($n = 5$ or 6 synapses) for 10 to 50 min after BDNF application. (**E**) Summary of effects of manipulating cytoplasmic cAMP signaling on potentiation induced by NT-3. Bars represent normalized MEPC frequency for 10 to 50 min after application of NT-3 (20 ng/ml) (low NT3) alone or in combination with 20 μM forskolin, or NT-3 (50 ng/ml) (high NT3) alone or in combination with 20 μM Rp-cAMPS. All values are mean ± SEM.

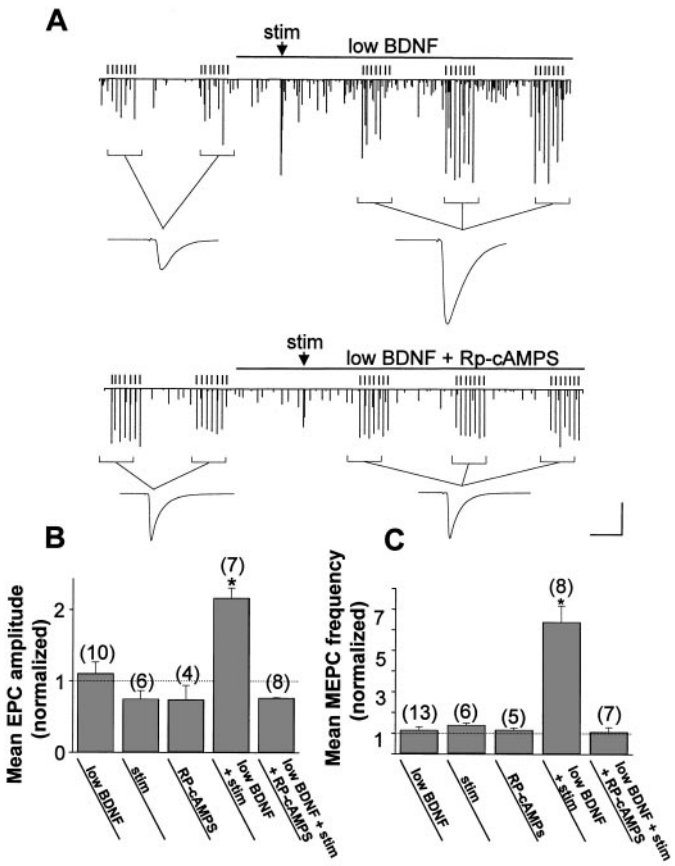


Fig. 3. Rp-cAMPS blocks the facilitatory effect of presynaptic activity on BDNF-induced potentiation. (**A**) Synapses exposed to brief presynaptic stimulation (15 pulses at 2 Hz) together with bath-applied BDNF (10 ng/ml) in the absence (top trace) or presence (lower trace) of Rp-cAMPS (20 μM). BDNF was applied after a 10-min control recording and the presynaptic neuron was stimulated 5 min after the onset of BDNF treatment. Average of EPCs is shown below. Scales: slow traces, 1 nA, 250 s; fast traces, 1 nA, 20 ms. (**B** and **C**) Summary of effects of various treatments on EPCs and MEPCs. Bars represent normalized values (mean ± SEM) for 10 to 50 min after the treatments. Low BDNF, 10 ng/ml; stim, suprathreshold stimulation of the presynaptic neuron for 15 pulses at 2 Hz; Rp-cAMPS, 20 μM. Significant difference from corresponding controls is indicated by an asterisk ($P < 0.05$; Student's t test).

neurotrophin receptors into the neuronal plasma membrane (*24*), and secretion of neurotrophins (*33*) and other peptides that act synergistically with BDNF. These processes could contribute to the enhanced potentiation of transmitter secretion in response to BDNF. Thus the synaptic response to neurotrophins is not a fixed neuronal phenotype but rather is determined by coincident signals received by the neuron. Gating of neurotrophin responses by cAMP-dependent processes may serve as a regulatory control for structural and functional plasticity in the nervous system.

References and Notes

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17. Cultures of *Xenopus* spinal neurons were prepared from the neural tube tissue of 1-day-old *Xenopus* embryos as described (*3–5*).
18. Whole-cell patch recordings were made in culture medium after 1 day of incubation at room temperature (20° to 22°C). Cultures were placed in a viewing chamber on a Nikon inverted microscope with a ×20 phase-contrast objective. The solution inside the whole-cell recording pipette contained 150 mM KCl, 1 mM NaCl, 1 mM $MgCl_2$, and 10 mM Hepes (pH 7.2). Unless indicated otherwise, when BDNF was applied it was added to the culture medium and the recording was made in the presence of BDNF throughout the experiment. The membrane current in all recordings was monitored by a patch-clamp amplifier (EPC-7, List Electronics, Great Neck, NY). Recordings were discarded if cell damage or substantial changes (>20%) in the series or input resistance were apparent. All other recordings were included for analysis in this work. The typical series resistance was 10 MΩ and the input resistance of the postsynaptic myocyte was in the range 50 to 200 MΩ. The estimated error in voltage clamp of the myocyte was therefore about 3 to 12 mV when $V_c = -70$ mV. The recorded currents were filtered at 10 kHz and stored on a videotape recorder for later analysis with the SCAN digital data analysis program. Evoked currents were elicited by extracellular stimulation of the presynaptic neuron at the soma (seven pulses at 0.02 Hz, 5-min interval) and were distinguished from spontaneous MEPCs by the presence of a clear stimulation artifact immediately preceding EPCs. Human recombinant BDNF (Promega, Madison, WI) was aliquoted at 100 μg/ml and stored at –20°C for up to 3 months before use. Working stock solutions of 1 μg/ml were prepared and used within 1 week. Rp-cAMPs and ACPD were purchased from RBI (Natik, MA) and aliquoted at 1 mg/ml; forskolin, okadaic acid, Sp-cAMPS, and KT5720 were purchased from Calbiochem (La Jolla, CA), aliquoted at 150 to 1000 times the working concentrations, and stored at −20°C for up to 6 months.
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