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Research Report

Chronic glutamate toxicity in mouse cortical neuron culture

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ABSTRACT

Two pathways for glutamate toxicity have been described, receptor-mediated excitotoxicity and non-receptor mediated oxidative glutamate toxicity. Here, we show that two distinct forms of receptor-mediated primary cortical neuronal death exist, chronic and acute glutamate toxicity, and that these depend on exposure time. *In vitro*, neuronal sensitivity to chronic glutamate exposure increased as neurons matured and the initial plating medium contributed as well. In immature neurons, high concentrations of glutamate induced neuronal death. The chronic glutamate toxicity was independent of neuronal density, whereas increased density potentiated acute glutamate toxicity. Activation of ionotropic glutamate receptors (iGluRs) contributed to induction of chronic and acute glutamate toxicity at similar rates at DIV14. Inactivation of the metabotropic glutamate receptors (mGluRs) by AIDA increased neuronal sensitivity to chronic glutamate exposure but not to acute exposure. Neuronal death by acute toxicity was much faster than by chronic toxicity in which activation of mGluRs was involved. These results suggest that acute glutamate toxicity is quite different from chronic toxicity, in which activation of mGluRs is associated with resistance to glutamate toxicity.

1. Introduction

Glutamate is the major fast excitatory neurotransmitter in the mammalian central nerve system. However, when excessively released and accumulated in the extracellular space of the brain, endogenous glutamate is associated with neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease (Choi, 1988; Simonian and Coyle, 1996). Thus far, two distinct glutamate-induced cell death pathways have been identified. The excitotoxic pathway relies on hyper-activation of glutamate receptors (Choi, 1992; Rothman, 1985), whereas the oxidative pathway involves breakdown of the glutamate/cystine anti-porter without glutamate receptor-mediation (Murphy et al., 1989; Sato et al., 1999). Recently, two pathways have been identified in a primary neuron culture, which suggests oxidative glutamate toxicity is a component of the excitotoxicity cascade (Schubert and Piasecki, 2001). Neuronal cell death mediated by prolonged exposure to NMDA and non-NMDA glutamate receptor agonists has been observed in cortical neuron culture (Dugan et al., 1995; Koh et al., 1990). However, neurotoxicity by chronic glutamate exposure may be considered a slow variant of acute glutamate toxicity (excitotoxicity) because the sensitivity to glutamate and receptor-specific agonists is similar in chronic and in acute toxicity. Generally, chronic glutamate toxicity has been overlooked in primary neuron culture, presumably because high glutamate concentrations (mM range) are required in cultures of immature neurons as in NMDA

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receptor-deficient HT22 cells, an excellent model for oxidative glutamate toxicity (Maher and Davis, 1996). However, the expression of iGluRs at early culture stages (DIV3–5) (Lesuisse and Martin, 2002; Sagara and Schubert, 1998) indicates that iGluR receptor-mediated toxicity possibly occurs by chronic and acute glutamate exposure. In this study, we analyzed neuronal responses to glutamate by comparing the sensitivities observed in chronic and acute glutamate exposure.

2. Results

2.1. *Glutamate sensitivity in chronic exposure*

First, to determine the sensitivity to glutamate during chronic exposure, cytotoxicity was monitored 24 h after exposure in cortical neuron culture at DIV7 and 14. When neurons were initially cultured in MEM with 10% serum, the neuronal sensitivity to glutamate was higher than in NB medium at DIV7 (Fig. 1A) but similar at DIV14 (Fig. 1B). In the NB medium, serum did not significantly alter neuronal glutamate sensitivity. In all tested culture mediums, neuronal sensitivity to chronic glutamate exposure increased as the neurons matured from DIV7 to DIV14. Neurons cultured in MEM supplemented with glucose showed increased sensitivity (data not shown). Along with these results, we suggest differential sensitivity to glutamate depending on culture conditions. Interestingly, neurons initially cultured in NB medium were highly resistant to chronic glutamate exposure at DIV7 and required more than 2 mM glutamate to achieve 80% cell death (Fig. 1A). To investigate whether neuronal glutamate resistance affects acute glutamate toxicity

Table 1 – Toxicity of glutamate and NMDA.

		DIV7	DIV14
Acute	Glutamate	42±3	14±1
	NMDA	63±3	54±3
Chronic	Glutamate	685±39	35±3
	NMDA	82±7	53±4

The EC_{50} concentrations (μM) were derived from concentration–cytotoxicity curves.

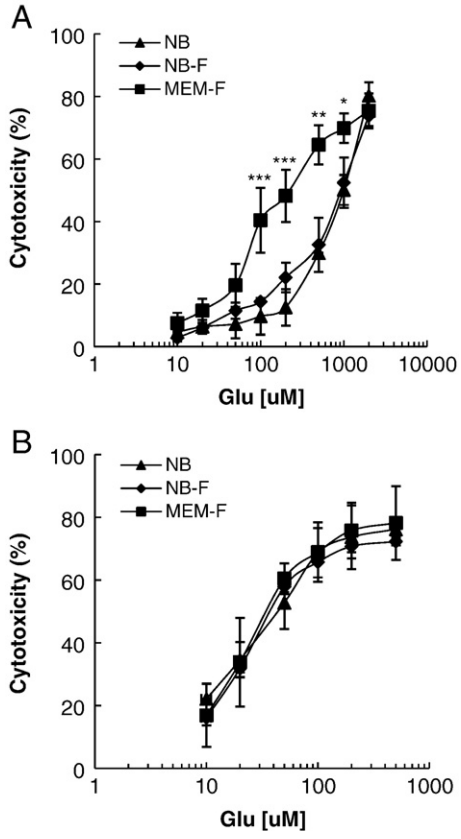

Fig. 1 – Neuronal sensitivity to chronic glutamate exposure depends on the initial plating medium and time in culture. Eagle's minimal essential medium (MEM) supplemented with 10% fetal bovine serum (MEM-F) or B27 neurobasal medium alone (NB) or containing 10% fetal bovine serum (NB-F) were used for 3 days and replaced with serum-free B27 neurobasal medium (NB) media. Neuronal (1×10^5 cells per well) cytotoxicity in chronic exposure was measured by LDH release at DIV7 (A) and DIV 14 (B). Results of triplicate experiments are shown. The values were expressed as means±SD. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ as compared with both NB and NB-F.

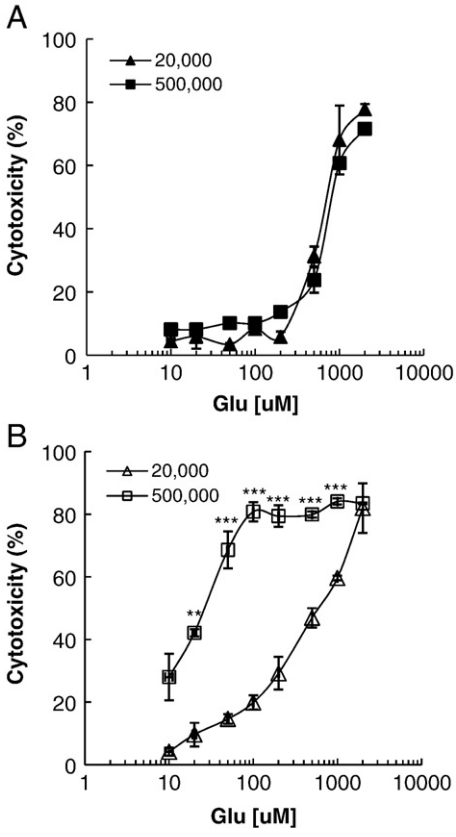

Fig. 2 – Neuronal sensitivity to glutamate depends on cell density in chronic and acute exposure at DIV7. Neurons were plated and cultured in serum-free NB medium at 2×10^4 and 5×10^5 cells per well. At DIV7, cytotoxicity was observed in chronic (A) and acute (B) glutamate exposure. The values were expressed as mean±SD. $^{**}p<0.01$ and $^{***}p<0.001$ *versus* 2×10^4 cells.