Impaired D-Serine-Mediated Cotransmission Mediates Cognitive Dysfunction in Epilepsy.

The modulation of synaptic plasticity by NMDA receptor (NMDAR)-mediated processes is essential for many forms of learning and memory. Activation of NMDARs by glutamate requires the binding of a co-agonist to a regulatory site of the receptor. In many forebrain regions, this co-agonist is d-serine. Here, we show that experimental epilepsy in rats is associated with a reduction in the CNS levels of d-serine, which leads to a desaturation of the co-agonist binding site of synaptic and extrasynaptic NMDARs. In addition, the subunit composition of synaptic NMDARs changes in chronic epilepsy. The desaturation of NMDARs causes a deficit in hippocampal long-term potentiation, which can be rescued with exogenously supplied d-serine. Importantly, exogenous d-serine improves spatial learning in epileptic animals. These results strongly suggest that d-serine deficiency is important in the amnestic symptoms of temporal lobe epilepsy. Our results point to a possible clinical utility of d-serine to alleviate these disease manifestations.

**Benchmark IV Progressing Nicely:**
Rational Pharmacotherapy May Address Cognitive Decline in Epilepsy

**Commentary**

NMDA-type glutamate receptors (NMDARs) have been front and center in synaptic plasticity (1) since they were first distinguished from other glutamate receptors pharmacologically in the 1980s. NMDARs are typically assembled from GluN subunits (GluN1, 2A-D) as tetrameric heteromers containing two GluN1 subunits and two, not necessarily the same, GluN2 subunits (2). Subunit “guidelines” have been proposed where GluN2A assemblies mediate long-term potentiation (LTP) and GluN2B assemblies mediate long-term depression (LTD). These are guidelines, as supporting evidence (3) is often contradictory (4) and dependent on the experimental details such as concentrations of antagonists used, techniques and, importantly, developmental age (5). Recent work highlights that the location of particular subunit assemblies, that is, synaptic versus extrasynaptic, is equally important (6). This distinction is rather important as activation of extrasynaptic versus synaptic NMDARs has been distinguished as mediating neuronal death versus survival (7). This specific, localized role and the mechanistic details in diseases has lately begun to emerge, for instance in Huntington disease (8).

Synaptic activation of NMDARs seems straightforward: glutamate is released from presynaptic terminals and diffuses to nearby receptors. Extrasynaptic receptors are activated by glutamate that spills over from the cleft or is not taken up by transporters on glia or neurons. However, NMDARs can also be activated by glutamate released from astrocytes (9). Further, NMDARs require glycine or D-serine as a co-agonist with glutamate in order to fully gate ionic currents; the efficacy of glutamate and glycine depend on the specific GluN2 assembly; GluN2B assemblies are more sensitive to both glutamate and glycine (10). The source and role of glycine and D-serine has recently been probed to suggest that astrocytically released D-serine is important for synaptic NMDARs and necessary for LTP, while glycine is important for extrasynaptic NMDARs and LTD (6). Again, these are not rules, only guidelines, as there is no clear boundary between the synapse, perisynapse, and extrasynapse. The ambient concentrations of glycine or D-serine are not saturating and can be modulated dynamically in order to influence NMDAR function and plasticity (11). The means to separate NMDARs pharmacologically is limited by the tools available. While NR2B relatively selective antagonists are available (such as ifenprodil and RO25-6981), antagonists for differentiating mixed assemblies (e.g., NR2A/NR2B) are lacking (12).

Thus, it seems logical that the role of NMDARs in mediating the cognitive deficits (13) associated with epilepsy should be probed. The authors utilized the pilocarpine model of temporal lobe epilepsy in adult rats and verified that only rats having spontaneous clinical seizures were used in the study, compared with sham controls. They utilized patch-clamp techniques in dorsal hippocampal slices, quantitative PCR, and behavioral studies. The goal was to determine functional...
abnormalities in synaptic and extrasynaptic NMDARs, whether these were modulated by the co-agonist D-serine and the impacts on learning and memory in the standard Morris water maze. They focused on the lateral perforant path (LPP) synapse in the dentate gyrus.

The authors found that synaptic NMDAR-mediated currents at the LPP synapse were comparatively smaller in epileptic rats based on increasing stimulus applied to LPP axons. Of importance, the authors compared the NMDAR-mediated synaptic current with the non-NMDAR-mediated synaptic current, the “A/N-ratio,” and found no differences. The NMDAR-mediated synaptic current, while smaller, was found pharmacologically to be mediated by a greater proportion of GluN2B-containing assemblies at the expense of NR2C/D. D-serine was then applied and found to enhance synaptic NMDARs to a greater extent in epileptic animals. The authors did not repeat the initial stimulus–response relationship in the presence of D-serine. Technical issues otherwise would not permit comparisons of the size of NMDAR-mediated synaptic currents between epileptic animals and controls.

The authors then blocked synaptic NMDARs with MK801 and investigated perisynaptic and extrasynaptic NMDARs activated by glutamate spillover. Again, they found that D-serine enhanced these NMDARs to a greater extent in epileptic animals. Extrasynaptic NMDARs mediate a tonic current that can be compared between epileptic animals and controls; this tonic current was greater in epileptic animals, but the effect of D-serine on this current was not examined.

LTP was examined at the LPP synapse and found to be greatly reduced in epileptic animals; this could be rescued by application of D-serine. In the presence of D-serine, LTP was then sensitive to a low concentration of a GluN2B antagonist or glycine-site antagonist in epileptic rats, while LTP in controls remained insensitive to these antagonists. Consistent with reduced LTP, the authors found impaired learning and memory in epileptic rats that was partially recovered after oral administration of D-serine. Of importance, adverse effects were not clinically observed, such as increased seizure frequency in the D-serine–treated epileptic rats. Using quantitative PCR, the authors observed that the enzyme that makes D-serine is reduced, while the degradation enzyme that reduces D-serine is increased, in epileptic rats.

The authors conclude that epileptic rats have greater expression of GluN2B at LPP synapses, but through loss of D-serine, these receptors are unable to be fully activated to mediate LTP, learning, and memory. These exciting studies provide an important breakthrough that addresses the National Institute of Neurological Disorders and Stroke (NINDS) Epilepsy Benchmark Area IVA: “Understand and limit adverse impacts of seizures on quality of life, including effects on neurodevelopment, mental health, intellectual disabilities, and other neurological and non-neurological functions” (14). Indeed, is a simple supplement like D-serine the answer to cognitive decline in epilepsy? As the authors point out, there are some caveats and, as with any good study, more questions often appear that must be addressed by future studies.

First, which came first, the loss of D-serine or greater expression of GluN2B? Biochemical demonstration of alterations in expression of NMDARs and their specific compartmentalization and phosphorylation status (15) will be important to explore in future studies. Which is compensating for the other, why and how? This is an important distinction as supplementation with D-serine may have negative long-term effects as it activates an abnormally larger pool or receptors. Specifically, greater activation of extrasynaptic NMDARs has been associated with apoptosis (7). LTD, likely even more greatly impacted by the alterations in extrasynaptic NMDARs, was not addressed here, along with associated behaviors (16). Thus, long-term consequences of D-serine may be greater injury, abnormal behavior and, perhaps, even worsening seizures. The authors recognize the need for not only longer-term studies, but more in-depth studies that utilize combined video-EEG to document true seizure frequency.

Second, while A/N ratios have been functionally maintained, increased expression of GluN2B suggests there is a downregulation of AMPARs, possibly compensatory. What is the mechanism of this homeostatic compensation? Is an alteration in A/N ratios unmasked by D-serine acutely? By chronically increasing functional activation of NMDARs with D-serine, what is the long-term impact on AMPAR function? Will further compensation provoke increased seizure frequency in epileptic rats? What other hippocampal circuits are involved?

Finally, what happened to glial function and regulation of the enzymes controlling D-serine production? D-serine supposedly regulates synaptic NMDARs, while glycine regulates extrasynaptic NMDARs (6), so what about glycine release? Glia normally coordinate with each other through gap junctions across a syncytium to release D-serine to hundreds of principal cells (11). The data therefore suggest that this syncytium may have been disrupted. This highlights the role of glia in epilepsy and cognition as another important avenue for future study.

by Tim Benke, MD, PhD

References


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