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Trafficking of NMDA receptors during status epilepticus: Therapeutic implications

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Summary

We used two models of status epilepticus (SE) to study trafficking of *N*-methyl-D-aspartate (NMDA) receptors. SE is associated with increased surface expression of NR1 subunits of NMDA receptors, and with an increase of NMDA synaptic and extrasynaptic currents suggesting an increase in number of functional NMDA receptors on dentate granule cells. The therapeutic implications of these results are discussed.

KEY WORDS: NMDA receptor, Receptor trafficking, Status epilepticus, Acute seizures, Cholinergic seizures, Epilepsy, Monotherapy, Hippocampus.

Despite the emergence of several new anticonvulsants, status epilepticus (SE) is still associated with significant morbidity and mortality. Pharmacoresistance to benzodiazepines and other drugs develops quickly (Mazarati et al., 1998). Trafficking and inactivation of synaptic $GABA_A$ receptors explains benzodiazepine pharmacoresistance (Naylor et al., 2005) but not the enhanced glutamatergic excitation or the effectiveness of NMDA antagonists in late stages of SE (Mazarati & Wasterlain, 1999).

Here we found during SE a relocation of *N*-methyl-D-aspartate (NMDA) receptor subunits to the cell surface with an increase in postsynaptic response in dentate granule cells, suggesting a potential mechanism for seizure maintenance during SE and for SE-related excitotoxicity.

We used a standard lithium/pilocarpine model of SE and a model of SE after intrahippocampal injection of neurokinin B (Naylor et al., 2013) to study NMDA receptor trafficking and its therapeutic implications. After 1 h of SE, rats were perfused/fixed for immunocytochemistry or hippocampal slices were prepared for whole-cell patch clamp of dentate granule cells (Naylor et al., 2013).

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NR1, the essential subunit of NMDA receptors, combines primarily with NR2A and/or NR2B subunits in the hippocampus. In granule and pyramidal cells from control hippocampi, NR1 subunit-like immunoreactivity (LI) localizes to the cell interior. After 1 h of SE, much of this NR1 subunit-LI has relocated to discrete puncta, which outline the cell membrane, and frequently colocalize with the synaptic marker synaptophysin-LI. In the soma of hippocampal granule cells (Fig. 1A), the number of overlaps between NR1 subunit-LI and synaptophysin-LI increases from 1.85 ± 0.18 per soma in controls to 7.15 ± 0.28 in SE ($p < 0.001$). Proximal dendrites, CA3, and CA1 pyramids show similar increases.

By electron microscopy, the number of asymmetric synapses in the dentate granule cell layer that were immunoreactive for NR1 increased from $8.4 \pm 2.8\%$ in controls to $26.9 \pm 4.7\%$ in SE (Fig. 1A–D), confirming the light microscopy results. The percentage of asymmetric synapses with more than one gold particle increased from $0.5 \pm 0.3\%$ in controls to $4.7 \pm 1.2\%$ after SE ($p < 0.05$; Fig. 1E). A greater proportion of NR1 particles was located in the periphery of asymmetric synapses after SE (Fig. 1F), suggesting the possibility of a perisynaptic location and of lateral entry of subunits into synapses ($45.3 \pm 3.2\%$ for controls versus $58.5 \pm 2.7\%$ for SE; $p < 0.05$).

In SE induced by intrahippocampal neurokinin B, similar increases were observed by immunocytochemistry. This

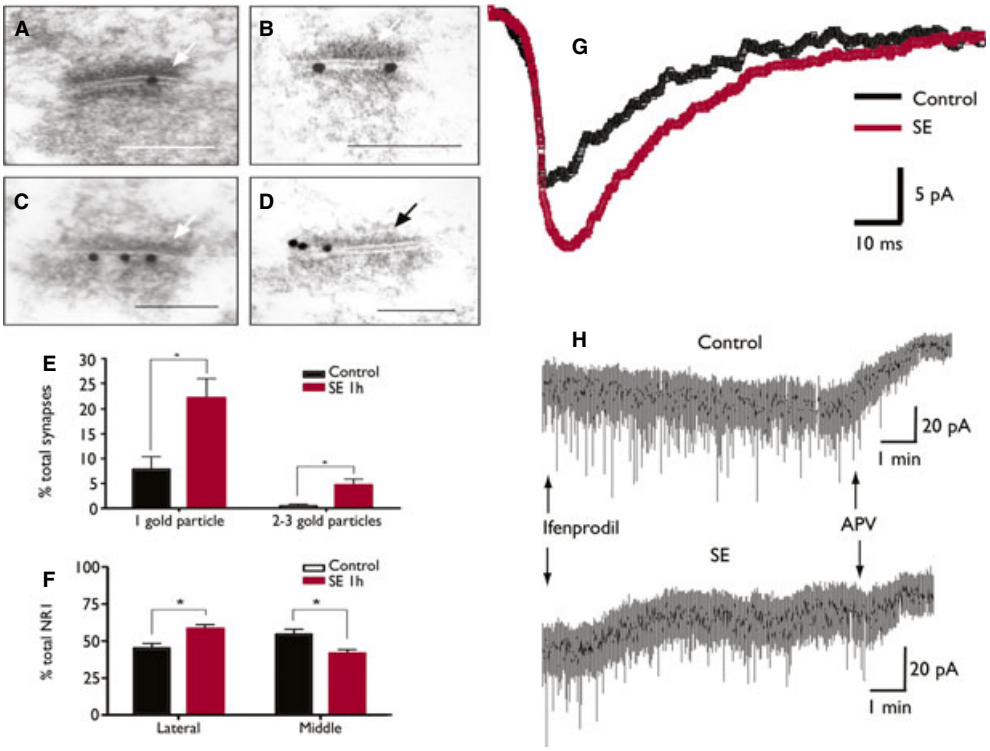

Figure 1.

A–**D**: Distribution of NR1-LI in asymmetric synapses of the granule cell layer of the dentate gyrus from control (**A, B**) and SE animals (**C**, **D**). Arrows indicate the postsynaptic density (bars = 150 nm). Asymmetric synapses show central/synaptic immunogold labeling (**A**, **C**) or a mix of central/synaptic and lateral/perisynaptic immunogold labeling of postsynaptic densities (**B**, **D**). SE increases the percentage of immunogold-labeled asymmetric synapses compared to controls (**E**). The lateral distribution of NR1 on the surface of synapses also increases with SE (**F**). (Error bars are ± standard error of the mean, SEM). **G**: Miniature excitatory postsynaptic NMDA currents (NMDA-mEPSCs) from representative dentate gyrus granule cells of SE (gray) and control (black) demonstrating larger amplitude and area under the curve (AUC) after SE. **H**: Ifenpodil (3 μM)-sensitive component of NMDA tonic currents of granule cells increases after SE. Both control and SE cells have significant baseline shifts with the subsequent addition of the nonselective NMDA receptor antagonist, APV.

increase in synaptic and/or perisynaptic NR1 subunits is likely to reflect movement of whole NMDA receptors to the membranes, since isolated subunits remain in the Golgi apparatus and lack the machinery for trafficking to the membrane.

To examine whether this immunocytochemical change did indeed reflect an increase in functional NMDA receptors on the cell surface, we recorded miniature excitatory NMDA currents (mEPSCs) from dentate granule cells in hippocampal slices obtained from animals in lithium-pilocarpine SE for 1 h (Fig. 1G). Mean traces of NMDA-mEPSCs recorded from dentate gyrus granule cells in slices from SE animals (Fig. 1G) displayed increased peak amplitude (−20.2 ± 2.7 pA for SE vs. −16.4 ± 0.64 pA for controls; $p < 0.001$).

To interpret the basis for the increase in amplitude and area under the curve (AUC) with SE, a model using mean-variance analysis of NMDA receptors suggested that the number of NMDA receptors increased during SE from 8 ± 1 per synapse in controls to 11 ± 2 per synapse with SE ($p < 0.001$).

In addition to synaptic relocation of NMDA receptors, we found relatively minor changes affecting NMDA-mEPSC kinetics, which predict that no change occurred in channel conductance with SE.

With ifenprodil in the perfusate, a positive tonic current baseline shift from of −76.0 ± 41.1 pA to −66.8 ± 34.5 pA ($p < 0.05$; $n = 17$) for SE granule cells held at −60 mV revealed a depolarizing tonic current of +9.2 pA, presumably mediated by NMDA receptors containing

NR2B subunits (Fig. 1H). No tonic current shift was observed in controls (-2.6 ± 12.9 pA; n.s.; n = 5).

After 1 h of SE, dentate granule cells display a 38% increase in the number of physiologically active NMDA receptors per somatic synapse, a twofold increase in the number of NR1 subunit-LI in the vicinity of the presynaptic marker synaptophysin, and a threefold increase in NR1-like immunoreactivity associated with postsynaptic densities. The NR1 immunoreactivity is likely a marker for functional heteromeric NMDA receptor complexes because all hippocampal NMDA receptors contain NR1 subunits, and solitary NR1 subunits are retained in the ER (Prybylowski et al., 2002). These changes could in part account for the enhanced glutamatergic excitation during SE (Wasterlain et al., 2000), and for the therapeutic efficacy of NMDA antagonists in some models of SE (Bertram & Lothman, 1990; Mazarati & Wasterlain, 1999). This movement of NMDA receptor subunits may reflect trafficking of NMDA receptor toward perisynaptic areas followed by lateral movement into synapses, and coincides with an internalization of synaptic γ-aminobutyric acid $(GABA)_A$ receptor. Both changes appear maladaptive in a seizing brain, and further exacerbate an imbalance between synaptic excitation and inhibition. We do not know whether physiologic recordings underestimate those changes because the slice rests for 90–120 min (without seizures) before recordings, or whether immunocytochemisty may detect extrasynaptic surface receptors that do not contribute to NMDA-mEPSC responses.

The increase of synaptic NMDA receptors may characterize an important transition when augmented synaptic excitation, coupled with a major loss of synaptic inhibition, provides the framework for progression from single seizures to self-sustaining SE. Although loss of inhibition may be important with initiation of SE, augmented glutamatergic excitation is important for the maintenance of SE (Mazarati & Wasterlain, 1999). These findings have therapeutic implications: the standard initial treatment of SE (benzodiazepines) is insufficient because increased glutamatergic excitation is untouched. Furthermore, benzodiazepines are unlikely to fully restore GABAergic inhibition given the dramatic reduction in number of postsynaptic receptors available for allosteric activation. Combinations of a benzodiazepine with an antagonist at NMDA receptors and another drug that increases inhibition at a nonbenzodiazepine site would more fully address the effects of SE-induced receptor trafficking. Indeed, such combinations seem to be more effective than monotherapy at stopping SE (Wasterlain et al., 2011).

Overactivity of NMDA receptors can increase excitotoxic neuronal death, and NMDA receptor blockade may also prove neuroprotective during SE (Fujikawa, 1995; Mazarati et al., 2000; Frasca et al., 2011) and modify SE-associated epileptogenesis (Mazarati et al., 2002) Because early treatment seems very effective in SE (Silbergleit et al., 2012), polytherapy initial treatment of SE with drug combinations including an NMDA antagonists deserves further study.

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Disclosure

The Authors have no conflict of interest to declare.

The authors confirm that they have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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