Activity-dependent Release of Brain-derived Neurotrophic Factor Underlies the Neuroprotective Effect of N-Methyl-D-aspartate*

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The molecular mechanism(s) of N-methyl-D-aspartate (NMDA) neuroprotective properties were investigated in primary cultures of cerebellar granule cell neurons. Granule cells express the neurotrophin receptor TrkB but not TrkA or TrkC. In these cells, the TrkB ligand brain-derived neurotrophic factor (BDNF) prevents glutamate toxicity. Therefore, we have tested the hypothesis that NMDA activates synthesis and release of BDNF, which may prevent glutamate toxicity by an autocrine loop. Exposure of granule cells for 2 and 5 min to a subtoxic concentration of NMDA (100 μM) evoked an accumulation of BDNF in the medium without concomitant changes in the intracellular levels of BDNF protein or mRNA. The increase in BDNF in the medium is followed by enhanced TrkB tyrosine phosphorylation, suggesting that NMDA increases the release of BDNF and therefore the activity of TrkB receptors. To examine whether BDNF and TrkB signaling play a role in the NMDA-mediated neuroprotective properties, neurons were exposed to soluble trkB receptor-IgG fusion protein, which is known to inhibit the activity of extracellular BDNF, and to K252a, a tyrosine kinase inhibitor. Both compounds blocked the NMDA-mediated TrkB tyrosine phosphorylation and subsequently its neuroprotective properties. We suggest that NMDA activates the TrkB receptor via a BDNF autocrine loop, resulting in neuronal survival.

Glutamate, the endogenous neurotransmitter required for normal physiological excitation, is also involved in the pathophysiology of hypoxic/ischemic neuronal injury (1–3). Although this neuropathological process can be mediated by any of the excitatory amino acid receptors, the N-methyl-D-aspartate (NMDA)1 glutamate receptor subtype plays a major role. In addition, NMDA receptors mediate adaptive responses important for synaptic plasticity during development (4, 5). The molecular mechanism(s) by which NMDA receptors mediate such opposing effects are not clear.

The role of NMDA receptors in glutamate-mediated excitotoxicity has been studied in rat cerebellar granule cells in vitro, in which overactivation of NMDA receptors results in neuronal cell death (6–8). Paradoxically, in these cells subtoxic concentrations of NMDA have been shown to protect vulnerable neurons against an excitotoxic concentration of glutamate (9). Moreover, recent studies have shown that the neuroprotective effect of NMDA is blocked by coinuciation with either the RNA synthesis inhibitor actinomycin D or the protein synthesis inhibitor cycloheximide (9). Thus, the neuroprotective effect of NMDA may involve a polypeptide(s) with neuroprotective properties.

Cerebellar granule cells are responsive to various neurotrophic factors and in particular to the neurotrophins (10, 11), a family of trophic factors related by primary amino acid sequence homology, whose members include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and NT-4/5 (12–17). The biological activity of neurotrophins depends upon the activation of high-affinity receptors (Trk), a family of structurally related receptors having a similar intrinsic protein-tyrosine kinase activity (18, 19), but different ligand binding properties. TrkA, TrkB, and TrkC are, respectively, the receptors for NGF, BDNF, and NT-3 (20–22).

Binding of neurotrophin to its receptor triggers a signal transduction pathway, with Trk phosphorylation being the primary event and induction of several genes as a late event (for review, see Ref. 23). These events may play a role in the trophic properties of neurotrophins, including the ability to attenuate excitotoxic cell death (24). Thus, expression of a specific Trk receptor may confer resistance against excitotoxicity depending on availability of the primary ligand. Among neurotrophins, BDNF has been shown to function in an autocrine fashion in the peripheral nervous system (25), suggesting that in cerebellar granule cells, which express BDNF (26, 27), this neurotrophin could exert neuroprotective properties by activating its own receptor in a similar autocrine loop. This mechanism could be unique for central nervous system neurons and might represent one of the molecular events in NMDA-mediated neuroprotection. In this study, we have tested the hypothesis that NMDA mediates neuronal survival by affecting the release of BDNF and therefore the functional state of the TrkB receptor. We report that NMDA exerts a neuroprotective activity by increasing BDNF release and TrkB receptor signaling.

EXPERIMENTAL PROCEDURES

Cell Culture—Granule cells were prepared from postnatal day 8 Sprague Dawley rat pups. Briefly, meninges-free cerebella were minced and recovered by centrifugation. The pellets from 20 cerebella were subjected to trypsinization, followed by inactivation of the trypsin by
the addition of soybean trypsin inhibitor. Cells were then dissociated by a series of triturations and recovered by centrifugation. The final pellet was reconstituted in basal Eagle’s medium containing glucose (2 m\text{M}), fetal calf serum (10\%), and potassium chloride (25 m\text{M}). No antibiotics were added, and the plating density was 1.8 \times 10^6 cells/ml. Cytosine arabinoside (10 \mu\text{M}) was added 18–24 h later to inhibit the proliferation of non-neuronal constituents (9). On day 3 in vitro, glucose (100 \mu\text{M} of a 100 \mu\text{M} solution) and sterile water (100 \mu\text{M}) were added to each 35-mm culture dish to maintain survival and to replace evaporative losses, respectively. Granule cell neurons were used on day 8 in vitro for all experiments unless otherwise indicated.

NIH3T3 cells overexpressing TrkB (a gift from Cephalon Inc.) were grown as monolayer cultures in Dulbecco’s modified Eagle’s medium supplemented with 10\% donor calf serum in the presence of genetin (150 \mu\text{g}/\text{ml}).

**Determination of NMDA-mediated Neuroprotection**—Cultured cerebellar granule cells were pretreated with NMDA (100 \mu\text{M}) with or without K252a (10 \mu\text{M}) or trkB-IgG (20 \mu\text{g}/\text{ml}) for 6 h. Medium was then removed and replaced with sister culture medium. Either inhibitor and glutamate (100 \mu\text{M}) was added, and neuronal viability was determined 24 h later using the fluorescein diacetate method as described previously (9). The viable neurons were quantified by counting fluorescein-positive cell bodies and expressed as percent of survival.

**TrkB Tyrosine Phosphorylation**—Analysis of TrkB tyrosine phosphorylation was carried out essentially as described (28). Briefly, cerebellar granule cells were exposed for 10 min to human recombinant BDNF (Promega, Madison, WI), NGF (Collaborative Research, Bedford, MA), or NT-3 (Genentech, San Francisco, CA) (100 ng/ml each) and disrupted in 1 ml of lysis buffer (1% Nonidet P-40, 20 mM Tris, pH 8.0, 137 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 0.15 units/ml aprotinin, 20 \mu\text{M} leupetin, 1 mM sodium vanadate) at 4 \text{C}. After removal of cellular debris by centrifugation, protein levels in the lysates were measured by the Bradford protein colorimetric assay and equalized accordingly. Lysates were incubated with pan-trk antibody (Santa Cruz Biotechnology, Santa Cruz, CA) followed by protein A-Sepharose precipitation (Amersham Pharmacia Biotech) at 4 \text{C} for 2 h. The immunoprecipitate was washed with lysis buffer and water before resuspension in 10 \mu\text{l} of sample buffer (2% SDS, 100 mM dithiothreitol, 10% glycerol, 0.25% bromphenol blue) followed by fractionation through 7.5% SDS-polyacrylamide gels. Gels were transferred to nitrocellulose membranes, and the immobilized proteins were incubated overnight at 4 \text{C} with anti-phosphotyrosine monoclonal antibody 4G10 (Upstate Biotechnology, Lake Placid, NY) diluted with Tris-buffered saline with a final concentration of 0.2\% Tween 20. Blots were analyzed by using enhanced chemiluminescence detection according to the manufacturer’s recommendations (Amersham Pharmacia Biotech).

**BDNF Two-site Enzyme Immunoassay**—The quantitative two-site enzyme immunoassay for the determination of BDNF was performed using an immunoassay system from Promega (BDNF Emax immunoassay system). In brief, neurons (1.8 \times 10^6 cells/ml) were exposed to NMDA (100 \mu\text{M}) for various times, and the medium was then collected and concentrated using a Centricon-10 (Amicon) concentrator as described previously (29). In a series of parallel experiments, neurons were lysed for the determination of intracellular BDNF levels.

**RNase Protection Assay**—Extraction of RNA and RNase protection assay were carried out as described (30). Levels of BDNF mRNA were determined using a 32P-labeled BDNF cRNA probe generated from plasmid C3 (a gift from Dr. P.J. Isackson, Mayo Clinic, Jacksonville, FL) comprising 387 nucleotides from the 5’ coding region of rat BDNF cDNA plus 59 nucleotides from the Bluescript polylinker region (31). This cRNA (specific activity, >1 \times 10^6 \text{cpm/\mu g}) of RNA was used to hybridize 20–25 \mu\text{g} of total RNA in 20 \mu\text{l} of hybridization solution. Hybridization was carried out at 50 \text{C} overnight. RNA was digested with RNase A (1 \text{unit/ml}) and T1 (200 \text{units/ml}) for 30 min at 37 \text{C}. The samples were recovered using isopropanol (1:1 \text{v/v}). The pellet containing the RNA:RNA hybrid was dissolved in loading buffer (80% formamide, 0.1\% xylene cyanol, 0.1\% bromphenol blue, 2 \text{M EDTA}) and boiled at 95 \text{C}, and RNA was separated on a 5% polyacrylamide/urea sequencing gel. The gel was dried, and protected mRNA fragments were visualized by autoradiography using x-ray film with a Cronex Quanta III intensifying screen. The content of BDNF mRNA was calculated by measuring the peak densitometry area of the autoradiograph analyzed with a laser densitometer (Hoefer GS 300 scanning densitometer) normalized by the peak densitometry area of the cyclophilin autoradiograph band as described previously (30).

**RESULTS**

**Cerebellar Granule Cells Express Active TrkB Receptors**—We first examined whether cerebellar granule cells, on day 8 in vitro, express an active form of TrkB or other Trk receptors by determining TrkB tyrosine phosphorylation in lysates from these cells. BDNF (100 ng/ml) but not NGF (100 ng/ml) elicited a strong increase in TrkB phosphorylation (Fig. 1, upper panel) without affecting Trk levels (Fig. 1, lower panel). NT-3 (100 ng/ml) induced a weak increase in TrkB phosphorylation (Fig. 1, upper panel) that was seen only after prolonged exposure. The low potency of NT-3 in inducing TrkB phosphorylation indicated the presence of a small amount of TrkC receptors in these neurons or cross-binding of NT-3 with TrkB receptors. Because activation of the Trk tyrosine kinase by neurotrophins represents an immediate and essential event in the neurotrophin signal cascade (18), TrkB appears to be the only functional neurotrophin receptor expressed by cerebellar granule cells at this day in culture.

**Neuroprotective Activity of BDNF**—In addition to TrkB, cerebellar granule cells produce BDNF mRNA, whose levels can be altered by NMDA receptor activation (26, 27). We hypothesized that NMDA-mediated neuroprotection may involve de novo synthesis of BDNF, which, in turn, activates its cognate receptor. To test whether NMDA may activate this “survival” loop, we first examined the neuroprotective properties of BDNF compared with those of NGF and NT-3. Addition of BDNF (100 ng/ml) to the culture medium for 24 h protected the neurons against a concentration of glutamate (100 \mu\text{M}), which in these cells is known to induce cell death (Table I). Moreover, the survival effect of BDNF was similar to that produced by a subtoxic concentration of NMDA (Table I). NGF and NT-3 (100 ng/ml) were ineffective in preventing glutamate-mediated cell death (Table I), consistent with the lack of expression of functional TrkA or TrkC receptors in these cells.

**NMDA Increases BDNF Synthesis and Release**—To determine whether the NMDA survival-promoting activity correlated with increased availability of BDNF, we examined the temporal kinetics of BDNF synthesis and release after NMDA treatment. BDNF de novo synthesis was estimated by measuring the steady-state content of BDNF mRNA and protein at various times after NMDA treatment. NMDA elicited a time-
failed to elicit this response (data not shown), indicating that increased by NMDA at 5 min (Table II). An increase in accuracy medium from NMDA-treated cells revealed an increase in the concentration of NMDA (100 μM) increased TrkB tyrosine phosphorylation after NMDA treatment. A subtoxic dose of NMDA (100 μM) increased TrkB tyrosine phosphorylation (Fig. 4, upper panel). This increase was weaker than that evoked by maximal concentrations of BDNF (100 ng/ml), but it lasted up to 6 h (Fig. 4, upper panel) and was blocked by the selective NMDA receptor antagonist MK-801 (1 μM) (data not shown). As a control for potential artifacts attributable to a nonspecific activation of TrkB by NMDA, TrkB tyrosine phosphorylation was determined in NIH3T3 cells overexpressing TrkB receptors but not responsive to NMDA. Although BDNF increased TrkB tyrosine phosphorylation, exposure of these cells to NMDA (100 μM) failed to elicit this response (data not shown), indicating that the presence of NMDA receptors was required for TrkB activation.

**Activation of TrkB by NMDA Requires BDNF**—The temporal similarity between TrkB activation and increased BDNF levels in the medium suggested that the rapid (within minutes) effect of NMDA on TrkB phosphorylation was most likely attributable to the activity-dependent release of BDNF. The release hypothesis was tested by using a trkB "receptor body," a chimeric protein generated by the fusion of the extracellular portion of TrkB with the Fc domain of human IgG heavy chain

**TABLE I**

| Treatment                  | Cell survival (%) |
|----------------------------|-------------------|
| No treatment               | 95 ± 4            |
| Glutamate (100 μM)         | 46 ± 6            |
| NMDA (100 μM) + glutamate (100 μM) | 82 ± 8*         |
| BDNF (100 ng/ml) + glutamate (100 μM) | 81 ± 5*         |
| NT-3 (100 ng/ml) + glutamate (100 μM) | 44 ± 11         |
| NGF (100 ng/ml) + glutamate (100 μM) | 49 ± 9          |

* p < 0.01 by analysis of variance and Student’s t test.

**FIG. 2.** NMDA elicits a time-dependent increase in BDNF mRNA. Cerebellar granule cells were incubated with NMDA (100 μM) or NMDA plus MK-801 (1 μM) for the indicated times. BDNF mRNA levels were determined by RNase protection assay as described (30, 47) using a rat BDNF cRNA probe (31). Cyclophilin mRNA levels were used to equalize the amount of RNA present in each lane. Data are expressed as means ± S.E. of two independent and separate experiments (n = 4). * p < 0.01 (analysis of variance and Dunnett’s test).

**FIG. 3.** Western blot analysis of BDNF immunoreactivity in cell medium. Neurons were exposed for 10 min to NMDA or medium alone, and then medium (4 ml) was collected and concentrated (29). Concentrated medium was then applied to a 7.5% SDS-polyacrylamide gels. 5 ng of recombinant BDNF was used as a control (lane 1). Protein was transferred to nitrocellulose and probed overnight with BDNF antibody (Promega). Lane 2, medium from control cells; lane 3, medium from NMDA-treated cells.

**TABLE II**

| Treatment                  | BDNF pg/ml |
|----------------------------|------------|
| Control 2 min              | 39.0 ± 2.8 |
| NMDA 2 min                 | 64.2 ± 15* |
| Control 5 min              | 43.5 ± 5.7 |
| NMDA 5 min                 | 84.0 ± 21* |
| Control 3 h                | 84.0 ± 9.3 |
| NMDA 3 h                   | 151.3 ± 31* |

* p < 0.05 versus control (Student’s t test).
(trkB-IgG), which has been shown to block different BDNF biological activities (32, 33). Pretreatment for 10 min of cerebellar granule cells with trkB-IgG (20 μg/ml) inhibited the BDNF-mediated increase in TrkB tyrosine phosphorylation and abolished the ability of NMDA to enhance TrkB tyrosine phosphorylation (Fig. 5). These data confirm that NMDA increases TrkB phosphorylation most likely by enhancing BDNF release.

**NMDA Neuroprotection Requires Activation of TrkB**—The ability of trkB-IgG to efficiently block TrkB signaling allowed us to further test our hypothesis that BDNF and TrkB are involved in the neuroprotective effect mediated by NMDA. Preincubation of cerebellar granule cells with trkB-IgG (20 μg/ml) blocked the protective properties of NMDA (Fig. 6). In fact, counting surviving neurons revealed that the ability of NMDA to reduce cell death from toxic concentrations of glutamate is dramatically reduced (Fig. 6), confirming that the survival-promoting effect of NMDA is mediated by its ability to enhance TrkB signaling. To examine the specificity of trkB-IgG, cells were also preincubated with the Fc portion of IgG (10 μg/ml) before NMDA. In these cells, NMDA exerted a neuroprotective activity against an excitotoxic concentration of glutamate (Fig. 6), indicating that the effect of trkB-IgG is not attributable to its immunoglobulin property. Moreover, trkB-IgG did not adversely affect neuronal survival in the presence or absence of NMDA and did not increase the excitotoxicity of glutamate, indicating that trkB-IgG is not an agonist, antagonist, or modifier of the NMDA receptor (Fig. 6). Furthermore, preincubation of cultured neurons with the tyrosine phosphorylation antagonist K252a (10 nM) inhibited the NMDA receptor-mediated neuroprotection (Fig. 6), further suggesting that neuronal survival evoked by NMDA requires activation of the TrkB receptor. Interestingly, both TrkB-IgG and K252a did not alter neuronal survival (Fig. 6), suggesting that TrkB activation is not required for basal survival of cerebellar granule cells.

**DISCUSSION**

Stimulation of the NMDA glutamate receptor subtype can result in neuronal protection from glutamate toxicity (9). The cellular and molecular mechanisms of such neuroprotection are unclear. In the present study, we have tested the hypothesis that NMDA exerts trophic activity by activating neurotrophin signaling. We first established that BDNF but not NGF or NT-3 is the only neurotrophin that protects cerebellar granule cells against glutamate toxicity. Consistent with our data, previous findings have shown that BDNF protects cerebellar granule cells against glutamate-mediated cell death (10). This is probably attributable to the fact that TrkB is the only functional neurotrophin receptor expressed in cerebellar granule cells at day 8 in vitro, as also demonstrated by the ability of BDNF to induce phosphorylation of phospholipase C-γ (34), a protein that interacts with activated Trk and is involved in neurotrophin signaling (35). Most importantly, we have shown that NMDA elicits a time-dependent, measurable increase in BDNF in the medium and TrkB tyrosine phosphorylation, suggesting that one of the mechanisms underlying the ability of NMDA to activate TrkB tyrosine phosphorylation might be the increased release of BDNF, which, in turn, may activate the

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**Fig. 4.** NMDA induces TrkB phosphorylation in cerebellar granule cells. Cerebellar granule cells were exposed to medium alone (C), BDNF (BD, 50 ng/ml) for 10 min, or NMDA (N, 100 μM) for 10 min and 3 and 6 h. The cultures were then lysed and precipitated with pan-trk antibody before immunoblotting with 4G10 anti-phosphotyrosine monoclonal antibody. Upper panel, tyrosine phosphorylation of TrkB induced by BDNF and NMDA is indicated by the arrow. Lower panel, the blot was stripped and incubated with an antibody specific for the extracellular domain of TrkB (48) to confirm that similar amounts of TrkB proteins were present in each lane.

**Fig. 5.** TrkB-IgG blocks the NMDA-mediated increase in TrkB tyrosine phosphorylation. Cerebellar granule cells were incubated with medium alone (C), BDNF (BD, 50 ng/ml), BDNF plus TrkB-IgG (20 μg/ml) TrkB-IgG alone, NMDA (N, 100 μM), or NMDA plus TrkB-IgG for 10 min. The cells were harvested, and TrkB tyrosine phosphorylation (indicated by the arrow) was determined as described in Fig. 1. This experiment was performed twice with comparable results.

**Fig. 6.** TrkB-IgG and K252a block the NMDA-mediated neuronal protection. Cerebellar granule cells were exposed to medium alone or medium containing glutamate (Glu, 100 μM), trkB-IgG, k252a, or IgG for 24 h. Cells were also exposed to a subtoxic concentration of NMDA (100 μM), with or without trkB-IgG (20 μg/ml), K252a (10 nM), or IgG (10 μg/ml) for 6 h, followed by the addition of glutamate as described (see “Experimental Procedures”). Viable neurons were quantified using the fluorescein diacetate assay (9). Data are presented as percent of neuronal survival (mean ± S.D.). The experiments were performed in triplicate using two different preparations of neurons. *, p < 0.01 versus glutamate alone; †, p < 0.01 versus NMDA plus glutamate (analysis of variance and Student’s t test).
tyrosine phosphorylation of its own receptor in an autocrine manner. This autocrine loop, although described for developing sensory neurons of the dorsal root ganglia (25), is novel for central nervous system neurons. Because TrkB tyrosine phosphorylation is an essential event in the BDNF signal cascade, our data suggest that the activity-dependent survival effect of NMDA may rely on its ability to activate TrkB signaling via the release of BDNF.

We used different tools to test this hypothesis. First, we determined whether NMDA activates release and synthesis of BDNF. We have obtained data to support the idea that NMDA induces the release of BDNF. We showed an early (by 2 min) accumulation of BDNF in the medium of neurons incubated with NMDA. This accumulation was greater by 5 min and was not attributable to an increase in intracellular BDNF levels or BDNF mRNA. An increased in BDNF levels in the medium was also observed after 3 h incubation with NMDA. By this time, the NMDA-mediated increase in BDNF in the medium was accompanied by a concomitant accumulation of BDNF mRNA, suggesting that at 3 h NMDA, in addition to the release, also affects BDNF synthesis. Therefore, we propose that NMDA elicits two temporally distinct responses: an early release of BDNF and a later increased BDNF synthesis and release.

Further support for the hypothesis that NMDA may regulate the release of BDNF was obtained by examining the functional state of the TrkB receptor. It has been established that BDNF binding to TrkB evokes a highly specific, rapid, and easily measurable receptor autophosphorylation. Thus, TrkB phosphorylation can be a useful response to estimate the biological effectiveness of BDNF. Our data show that in cerebellar granule cells NMDA elicits a rapid increase in TrkB tyrosine phosphorylation (within 10 min). NMDA failed to increase TrkB phosphorylation in trkB-transfected NIH3T3 cells, which do not express NMDA receptors. Furthermore, the NMDA-mediated increase in TrkB phosphorylation was blocked by the NMDA receptor antagonist MK-801. Thus, TrkB activation by NMDA is a receptor-mediated event that occurs in cells responsive to glutamatergic input rather than a nonspecific effect of NMDA. Our data are also consistent with recent findings showing that glutamate enhances tyrosine phosphorylation of mitogen-associated protein 2 kinase (36), one of the downstream target proteins activated by neurotrophins (35) in neurons. By increasing BDNF release and activating TrkB receptors, NMDA may therefore indirectly exert trophic activity.

Consistent with the hypothesis that NMDA activates a BDNF autocrine loop, NMDA failed to increase TrkB tyrosine phosphorylation when neurons were exposed concomitantly to the trkB-IgG receptor body. TrkB-IgG has a binding affinity and specificity similar to TrkB and by being a soluble protein can sequester BDNF. Accordingly, it has been used as a new tool to block different biological activities of endogenously produced BDNF (32, 33). We showed that the ability of NMDA to prevent glutamate cell death was blocked when cerebellar granule cells were exposed to the trkB-IgG fusion protein. In contrast, the ability of NMDA to prevent glutamate toxicity was not blocked when cells were preincubated with IgG, indicating that the TrkB portion of the molecule inhibits the activity of NMDA, thus eliminating a possible immunoglobulin effect of trkB-IgG. Moreover, the important role of BDNF and TrkB signaling in NMDA-mediated neuroprotection is further supported by data showing that NMDA fails to prevent glutamate-mediated cell death when neurons were preincubated with K252a, a tyrosine kinase inhibitor used in several studies as a tool to inhibit neurotrophin activity. Together these data provide support for the hypothesis that the neuroprotective properties of NMDA may involve a BDNF autocrine loop.

The increase in TrkB tyrosine phosphorylation by NMDA is weaker than that elicited by BDNF. This apparent lack of correlation between the amount of tyrosine phosphorylation and neuronal protection is not surprising. In cultured cerebellar granule cells, a natural ganglioside, GM1 (6), as well as other synthetic gangliosides, block glutamate-mediated cell death without affecting the function of NMDA or other glutamate-operated cation channels (37). Interestingly, GM1, which does not affect synthesis of neurotrophins, has been shown to mimic neurotrophin activity by increasing TrkB function (28, 38, 39). When compared with BDNF, both GM1 and NMDA elicit a modest increase in TrkB tyrosine phosphorylation; however, both compounds prevent glutamate excitotoxicity, suggesting that there could be cross-talk between two or more signaling pathways resulting in a synergism or potentiation of the effect, perhaps via different substrates. In addition, the neuroprotective effect of NMDA may also involve other neurotrophic factors. A most likely candidate is basic fibroblast growth factor, a trophic factor known to prevent glutamate toxicity (40) whose expression is induced in cerebellar granule cells (41). Therefore, the BDNF autocrine loop may not account solely for NMDA-mediated neuroprotection. Future experiments will examine the possible role of other neurotrophic factors in NMDA-mediated neuronal protection.

In addition to the neuroprotective activity, BDNF also modulates both acute and long-term synaptic function (42–44), as well as NMDA receptor phosphorylation and expression (45, 46). Our observation that NMDA increases BDNF release and the consequent TrkB activation suggests a correlation between activity-dependent synaptic remodeling, neuronal growth, and differentiation (4, 5) with neurotrophic factor receptor activation. It will be important to establish whether similar mechanisms are operative in fully mature neurons and whether activation of other neurotransmitter receptors, such as β-adrenergic receptors, which are known to increase neurotrophin synthesis (47), can alter neuronal plasticity and therefore contribute to synaptic reorganization.

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