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Rapid communication

Selective NR2B NMDA receptor antagonists are protective against staurosporine-induced apoptosis ☆

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Abstract

Staurosporine-induced apoptosis was associated with a 20% cellular survival rate in primary rat forebrain cultures. Treatment with the NR2B subunit-selective NMDA receptor antagonist conantokin-G (0.1–1 μM) increased the survival rate up to 78%. No protection was provided by the nonselective NMDA receptor antagonist dizocilpine (0.01–10 μM) but 34–64% cellular survival was provided by ifenprodil (0.01–10 μM), another NR2B subunit-selective antagonist. These results suggest a novel anti-apoptotic mechanism linked to the NR2B receptor subunit.
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Keywords: Conantokin-G; Neuronal culture; Staurosporine

The development of NMDA receptor antagonists for the treatment of brain injury has been limited due to resultant side effects and the narrow therapeutic treatment window of these compounds (Steinberg et al., 1995). It is likely that the limited therapeutic window seen with NMDA receptor antagonists relates to their relative inability to interfere with delayed injury processes such as apoptosis. Indeed, it has been previously reported that the NMDA receptor antagonist dizocilpine (MK-801) is not effective against staurosporine-induced apoptosis in cultured neurons (Koh et al., 1995) and the question of NMDA receptor involvement in apoptosis remains largely unresolved.

Recently, the development of a novel class of highly potent, neuroprotective NMDA receptor antagonists has been identified and studied pre-clinically as a potential therapy for a variety of central nervous system (CNS) disorders (Prorok and Castellino, 2001). The prototype compound, CGX-1007 (conantokin-G), has picomolar affinity for the NMDA receptor, specifically binds the NR2B subunit (Donevan and McCabe, 2000) and is distinguished as a potent neuroprotective agent (Williams et al., 2000). Here, we report the effect of CGX-1007 (as compared to MK-801 or ifenprodil) to reduce staurosporine-induced apoptosis in primary cultures and provide the first evidence for NR2B mediation of apoptotic cell death.

Enriched neuronal cultures were prepared from 15-day old Sprague–Dawley rat embryos as described previously (Williams et al., 2000). Briefly, cells were isolated, dissociated and were plated at a density of 5×10^5 cells/well in 48-well culture plates pre-coated with poly-L-lysine. Cultures were maintained in a medium containing equal parts of Eagle's basal media (without glutamine) and Ham's F12 k media supplemented with 10% heat-inactivated horse-serum, 10% fetal bovine serum, glucose (600 μg/ml), glutamine (100 μg/ml), penicillin (50 units/ml), and streptomycin (50 μg/ml). After 48 h, cytosine arabinoside (10

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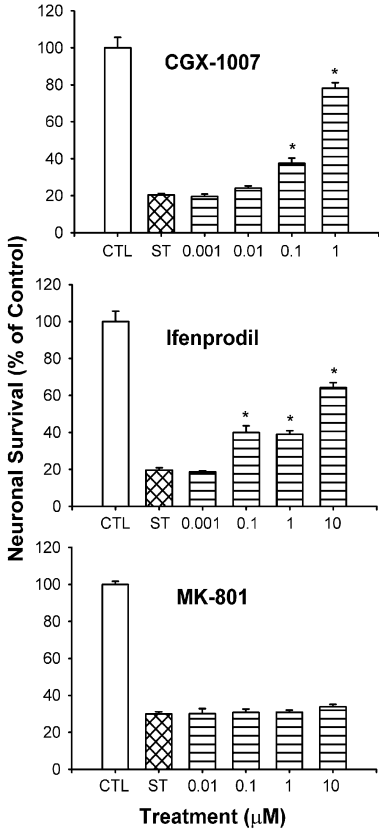

Fig. 1. Dose–response effect of CGX-1007, ifenprodil, and MK-801 against staurosporine (ST)-induced apoptosis. Each treatment response is compared to the untreated control (CTL) group and ST-only treated group. Values are given as the mean ± S.E.M. $*P<0.01$ as compared to ST-treated group.

μM) was added to inhibit nonneuronal cell division. Cells were used in experiments after 7 days in culture.

Cells were exposed to staurosporine (1 μM) for 45 min and co-treated with CGX-1007 (0.001–1 μM, Cognetix, Salt Lake City, UT), ifenprodil (0.01–10 μM, RBI, Natick, MA), or MK801 (0.01–10 μM, RBI, Natick, MA) in Locke's solution. At the conclusion of the staurosporine exposure, the condition media (original) was replaced and 24 h later the morphological and cell viability assessments were made. Cell damage was quantitatively assessed using a tetrazolium salt colorimetric assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT); Sigma, St. Louis, MO) (Williams et al., 2000). Significant differences in cell viability among treatment groups were determined using one-way analysis of variance and the Newman–Keuls test.

Staurosporine produced apoptotic cell death morphologically defined by cell shrinkage, chromatin condensation, and appearance of apoptotic bodies (not shown), reducing cellular survival to 20–30% as compared to control cell cultures. When cells were co-treated with increasing doses of CGX-1007 (Fig. 1, upper panel), ifenprodil (Fig. 1, middle panel), or MK-801 (Fig. 1, lower panel) differential neuroprotective effects were measured. Co-treatment with CGX-1007 provided significant neuroprotection with cellular survival rates of up to 78%. Co-treatment with ifenprodil was also neuroprotective, providing up to 64% neuronal survival at the highest dose tested. In contrast, the NMDA receptor antagonist MK-801 was ineffective at doses, which have been shown to be highly neuroprotective against other forms of neurotoxic insults.

Neurodegeneration from a variety of CNS insults has been described as a necrotic/apoptotic continuum of cell death (Martin et al., 1998). Thus, neuroprotection strategies that target both the acute (necrotic) and delayed (apoptotic) cell death responses may possess an improved neuroprotective potential for the treatment of ischemic brain injury as opposed to agents that target only one type of cell death pathway. Our results suggest that CGX-1007, which specifically bind the NR2B subunit of the NMDA receptor (Donevan and McCabe, 2000), and inhibits necrotic cell death (Williams et al., 2000), is highly effective at blocking the apoptotic damage induced by the protein kinase inhibitor staurosporine and is more effective than ifenprodil (78% vs. 64% cell survival). Importantly, the nonsubunit-selective NMDA receptor antagonist MK-801 had no effect against staurosporine-induced injury. These data indicate that neuronal apoptosis can be attenuated with compounds that specifically target the NR2B subunit, at least as defined in this in vitro model. It remains to be determined how the modulation of the NR2B subunit can affect the intracellular signaling cascades involved in the induction of apoptosis.

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