

NEUROPROTECTION: UNDERSTANDING THE MODUS OPERANDUM OF GLUTAMATE EXCITOTOXICITY

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The following review describes research using the Promega BDNF E_{max}[®] ImmunoAssay System in a continuous medium-withdrawal method for measuring BDNF release from NMDA-treated neurons. This method was initially developed by Ann M. Marini and colleagues in 1998 (5) in a landmark paper for ELISA-based neurotrophin detection.

Introduction

Glutamate is an excitatory amino acid neurotransmitter that is involved in neuronal plasticity within the brain during development (1) and is required for normal physiological excitatory responses of the mammalian central nervous system (CNS;2). In addition, the NMDA/glutamate receptor promotes neuronal survival—an effect that is counteracted by NMDA/glutamate receptor antagonists (1). As Daming Zhu and colleagues indicate, understanding how NMDA exerts neuroprotection might provide avenues for treatment strategies against neurodegenerative disorders (2).

Expression of the NMDA/glutamate receptor is quite complex and involves several different receptor subunits at various stages of development. NMDA/glutamate receptor activation results in neuroprotection of cerebellar granule cells, which in vivo might act to prevent neurodegenerative processes in the cortex of the brain (2). Robert H. Lipsky and colleagues have shown that NMDA induces a time-dependent increase in the amount of BDNF mRNA that lasts at least 12 hours (possibly as long as 24 hours) post induction and that NMDA provides protection against the damaging effects of excitotoxic concentrations of glutamate (2,3). Nearly 100% protection was achieved using 100 μ M NMDA for cells treated with 100 μ M glutamate. As Lipsky and colleagues have shown in their gel retardation assays, this induction of BDNF expression is mediated via the activation of the NF- κ B transcription factor (3). Competition experiments with an unlabeled oligo have shown this interaction to be specific to BDNF. Treatment of these same cells with the NMDA receptor antagonist MK-801 completely eliminated the NF- κ B DNA binding activity, confirming that the activation of BDNF expression was specific to the action of NMDA. This result has been confirmed by Jiang and colleagues in their measurements of BDNF mRNA in MK-801-treated cells (1).

Continuous Medium-Withdrawal Assay

Using the BDNF E_{max}[®] Immunoassay System in a continuous medium-withdrawal assay to quantify the amount of BDNF released into the medium from NMDA-treated neurons, Jiang and colleagues have also reported on how

NMDA/glutamate receptor stimulation produces an increased expression of BDNF and TrkB receptor phosphorylation (1). In further experiments, Jiang and colleagues showed that glutamate concentrations above 20 μ M were toxic to their hippocampal neuron cultures (1). The NMDA/glutamate receptor clearly plays a role in this excitotoxicity since the NMDA receptor antagonist, MK-801, markedly reduced this effect. Moreover, sensitivity to excitotoxicity varied, depending on the embryonic stage from which culture neurons were prepared (1). By pretreating neurons with subtoxic concentrations of either glutamate (<10 μ M; 1) or NMDA (<100 μ M; 2), glutamate excitotoxicity was reduced.

Wu and colleagues have provided evidence that demonstrates how the drugs AMPA and aniracetam exert neuroprotective activity through an increased expression of BDNF (4). As part of their study, Wu and colleagues used the BDNF E_{max}[®] Assay, again in a continuous medium-withdrawal assay, to ascertain the amount of BDNF protein released into the medium of cerebellar granule cell cultures exposed to AMPA and aniracetam (4). In treated cultures, qRT-PCR experiments and BDNF immunoassays showed an increase in BDNF mRNA and protein levels, respectively, over untreated cultures. Using antibodies against the phosphorylated forms of ERK1 and ERK 2, Zhu and colleagues have shown that a neuroprotective concentration of NMDA (100 μ M) in cultured cerebellar granule cells leads to phosphorylation of both of these proteins (2).

What the Future Holds

Glutamate plays an important role in certain types of neuronal damage, in particular hypoxic-ischemic neuronal damage and cell death. Understanding the downstream mechanisms through which neurons protect themselves against the effects of excitotoxic concentrations of glutamate is a critical aspect of potential therapy development against such damage. A detailed characterization of the molecular aspects of disease onset could play an essential role in the future development of what Jiang and coworkers call “targeted therapeutic interventions” (6).

Glutamate Excitotoxicity

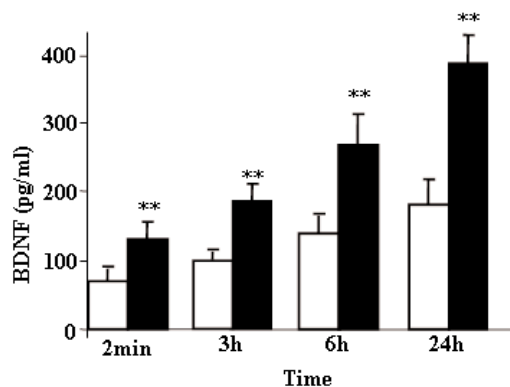


Figure 1. NMDA evokes the release of BDNF in hippocampal medium. Hippocampal cultures were exposed for 2 minutes, 3 hours, 6 hours and 24 hours to medium alone (white bars) or to medium containing NMDA (50 μ M, black bars) beginning on DIV 8. Medium (2 ml) was collected, concentrated to 100 μ l, and assayed by the ELISA two-site immunoassay. Data are expressed as the mean \pm SD; n = 5; **p < 0.05 versus medium alone by Anova. Figure and legend reprinted with the kind permission of A. Marini and *J. Neurochemistry* from reference 1.

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Protocol

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Ordering Information

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