Published in final edited form as:

Epilepsia. 2010 July ; 51(Suppl 3): 102–105. doi:10.1111/j.1528-1167.2010.02621.x.

Inverse relationship between seizure expression and extrasynaptic NMDAR function following chronic NMDAR inhibition

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Summary

We showed previously that electrographic seizures involving dentate granule cells in organotypic hippocampal slice cultures were dramatically reduced following chronic treatment with the NR2B-selective antagonist, Ro25,6981, but were increased following chronic treatment with the high-affinity competitive antagonist, APV. To begin to investigate the potential mechanisms underlying the differential effects of NMDAR antagonists on seizures, electrophysiological experiments were conducted in dentate granule cells in hippocampal slice cultures treated for the entire 17–21 day culture period with vehicle, Ro25,6981 or D-APV. Initial experiments revealed a lack of an association between mEPSC measures and seizures suggesting that shifts in mEPSC were unlikely to account for the differential effects of D-APV and Ro25,6981 on seizures. However, the amplitude of tonic NMDAR-mediated currents was reduced in cultures treated chronically with D-APV and dramatically enhanced in cultures treated chronically with Ro25,6981. Since tonic NMDAR currents are mediated primarily by extrasynaptic NMDAR, these data show an inverse relationship between changes in extrasynaptic NMDAR function and alterations in seizure expression.

Keywords

dentate gyrus; NR2B; Ro25; 6981; organotypic hippocampal slice culture; tonic current

Introduction

Too much *N*-methyl-D-aspartate receptor (NMDAR) activation is associated with pathophysiology in epilepsy, traumatic brain injury and a variety of other nervous system disorders (Dingledine et al., 1990; Lipton, 2007). Too little NMDAR activation also causes pathophysiology including cognitive and psychotomimetic dysfunction, neuronal death, paradoxical seizure exacerbation and increased synaptic connectivity and ionotropic glutamate receptor expression (Olney et al., 1989; Sveinbjornsdottir et al., 1993; Muir and Lees, 1995; Loscher, 1998; Lin and Constantine-Paton 1998; McKinney et al. 1999; Parsons

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Disclosure: None of the authors has any conflict of interest to declare.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this article is consistent with those guidelines.

et al., 1999). Therefore, NMDAR antagonists that reduce pathological over-activation while maintaining physiological levels of NMDAR function therefore may have clinical utility. Allosteric antagonists that selectively target NMDAR containing the NR2B subunit inhibit NMDAR in a use-dependent manner and maintain some level of NMDAR function even under conditions of maximal binding. The greatest inhibition (~80%) occurs at very high glutamate concentrations, while a slight potentiation manifests at very low glutamate concentrations due to increased glutamate affinity for NMDAR (Kew et al., 1996). NR2B-selective NMDAR antagonists are still in clinical trials, but are well-tolerated in broad patient populations (Muir and Lees, 1995; Palmer, 2001; Kemp and McKernan, 2002).

Experimental approach

To examine the effects of NR2B-selective antagonists on epileptogenesis, excitatory neurotransmission and glutamate receptor function we used an *in vitro* model of epileptogenesis. Organotypic hippocampal slice cultures isolated from postnatal day 10–11 Sprague-Dawley rats maintain intrinsic hippocampal networks and layer-specific projections but experience trauma, deafferentation, neuronal loss and neuronal circuitry rearrangements similar to the events leading to acquired epilepsy in humans and animal models. They also exhibit a latent period followed by onset of seizures, a hallmark of acquired epilepsy. Multiple, recurrent electrographic seizures precipitated by acute application of the GABA_A receptor antagonist, bicuculline methiodide (BMI, 10 μ M) occur in the normally seizure resistant dentate granule cell layer in 0% of cultures at <5 days *in vitro* (DIV), 67% at 10–13 DIV, and 100% at $\geq$ 17 DIV (see Bausch 2009). These data provide support for an ongoing process of epileptogenesis and for using hippocampal slice cultures as a relatively simple first step to investigate the relationship between seizure expression and functional changes following chronic treatment with NMDAR antagonists. Hippocampal slice cultures were treated chronically for the entire 17–21 day culture period with the NR2B-selective NMDAR antagonist, Ro25,6981 (1 μ M) or the high-affinity competitive NMDAR antagonist, D(-)-2-amino-5-phosphonopentanoic acid (D-APV, 50 μ M). D-APV was included to examine the effects of complete NMDAR blockade.

Effects of NMDA receptor inhibition

We showed previously that the total duration of electrographic seizures induced by acute application of a GABA_A receptor antagonist (BMI, 10 μ M) or removal of Mg²⁺ from the recording buffer was significantly increased in D-APV- compared to vehicle-treated cultures (Fig. 1 and Wang and Bausch, 2004; Dong and Bausch, 2005). This finding was consistent with the seizure exacerbation reported by some epilepsy patients following chronic treatment with another high-affinity competitive NMDAR antagonist, D-CPP-ene (Sveinbjornsdottir et al. 1993). Cultures treated similarly with Ro25,6981 exhibited significantly fewer seizures and a significantly lower total seizure duration (Fig. 1 and Wang and Bausch, 2004; Dong and Bausch, 2005). The effects of D-APV and Ro25,6981 were long-lasting and not due to incomplete washout of antagonists prior to recordings. Results were similar when D-APV or Ro25,6981 were removed from the culture media 24–48 hr prior to recordings and no significant effects on electrographic seizures were noted when Ro25,6981 was acutely applied to vehicle-treated cultures (Wang and Bausch 2004 and data not shown).

To begin to discern potential mechanisms contributing to opposite effects of chronic D-APV and Ro25,6981 treatment on seizure expression, we focused on excitatory networks because of published reports describing effects of chronic NMDAR blockade on excitatory neurotransmission (Rao and Craig, 1997; Liao et al., 1999). We hypothesized that chronic treatment with D-APV would promote plasticity that increased, while Ro25,6981 would

support plasticity that decreased excitatory transmission in dentate granule cells. Consistent with our hypothesis, whole-cell voltage clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) recorded in the presence of bicuculline and tetrodotoxin revealed that chronic NMDAR blockade with D-APV dramatically increased mEPSC amplitude and charge transfer. Miniature EPSCs were measured at a -70 mV holding potential and were abolished by CNQX, suggesting an upregulation of AMPAR/KAR (Bausch et al. 2006 and data not shown). Granule cell recordings from Ro25,6981-treated cultures revealed intermediate increases in the cumulative probability plots of mEPSC amplitude and charge transfer compared to D-APV and vehicle-treated cultures (not shown). The lack of an association between mEPSCs and seizures suggests that shifts in mEPSC amplitude and by extension, postsynaptic AMPAR function were unlikely to account for the differential effects of D-APV and Ro25,6981 on seizures.

We next examined potential changes in NMDAR-mediated transmission. Minimal changes in NMDAR-mediated mEPSC (mEPSC_{NMDAR}) amplitude and frequency were noted in cultures treated chronically with D-APV or Ro25,6981 (not shown). However, the amplitude of tonic NMDAR-mediated currents was reduced in cultures treated chronically with D-APV and dramatically enhanced in cultures treated chronically with Ro25,6981 (Fig. 2). Tonic currents were mediated by NMDAR because they were blocked by acute application of D-APV during recordings (not shown). Since tonic NMDAR currents are mediated primarily by extrasynaptic NMDAR (Le Meur et al., 2007), these data show an inverse relationship between changes in extrasynaptic NMDAR function and alterations in seizure expression.

Discussion

The consequences of changes in tonic NMDAR-mediated currents are unclear. Tonic NMDAR currents mediated by ambient glutamate levels can enhance neuronal excitability (Sah et al., 1989), which could lead to depolarization-induced block of action potential generation. Conversely, extrasynaptic NMDAR activation may lead to a shunting effect, analogous to shunting inhibition observed following extrasynaptic GABA_A receptor activation, which could lead to impaired excitatory synaptic input integration and reduced action potential generation (Semyanov et al., 2004) when dendrites are depolarized to near the NMDAR reversal potential during seizures. Lastly, altered tonic NMDAR-mediated currents could represent homeostatic changes that have little or no effect on seizure expression. Further studies are needed to differentiate between these possibilities.

Acknowledgments

We thank Dr. Angelique Regnier, Ms. Norah Harwood, and Mr. Craig Budinich for assistance. Work was supported by the Defense Brain and Spinal Cord Injury Program and National Institute of Neurological Disorders and Stroke grant NS045964. All treatment of animals was consistent with institutional, Department of Defense and National Institutes of Health guidelines. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or Uniformed Services University.

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Figure 1.

Chronic treatment of organotypic hippocampal slice cultures with the NR2B-selective antagonist, Ro-25,6981 decreased while D-APV increased the total duration of electrographic seizures induced by (A) acute application of bicuculline methiodide (BMI, 10 μ M) or (B) removal of Mg^{2+} from the recording buffer. Extracellular field potentials were recorded in the granule cell layer from hippocampal slice cultures treated chronically for the entire 17–26 day culture period with the allosteric NR2B-selective NMDAR antagonist, Ro25,6981 (1 μ M) or the competitive non-subunit-selective NMDAR antagonist, D-APV (50 μ M) as described previously (Wang and Bausch 2004). Seizures were defined as a burst of rhythmic activity ≥ 3 s in duration that evolved over time and exhibited an abrupt onset and abrupt termination (Bausch and McNamara, 2000). Bars indicate mean $\pm$ SEM. Numbers of cultures are indicated in parentheses. * $p < 0.05$, different than vehicle, ANOVA by Ranks with Dunn's *post hoc* comparison.

Figure 2.

Representative traces show that chronic treatment of hippocampal slice cultures with Ro-25,6981 increased while D-APV decreased tonic NMDA receptor-mediated currents in granule cells. Recordings were conducted at a -70 mV holding potential in room temperature 0 mM Mg^{2+} recording buffer containing TTX (1 μ M), BMI (10 μ M) and NBQX (10 μ M). Note the change in holding current and increase in noise following application of the highly selective excitatory amino acid transporter blocker, DL-TBOA (50 μ M, bar). Recording pipettes were filled with a Cs-methanesulfonic acid-based solution. Scale bar is for all traces.