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Neurotrophin Modulation of NMDA Receptors in Cultured Murine and Isolated Rat Neurons

C. R. JARVIS,¹ Z.-G. XIONG,² J. R. PLANT,¹ D. CHURCHILL,¹ W.-Y. LU,² B. A. MACVICAR,¹
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¹NeuroScience Research Group, Faculty of Medicine, University of Calgary, Alberta T2N 4N1; and ²Departments of Physiology and Pharmacology, University of Toronto, Medical Sciences Building, Toronto, Ontario M5S 1A8, Canada

Jarvis, C. R., Z.-G. Xiong, J. R. Plant, D. Churchill, W.-Y. Lu, B. A. MacVicar, and J. F. MacDonald. Neurotrophin modulation of NMDA receptors in cultured murine and isolated rat neurons. *J. Neurophysiol.* 78: 2363–2371, 1997. Patch-clamp and calcium imaging techniques were used to assess the acute effects of the neurotrophins, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and nerve growth factor (NGF), on the responses of cultured and acutely isolated hippocampal and cultured striatal neurons to the glutamate receptor agonist *N*-methyl-D-aspartic acid (NMDA). The effects of BDNF on NMDA-activated currents were examined in greater detail. Currents evoked by NMDA, and the accompanying changes in intracellular calcium, were enhanced by low concentrations of the neurotrophins (1–20 ng/ml). The potentiation by the neurotrophins was rapid in onset and offset (<1 s). The neurotrophins also reduced desensitization of these currents in most cells. The enhancement of NMDA-activated currents by BDNF was observed using both perforated and whole cell patch recording techniques and could be demonstrated in outside-out patches. Furthermore, its effects were not attenuated by pretreatment with the protein kinase inhibitors genistein or 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7). Therefore, the actions of BDNF do not appear to be mediated by phosphorylation. Similar enhancements were observed with NT-3 and NT-4 and with NGF despite the fact that hippocampal neurons lack TrkA receptors. All together this evidence suggests that the enhancement of NMDA-evoked currents is unlikely to be mediated through the activation of growth factor receptors. Modulation of NMDA responses by BDNF was dependent on the concentration of extracellular glycine. The most pronounced potentiation by BDNF was observed at low concentrations, whereas no potentiation was observed in saturating concentrations of glycine, suggesting that BDNF may have increased the affinity of the NMDA receptor for glycine. However, the competitive glycine-site antagonist 7-chloro-kynurenic acid blocked the enhancement by BDNF without shifting the dose-inhibition relationship for this antagonist, and Mg²⁺ consistently depressed the potentiation of NMDA-evoked currents by BDNF, indicating that BDNF does not alter glycine affinity. BDNF also reversibly increased the probability of opening of NMDA channels recorded from outside-out patches taken from cultured hippocampal neurons. Other unrelated peptides including dynorphin and somatostatin also caused a glycine-dependent enhancement of NMDA currents and depressed the currents in saturating concentrations of glycine. In contrast, a shortened analogue dynorphin (6-17), which lacks N-terminus glycine residues, and another peptide met-enkephalin were without effects on NMDA currents recorded in low concentrations of glycine. Our results suggest that neurotrophins and other peptides can serve as glycine-like ligands for the NMDA receptor.

INTRODUCTION

Neurotrophins are small (~13 kDa) basic endogenous proteins that increase the growth and differentiation in sev-

eral types of central neurons, including striatal and hippocampal neurons. High-affinity neurotrophin receptors possess intrinsic tyrosine kinase activity. Neurotrophin receptors with high affinity for brain-derived neurotrophic factor (BDNF), neurotrophin-4 (NT-4; TrkB) and neurotrophin-3 (NT-3; TrkC) are distributed widely throughout the CNS and have been localized to hippocampal and striatal neurons, whereas receptors for nerve growth factor (NGF; TrkA), although present in cholinergic neurons of the striatum (Bothwell 1995; Holtzman et al. 1995; Lindsay et al. 1994), appear to be absent in hippocampal neurons and GABAergic striatal neurons (Ip et al. 1993). The administration of neurotrophins can protect neurons against metabolic stress and excitotoxicity (Cheng and Mattson 1994). However, Koh et al. (1995) have reported recently that in cultured cortical neurons BDNF, NT-3, or NT-4 but not NGF exacerbated the toxicity associated with activation of the *N*-methyl-D-aspartate (NMDA) subclass of glutamate receptors. Administration of NMDA also has been associated with an enhanced expression of the neurotrophins themselves, likely as a compensatory response to excitotoxicity (Hughes et al. 1993).

The NMDA receptor also appears to play a trophic-like role in the development of synaptic connections in the CNS (Constatine-Paton 1990; Komuro and Rakic 1993), and, in low concentrations, NMDA can enhance the outgrowth and branching of neurons in culture (Brewer et al. 1993). The influx of calcium through this channel likely contributes both to its excitotoxic as well as its trophic-like activities. Although it is unlikely that Trk receptor activation would lead directly to phosphorylation of the NMDA receptor, it is possible that subsequent activation of other protein kinases might modulate the activity of this channel (Raymond et al. 1993). However, we report that neurotrophins can enhance rapidly and reversibly the activity of NMDA receptors by a mechanism that involves neither Trk receptors nor activation of protein kinases. Instead, neurotrophins as well as other peptides, can act to directly modulate the activity of the NMDA receptor.

METHODS

Cell culture and acute dissociation

Hippocampal and striatal tissue were obtained from mouse embryos at E14 or E13, respectively. Cells were plated (1×10^6 cells per milliliter) and grown in the presence of serum (hippocampal neurons) or in defined medium (striatal neurons) for 12–20 days as previously described (MacDonald et al. 1989; Weiss et al.

1993). CA1 pyramidal neurons were acutely isolated from 2-wk-old rats according to the technique described by Wang and MacDonald (1995). Briefly, Wistar rats (2 wk old) were anesthetized with ether and killed by decapitation using a guillotine. The brain then was removed rapidly and rinsed with cold extracellular solution (see below). Each hippocampus was isolated surgically and then cut into 300- to 500- μm -thick slices by hand using a razor blade. The slices were treated for 20–30 min with a solution containing 0.3–0.5 mg/ml papain (EC 3.4.22.2–1.1 units/mg solids, derived from papaya latex, Sigma, P-3250), and neurons then were isolated mechanically from the CA1 region of the slice using two fire-polished glass pipettes.

Electrophysiology

Recording electrodes with resistances of 3–5 M Ω were constructed from thin-walled borosilicate glass (1.5 mm diameter, WPI). Patch-clamp recordings were made in the perforated, whole cell, and outside-out patch configurations using an Axopatch 1-D amplifier (Axon Instruments, Foster City, CA). For perforated and whole cell recordings, data were digitized, filtered (2 kHz), and acquired on-line using the program pClamp 5.5.1 (Axon Instruments). For outside-out membrane patch recordings, single-channel events first were recorded on video tape using a digital data recorder (VR-10, Instrutech, Mineola, NY) and later played back and acquired using the pClamp 6 program (Axon Instruments). The single-channel open probability was determined from the ratio of the time spent in the open state to the duration of recording: $P_o = (t_1 + t_2 + \dots + t_n)/N_{tot}$, where t is the amount of time that n channels are open and the N is the maximum number of levels observed in the patch. Data were expressed as the means \pm SE, and the Student's t -test was used to examine the statistical significance of differences between groups.

The extracellular solutions contained (in mM) 140 NaCl, 1.3 or 2 CaCl₂, 5.0 or 5.4 KCl, 10 or 25 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 33 or 10 glucose, and 0.0005–0.001 tetrodotoxin (TTX; pH 7.4 using NaOH; 320–335 mOsm). Perforated patch electrodes were filled with (in mM) 120 KCl, 5 MgCl₂, 40 HEPES, and 10 ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) plus 0.45 mg/ml nystatin and a few crystals of pluronic acid. Whole cell patch electrodes contained (in mM) 140 CsF or 120 KCl, 35 CsOH, 10 or 40 HEPES, 2 or 5 MgCl₂, 11 or 10 EGTA, 2 tetraethylammonium, 1 CaCl₂, and 4 Na₂ATP or 2 MgATP (pH 7.3 using CsOH or NaOH; 300 mosM). All experiments were performed at room temperature (20–22°C). Various concentrations of glycine (Sigma), NMDA (Sigma), MgCl₂ (Aldrich Chemical, Milwaukee, WI), BDNF, NGF, NT-3, NT-4 (Pepro Tech, Rocky Hill, NJ and Intergen, NY) and 7-chloro-kynurenic acid (Tocris, Bristol, UK) were added to give the concentrations indicated in the text and figure legends. In some experiments, we also applied somatostatin, met-enkephalin, dynorphin (1-17), and dynorphin (6-17) (Sigma and/or Peninsula). A multibarrel perfusion system was employed to achieve a rapid exchange of solutions.

Calcium imaging

Cultured striatal neurons were incubated in fura-2-acetoxymethyl ester (6 μM) dissolved in dimethyl sulfoxide with pluronic acid in minimal essential media for 30–40 min at 37°C as described previously (Weiss et al. 1993). Coverslips with fura-2-loaded cells were transferred to a perfusion chamber on an inverted microscope (Zeiss, Axiovert). Cells were illuminated using a xenon lamp (75 W) and were observed using a $\times 25$ plan-neofluar objective on an inverted microscope (Zeiss). Recordings were done at room temperature. Video images were obtained using a CCD camera (Cohu 4982) coupled to an image intensifier (Videoscope KS 1381). Digitized images were acquired by averaging 32 frames at

video rates using an image processing board (DT2867) in a PC-type computer controlled by Axon Imaging Workbench software (AIW, Axon Instruments). The shutter and filter wheel also were controlled by AIW to allow timed illumination of cells at 340 and 380 nm excitation wavelengths. Ratio images were analyzed by averaging pixel ratio values in circumscribed regions of all responding cells in the field of view. The values were exported from AIW to a spreadsheet program (Excel 5.0) and plotted in GraphPad.

RESULTS

BDNF potentiates NMDA-evoked responses

We initially employed the perforated patch technique to examine the effects of BDNF on NMDA-induced currents while minimally disturbing the cytosol of the cells (Fig. 1,

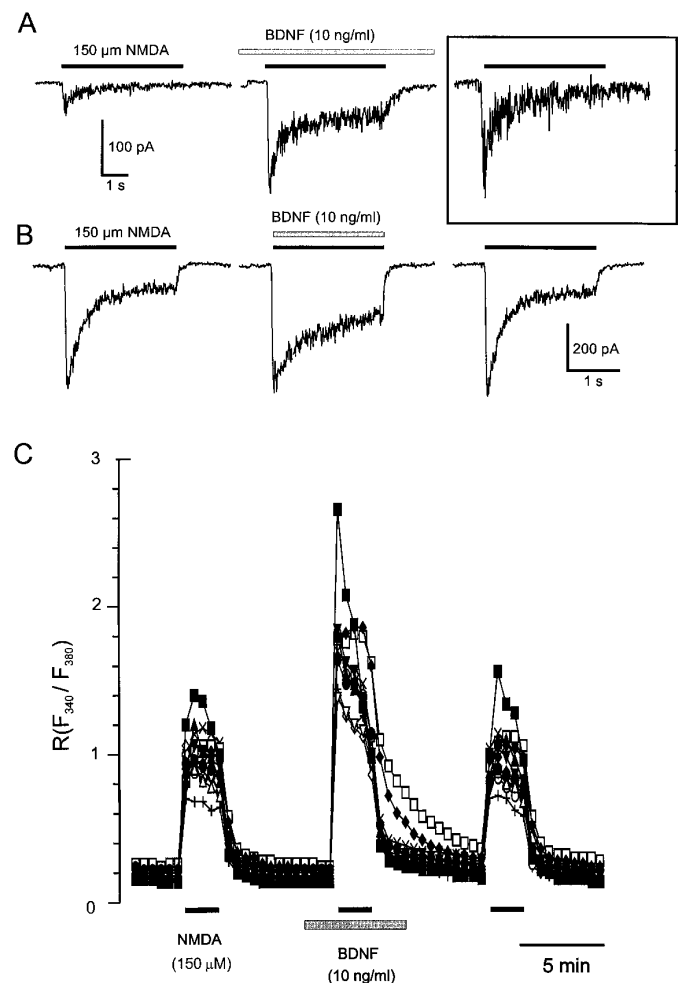


FIG. 1. Brain-derived neurotrophic factor (BDNF) potentiates *N*-methyl-D-aspartate (NMDA)-activated currents and NMDA-induced increases in intracellular calcium levels. Perforated patch recordings demonstrated that BDNF enhanced NMDA-activated currents in cultured striatal neurons (A). Holding potential, -70 mV. Inset: control response to NMDA scaled to the peak of the response observed with BDNF. In whole cell recordings from isolated CA1 hippocampal pyramidal neurons, BDNF decreased the desensitization of NMDA-evoked currents (B). Holding potential, -60 mV. Fura-2, AM ratiometric imaging of intracellular calcium concentration in striatal neurons showed that BDNF increased the magnitude of the NMDA-activated calcium influx (C). Shown are superimposed data traces from 10 cells. All experiments were performed in the absence of added glycine.

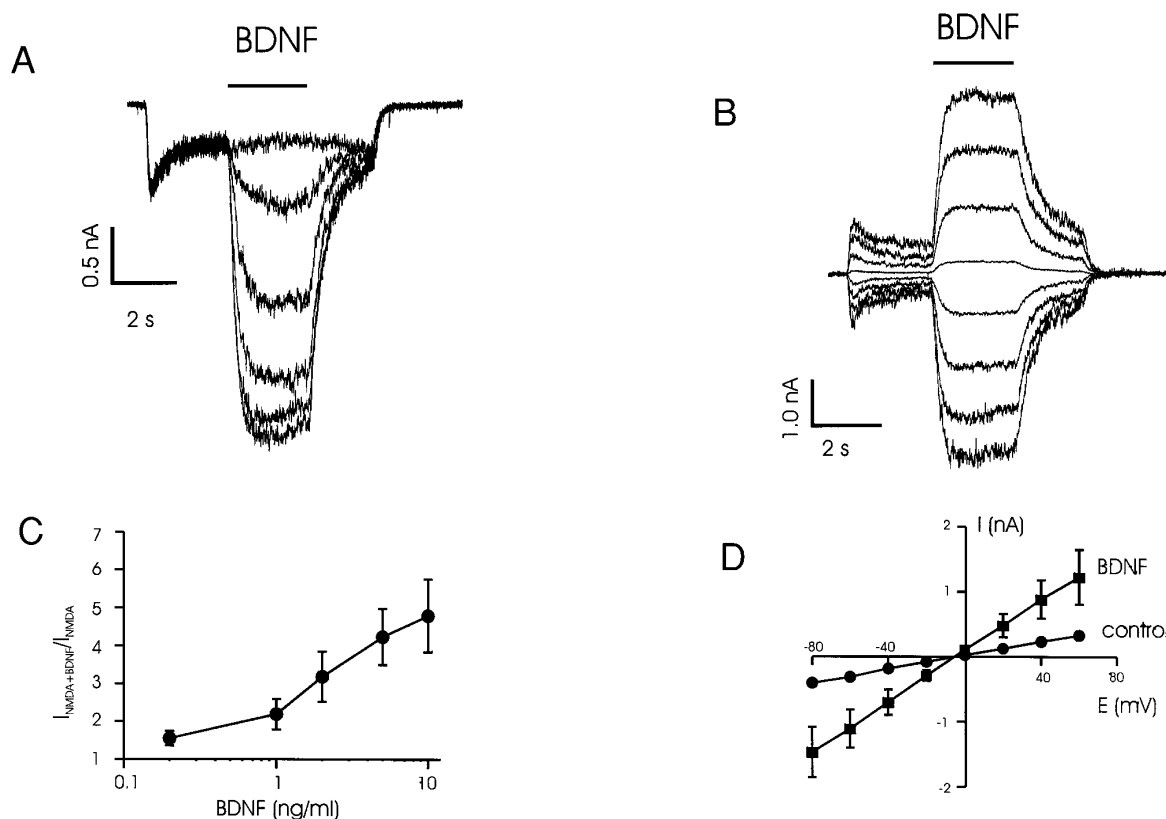


FIG. 2. BDNF effects are dose dependent and voltage independent. Recordings were made from cultured hippocampal neurons using the whole cell patch-clamp technique. Applications of different concentrations of BDNF were superimposed on responses to NMDA (100 μ M) recorded in the absence of added glycine and Mg^{2+} (A). Dose-dependent enhancement by BDNF is plotted in C for a series of 6 cells. Maximal effect was not determined, and therefore the data were not further analyzed. In another neuron, an application of BDNF (10 ng/ml) was superimposed on an application of NMDA (100 μ M) and then repeated at a series of different holding potentials (B). No apparent voltage-dependence to the enhancement by BDNF was observed and the plots (D) of NMDA (control) and NMDA + BDNF currents were near linear ($n = 8$).

A). The extracellular solution did not contain added glycine or Mg^{2+} . In previous experiments, we have estimated the concentration of contaminating glycine in our solutions to be ~ 20 nM (Wang and MacDonald 1995) hence responses to NMDA can be observed readily in solutions containing no added glycine. In cultures, the cells release additional glycine such that the concentrations of glycine can vary substantially from dish to dish. In cultured hippocampal and striatal neurons (no added glycine), BDNF (1–20 ng/ml) caused an enhancement of NMDA-evoked currents (Fig. 1A). BDNF also enhanced the currents recorded from acutely isolated CA1 pyramidal neurons of the rat hippocampus (Fig. 1B). The enhancement of NMDA-activated currents was observed when the neurons were either preexposed to the neurotrophin (Fig. 1A) or when the neurotrophin was simply included with NMDA itself (Fig. 1B). The enhancement by BDNF was readily demonstrable using whole cell patch recordings, and no “run-down” of the potentiation of NMDA currents was observed during prolonged recordings lasting >1 h (not shown). The enhancement of NMDA-evoked currents by BDNF was manifested in two different ways. In most cultured cells, the peak and steady state currents were potentiated, and desensitization was reduced (e.g., Fig. 1A), whereas in some cultured striatal cells and in all isolated neurons examined, the major action was a reduction of desensitization reflected as an increase in the time con-

stants of desensitization (cultured neurons $\tau_{\text{control}} = 594 \pm 76$ ms; $\tau_{\text{BDNF}} = 908 \pm 125$ ms, $n = 10$, $P < 0.05$; isolated hippocampal neurons $\tau_{\text{control}} = 290 \pm 25$ ms; $\tau_{\text{BDNF}} = 433 \pm 43$ ms, $n = 9$, $P < 0.01$) with little or no change in the amplitude of the peak current (e.g., Fig. 1B). The effects of BDNF were observed consistently in cultured hippocampal neurons grown in the presence of serum (53 out of 59), but the response was observed less frequently in murine striatal cells grown in serum free medium (71 out of 102). We attribute the lack of responsiveness in some cases to the variable concentrations of free glycine found in such cultures (also see below). The enhancement by BDNF was dose-dependent (Fig. 2, A and C) and was similar at positive and negative membrane potentials (Fig. 2B). Plotting current versus holding potential (between -80 and $+60$ mV) demonstrated that the effect of BDNF was voltage independent (Fig. 2D). The BDNF effect was rapid in onset and offset (Fig. 2, A and B), and these parameters did not vary significantly at different holding potentials (not shown).

We then used calcium imaging techniques to monitor intracellular calcium levels with the objective of examining the effect of BDNF on NMDA-evoked increases in intracellular calcium. In cultured striatal neurons, BDNF (10 ng/ml or approximate equivalent of 1 nM) reversibly enhanced the Ca^{2+} -response induced by applications of NMDA (Fig. 1C). BDNF had no effect on the Ca^{2+} -response elicited by the

application of solutions containing high K^+ (50 mM; not shown). These results are in agreement with our results from electrophysiological studies.

NT-3 and NGF also potentiate NMDA-evoked responses

Noncholinergic neurons of the hippocampus and striatum express TrkB and TrkC receptors that preferentially bind BDNF and NT-3. In contrast, TrkA receptors, which preferentially bind NGF, are absent (Ip et al. 1993). To test if the enhancement by BDNF was mediated through the selective activation of a Trk receptor, we examined the actions of the different neurotrophins (Fig. 3). Each of the neurotrophins (BDNF, NGF, and NT-3) enhanced NMDA-activated currents (Fig. 3, A and C) and increased calcium responses to NMDA (Fig. 3, B and D). There were no significant differences in the magnitude of the effect of BDNF, NGF, and NT-3. In preliminary experiments, NT-4 also enhanced NMDA-evoked currents and calcium responses (data not

shown). These data suggest that the potentiation of NMDA-evoked responses by the neurotrophins is unlikely to be mediated by the activation of a specific Trk receptor because TrkA receptors that preferentially bind NGF are absent. The involvement of p75 receptors in the neurotrophin response is also unlikely due to the rapid onset of the response (<1 s).

Effects of BDNF are not blocked by protein kinase inhibitors

Neurons were treated with the tyrosine kinase inhibitor genistein to test whether or not tyrosine phosphorylation was involved in the response to BDNF. Pretreatment of cultured hippocampal neurons with genistein, which should have blocked the intrinsic tyrosine kinase activity of the growth factor receptors (50 μ M, $n = 5$), failed to alter the enhancement of NMDA-evoked currents (not shown). Furthermore, applications of the relatively nonspecific kinase inhibitor

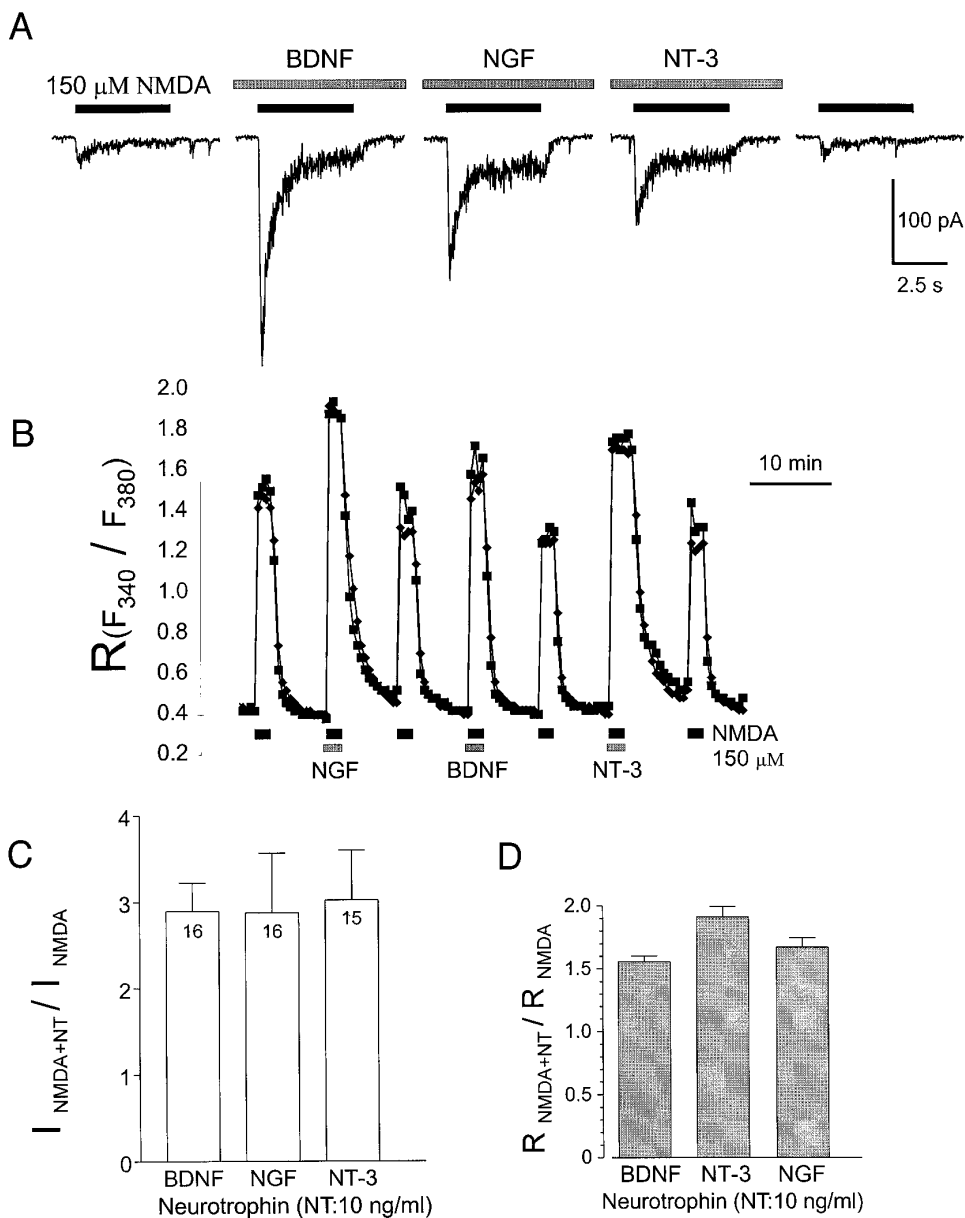


FIG. 3. Other members of the neurotrophin family also enhance NMDA-activated currents and calcium signals. Whole cell recordings revealed that BDNF, nerve growth factor (NGF), and neurotrophin-3 (NT-3) each potentiated NMDA-activated currents in a cultured striatal neuron (A). Holding potential, -70 mV. Ratiometric calcium imaging techniques demonstrated that NGF, BDNF, and NT-3 also increased the magnitude of NMDA-evoked calcium influx in cultured striatal neurons (B). Neurotrophins (BDNF, NGF, and NT-3) enhanced NMDA-mediated currents in the absence of added glycine. Summary data from 15 or 16 cells is shown (C). Normalized calcium imaging data from 28 cells is also shown (no added glycine) (D).

1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7; 50 μ M, $n = 4$) were ineffective in modulating the effects of BDNF. These results suggest that the enhancement of NMDA-activated currents by BDNF was not mediated by protein kinase activation.

Enhancement of NMDA-evoked currents by BDNF is glycine dependent

We then examined the effects of changing the extracellular concentration of glycine on the response to BDNF. The actions of BDNF were found to be strongly dependent on the concentration of added glycine (Fig. 4). The greatest potentiation was observed in the absence of added glycine (Fig. 4A), whereas a small depression of NMDA-evoked responses was observed in saturating concentrations of this coagonist (Fig. 4B; $n = 5$, mean = $5.7 \pm 2.0\%$). This differs from the effect of polyamines and divalent cations, which enhance NMDA currents in the presence of saturating concentrations of glycine (Benveniste and Mayer 1993; Paoletti et al. 1995).

The dependence of the effects of BDNF on the concentration of glycine together with its ability to reduce desensitization suggests that neurotrophins may enhance the affinity of the NMDA receptor for glycine. For example, polyamines (McBain and Mayer 1994) and divalent cations (Paoletti et al. 1995; Wang and MacDonald 1995) potentiate NMDA-evoked currents by this mechanism. One way to demonstrate such an increase in the affinity for glycine is to examine whether or not a substance can influence the effectiveness of competitive antagonists of the glycine site. For example, spermine (Benveniste and Mayer 1993) and Mg^{2+} (Wang and MacDonald 1995) can shift appropriately the dose-inhibition curves for 5,7-dichloro-kynurenic and 7-chloro-kynurenic acids, respectively. However, we were unable to reproduce any such shift in the presence of 7-chloro-kynurenic acid as the response to BDNF was blocked completely (Fig.

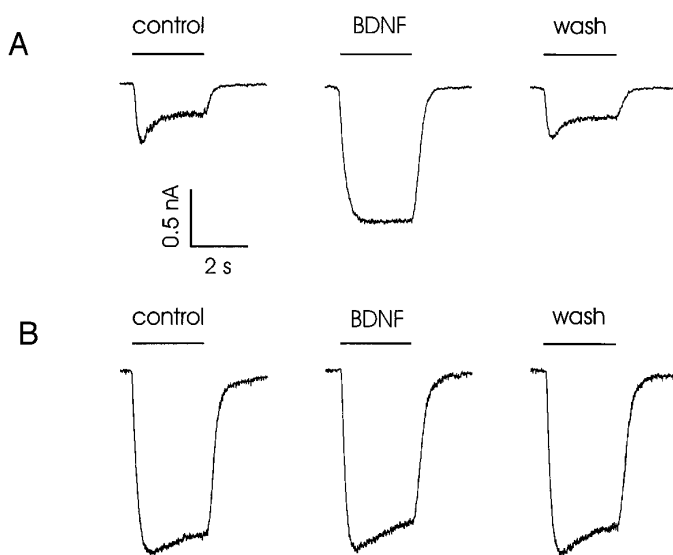


FIG. 4. Effect of BDNF on NMDA-induced currents is dependent on the extracellular concentration of glycine. In the absence of added glycine, BDNF (10 ng/ml) substantially enhanced NMDA currents recorded in a cultured hippocampal neuron. However, in the presence of 10 μ M glycine, BDNF did not enhance this current.

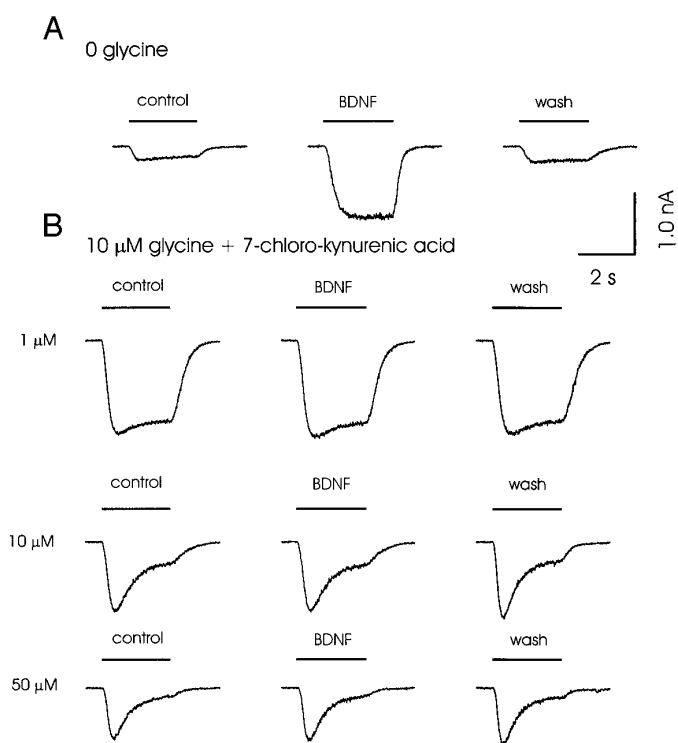


FIG. 5. BDNF does not alter the affinity of the NMDA receptor for glycine. An example recording from a cultured hippocampal neuron is shown. In low concentrations of glycine, BDNF substantially enhanced the NMDA-activated current (100 μ M) in this neuron (A). Holding potential, -60 mV. Increasing concentrations (1–50 μ M) of the competitive glycine-site antagonist 7-chloro-kynurenic acid progressively depressed NMDA-evoked currents (reduced the peak and increased desensitization; B). These experiments were carried out in the presence of a saturating concentration (10 μ M) of glycine. If BDNF were to increase the affinity of glycine for its binding site, it would have been anticipated that it also would reduce the effectiveness of 7-chloro-kynurenic acid. However, in all of the neurons tested BDNF had no effect on NMDA-evoked currents at any of the antagonist concentrations tested ($n = 6$).

5) at all concentrations of the antagonist tested (1–50 μ M). We also examined the effect of BDNF on the glycine concentration-response relationship in isolated neurons avoiding the higher level of glycine concentrations associated with neurons in cultures (Wang and MacDonald 1995). However, we could not detect a change in the apparent affinity of the receptor for glycine (control $EC_{50} = 496 \pm 99$ nM; BDNF $EC_{50} = 481 \pm 134$, $n = 5$, $P > 0.05$). These results suggest that BDNF does not increase the affinity of the receptor for glycine.

Mg^{2+} depresses the BDNF-induced enhancement of NMDA-evoked currents

High-performance liquid chromatography (HPLC) measurements of extracellular recording solutions revealed that contaminating levels of glycine and serine were both below the level of detectability (4 μ M). Glycine concentrations were also below the level of detectability in concentrated solutions (100 ng/10 μ l) of the neurotrophins. Hence, we estimate that the contamination contributed by the BDNF solution only could have raised the nominal glycine concentration by <4 nM. This is considerably lower than the likely contamination of all our solutions by glycine from other

sources (Johnson and Ascher 1992; Wang and MacDonald 1995). Nevertheless, to further determine if the effects of BDNF could be attributed to contamination of the BDNF with glycine, we investigated the effects of extracellular Mg^{2+} on BDNF- versus glycine-induced enhancements of NMDA-activated currents. In cultured and isolated hippocampal neurons, Mg^{2+} enhances responses to glycine (Paoletti et al. 1995; Wang and MacDonald 1995) in part by increasing the affinity of the receptor for glycine. If BDNF were contaminated with glycine, we would anticipate that this divalent cation also would enhance the response to BDNF. However, responses to BDNF were depressed substantially (Fig. 6, A and C) even though Mg^{2+} consistently enhanced responses to glycine (Fig. 6B) (also Paoletti et al. 1995; Wang and MacDonald 1995).

BDNF increases the open probability of NMDA channels

In outside-out patches taken from cultured hippocampal neurons and held at -60 mV, BDNF increased the open probability of NMDA channels (Fig. 7). In control solutions ($5 \mu M$ NMDA; 100 nM glycine), the probability of NMDA channels being in the open state was 0.017 ± 0.003 compared with 0.043 ± 0.007 ($n = 5$; $P < 0.05$) in the presence of BDNF (10 ng/ml). In contrast, single-channel amplitude and open time were unaltered by BDNF (Fig. 7, C and D).

Our results suggest that BDNF and other neurotrophins can interact directly with the glycine site of the NMDA receptor. Other peptides also may interact in a similar manner. For example, Brauneis et al. (1996) recently reported that the peptide dynorphin (dynorphin 1-13) caused a glycine-dependent enhancement of recombinant NMDA receptor-induced currents expressed in *Xenopus* oocytes. This enhancement changed to a depression when the solutions contained saturating concentrations of glycine. However, the shortened peptide (dynorphin 4-13), which lacks terminal glycine residues, was without effect on NMDA currents, suggesting that these residues could substitute for free glycine. For this reason, we compared the effects of dynorphin (1-17) and dynorphin (6-17) with those of BDNF. Dynorphin (1-17) caused a glycine-dependent enhancement of NMDA currents, and, in high concentrations of glycine, this peptide depressed NMDA currents (Fig. 8). Another peptide somatostatin also caused a glycine-dependent enhancement

of NMDA-induced currents. In contrast, the shorter form of dynorphin (6-17) (Fig. 8C) and met-enkephalin (not shown) failed to enhance NMDA-activated currents recorded in low concentrations of glycine.

DISCUSSION

Our results demonstrate that neurotrophins can rapidly and reversibly enhance NMDA-evoked currents in striatal and hippocampal neurons. This response was observed in cultured and isolated neurons using either perforated or whole cell patch techniques as well as in outside-out patches. The increase in the probability of NMDA channel opening associated with applications of BDNF to outside-out patches suggests that second messengers are not likely to be involved. In addition, all of the neurotrophins enhanced NMDA receptor-mediated responses, suggesting that the actions of neurotrophins were not mediated through the selective activation of a given Trk receptor. Furthermore, NGF was effective even though TrkA receptors are not expressed in cultured hippocampal neurons (Ip et al. 1993). The failure of the kinase inhibitors to block the enhancement also demonstrates that tyrosine kinases as well as serine-threonine protein kinases were unlikely to have been responsible for this response. A more likely explanation is that the neurotrophins act directly on the NMDA receptor itself. However, we could not demonstrate a shift in the affinity of the receptor for glycine by BDNF. Because both BDNF and glycine potentiate NMDA-evoked currents, reduce their desensitization, and increase the probability of channel opening, we suggest that BDNF is capable of substituting for the coagonist glycine. On the other hand, our results demonstrate that the enhancement of NMDA-evoked currents by BDNF is depressed by Mg^{2+} even though this divalent cation increases the affinity for glycine and potentiates the response to this amino acid. This suggests that BDNF is unlikely to act simply as a partial agonist at the glycine site.

Is the enhancement by BDNF simply due to contamination of the BDNF solution with glycine? Experiments investigating the effects of extracellular Mg^{2+} on BDNF- versus glycine-induced enhancements of NMDA-evoked currents revealed that this was not the case. As previously reported by Wang and MacDonald (1995), extracellular Mg^{2+} enhances the affinity of the NMDA receptor for glycine and increases

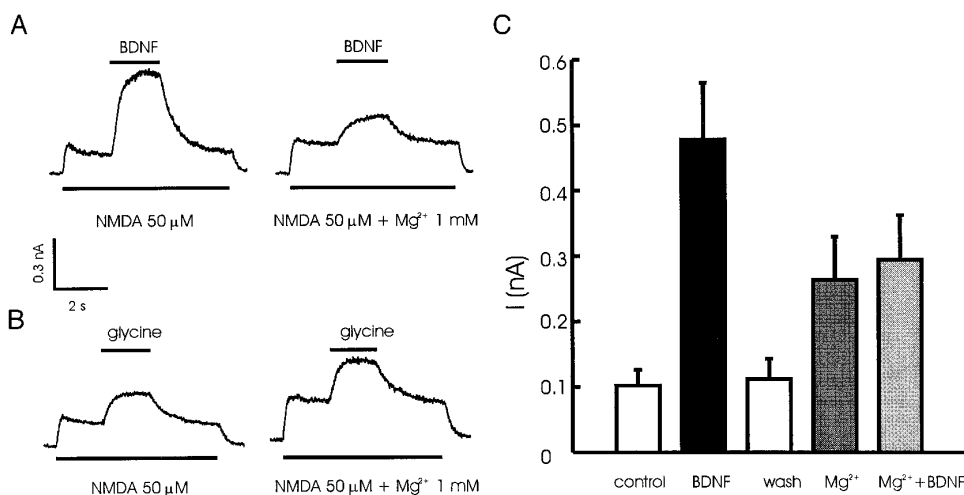


FIG. 6. Mg^{2+} depresses the enhancement of NMDA-evoked currents induced by BDNF (A) or glycine (B) potentiated NMDA currents in cultured hippocampal neurons. Coapplications of NMDA and Mg^{2+} also enhanced these currents. However, Mg^{2+} consistently depressed the potentiation induced by BDNF alone while enhancing the response to glycine. Holding potential, $+30$ mV. Summary data are presented below the example recordings (C). In 10 neurons, BDNF caused an almost fourfold enhancement of NMDA-activated currents (control, 102 ± 24 pA; BDNF, 487 ± 87 pA; $P < 0.001$, no added glycine). Mg^{2+} enhanced NMDA-evoked currents (263 ± 66 pA, < 0.001) but also strongly depressed the BDNF-induced potentiation as compared with the effect of BDNF alone (Mg^{2+} + BDNF, 294 ± 68 pA, $P < 0.05$).

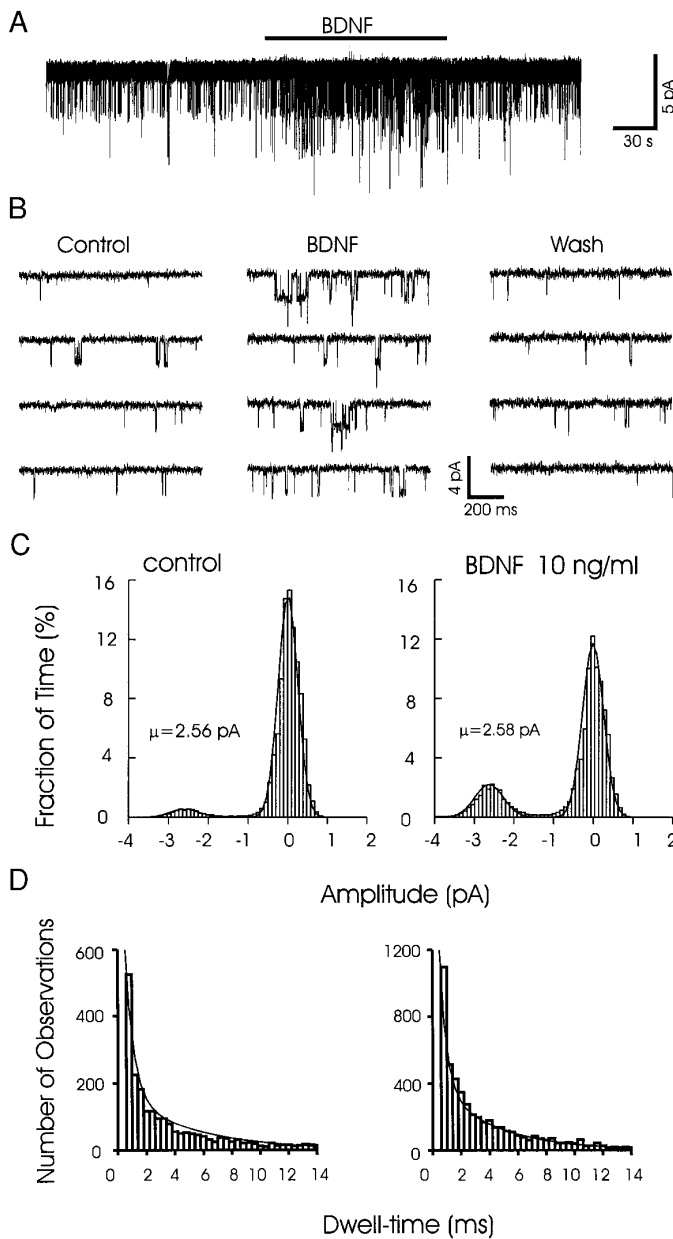


FIG. 7. BDNF increases the open probability of NMDA channels in an outside-out patch taken from a cultured hippocampal neuron. An example record showing the NMDA channel activity in an outside-out patch before, in the presence of, and after washout of BDNF (A; 10 ng/ml) is shown. In this example, NMDA evoked multiple openings, indicating the presence of more than one channel in the patch. Solid bar indicates the duration of the BDNF application. Holding potential was -60 mV, and NMDA ($5 \mu\text{M}$) and glycine (100 nM) were included in the extracellular solution. Portions of the record are displayed at an expanded time scale (B). Probability of NMDA channels being in the open state was increased by BDNF 10 ng/ml (control, 0.017 ± 0.003 ; BDNF, 0.043 ± 0.007 , $n = 5$; $P < 0.05$). Amplitude histograms in the absence and in the presence of BDNF are shown (C). BDNF did not change the amplitude of single channel currents (control, 2.4 ± 0.8 pA; BDNF, 2.6 ± 0.6 pA; $n = 5$, $P > 0.05$). Open time histograms in the absence and in the presence of BDNF (10 ng/ml; D). Open time histograms were well fit with 2 exponentials. Both short and long open times were unaffected by BDNF (control $\tau_{\text{short}} = 0.52 \pm 0.06$ ms, $\tau_{\text{long}} = 4.9 \pm 0.32$ ms, $n = 6$; BDNF $\tau_{\text{short}} = 0.47 \pm 0.08$ ms, $\tau_{\text{long}} = 4.4 \pm 0.037$ ms, $n = 6$).

glycine's action on NMDA-activated currents. However, in the present study, Mg^{2+} depressed BDNF-induced enhancement of NMDA-evoked currents. If the enhancement by BDNF was due to glycine contamination, then Mg^{2+} would be expected to increase BDNF's effect as it does for glycine itself. Moreover, glycine concentrations in BDNF solutions were estimated to be ≤ 4 nM as measured by HPLC.

We and others (Brauneis et al. 1996) have shown that several peptides including neurotrophins, somatostatin and dynorphin, can cause a glycine-dependent potentiation of NMDA-activated currents. In the case of dynorphin, the enhancement depends on the presence of several glycine residues in the N terminus of the peptide because the shortened form of dynorphin (4-13), which lacks these glycines, is ineffective (Brauneis et al. 1996). It was suggested that the peptide containing these residues (Brauneis et al. 1996) may have substituted for free glycine at the NMDA receptor. We have confirmed this observation in that dynorphin (1-17) – enhanced NMDA-induced currents in a glycine-dependent fashion, whereas a shortened form of dynorphin (6-17) was without effect. This suggests that neurotrophins dynorphin and somatostatin may have acted via a similar mechanism to potentiate NMDA currents. However, the dynorphin

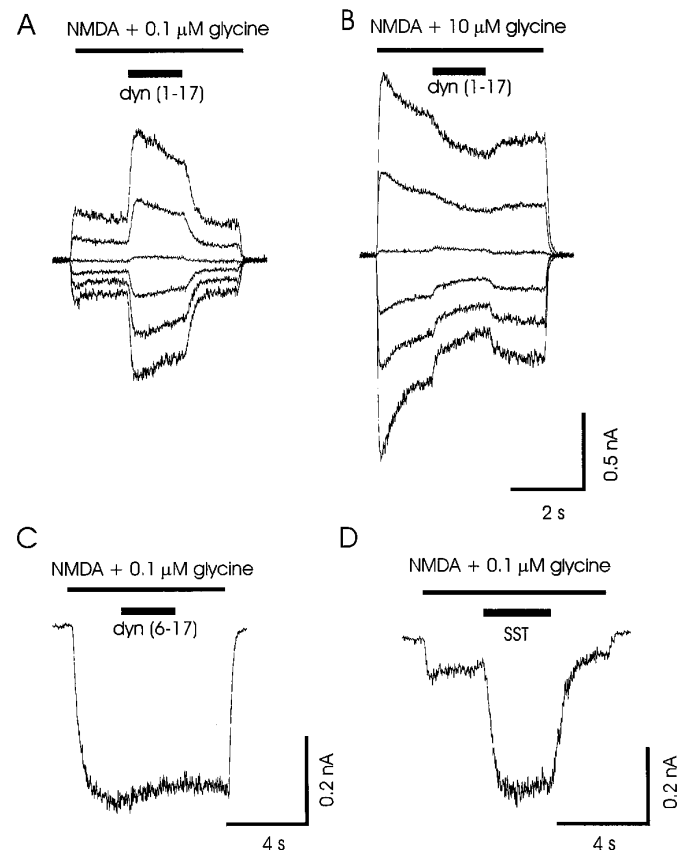


FIG. 8. Other peptides also cause a glycine-dependent enhancement of NMDA currents. In an isolated CA1 pyramidal neuron, application of dynorphin (1-17) ($5 \mu\text{M}$) caused a voltage-independent potentiation of NMDA-evoked currents (A). However, in the same cell, when glycine was increased to saturating concentration, the enhancement was replaced by a depression (also voltage-independent; B). These results were confirmed in 5 cells. Similar results (D) were observed with applications of somatostatin (SST, $5 \mu\text{M}$, $n = 5$). In contrast, the shortened form of dynorphin (6-17) was unable to potentiate NMDA-evoked currents recorded in low concentrations of glycine ($n = 5$; C).

(6-17) and met-enkephalin were unable to enhance NMDA-induced currents, demonstrating that the enhancement is not an entirely nonspecific action of peptides.

The neurotrophins, including BDNF, NT-3, and NT-4/5, have been reported to cause a long-lasting enhancement of synaptic transmission at synapses in the adult hippocampus (Kang and Schuman 1995), suggesting that neurotrophins may play a key role in some forms of synaptic plasticity. The acute effect of neurotrophins on excitatory synaptic transmission may result from the enhanced release of glutamate from presynaptic terminals. For example, BDNF and NGF acutely enhanced the probability of glutamate release at a subpopulation of excitatory synapses in the rat visual cortex (Carmignoto et al. 1997), and BDNF and NT-4/5 transiently potentiated excitatory synaptic transmission in cultured hippocampal neurons (Lessmann et al. 1994). However, a tyrosine phosphorylation-dependent enhancement of postsynaptic glutamate receptors also has been reported (Levine et al. 1995). The direct neurotrophin-induced enhancement of NMDA receptor function we have observed may have contributed to the transitory effects in cultured hippocampal neurons but could not have accounted for the long-lasting enhancement in the adult hippocampus as the latter was insensitive to NMDA receptor antagonists. On the other hand, the glycine-like actions of the neurotrophins might have contributed to the neurotrophin-induced excitotoxicity reported in cultured neurons (Koh et al. 1995).

The participation of the NMDA receptor in excitatory transmission requires the cobinding of glycine. If the glycine site is unoccupied, little or no activation of the receptor is possible (McBain and Mayer 1994), whereas if the concentration of glycine is near-saturating, modulation of the receptor by neurotrophins would not be observed. Although the extracellular concentration of glycine in the synaptic cleft is unknown, high-affinity glycine transporters may maintain its concentration below saturating values (McBain and Mayer 1994; Supplisson and Bergman 1977; Wood 1995), suggesting that the neurotrophins might be able to modulate the function of NMDA receptors. For example, during the development of the cortex, activation of NMDA receptors is thought to play a paracrine role in the migration of neurons and in the guidance of synapse formation (Rakic and Komuro 1995; Rossi and Slater 1993). Neurotrophins released from cells might enhance NMDA channel activity and contribute in this way to the guidance of migrating neurons. Neurotrophins also can act through Trk receptors to enhance the expression of NMDA receptors. For example, NGF acting via the TrkA receptor stimulates promoter activity for the expression of the NMDAR1 subunit (Bai and Kusiak 1997). The expression of neurotrophins in turn is enhanced by NMDA receptor-dependent mechanisms after long-term potentiation (Bramham et al. 1996), electrical stimulation (Springer et al. 1994), and epileptogenic seizures (Mudo et al. 1996). This evidence suggests that there is a complex interdependence between neurotrophins and the function of the NMDA receptor.

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