

MOLECULAR DEFECT IN KINDLING EPILEPSY

Accumulation of 7S SNARE Complexes in Hippocampal Synaptosomes from Chronically Kindled Rats

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Kindling is a model of complex partial epilepsy wherein periodic application of an initially subconvulsive stimulus leads to first limbic and then generalized tonic-clonic seizures. Several laboratories have reported that augmented neurotransmitter release of L-glutamate is associated with the chronically kindled state. Neurotransmitter release requires membrane proteins called SNAREs, which form transmembrane complexes that participate in vesicle docking and are required for membrane fusion. We show here that kindling by entorhinal stimulation is associated with an accumulation of 7S SNARE complexes in the ipsilateral hippocampus. This increase of 7S SNARE complexes appears to begin early in the kindling process, achieves a peak with full kindling, and remains at this level for at least a month after cessation of further kindling stimuli. The increase is focal and permanently limited to the ipsilateral hippocampus despite progression to generalized electrographic and behavioral seizures. It is not seen in animals that receive electroconvulsive seizures, suggesting it is related to the kindling process itself. The duration and focality of increased 7S SNARE complexes with entorhinal kindling suggest that this is an altered molecular process associated with epileptogenesis.

Matveeva et al. postulated that the increased calcium-dependent glutamate release from presynaptic terminals, which could be the substrate for high-frequency network discharges associated with seizures, might derive from the modulation of elements fundamental to presynaptic release. Specifically, the authors examined whether changes in the levels of 7S SNARE complex formation correlated with enhanced neurotransmitter release in the chronic epileptic state.

The target being measured, 7S SNARE, is a stable ternary 7S soluble *N*-ethylmaleimide sensitive factor (NSF) attachment protein–receptor complex composed of the vesicular protein synaptobrevin (*v*-SNARE) and the plasma membrane proteins syntaxin and SNAP-25 (*t*-SNAREs). The three proteins arrange as a four-helical, coiled-coil that spans the two fusing membranes (the *trans* configuration). After fusion, the 7S complex, now in a *cis* configuration, is disassembled by NSF, which acts as a protein helicase to unwind the spent 7S complexes—a process essential to avoiding accumulation of *cis*-complexes on either the vesicular or plasma membrane, as well as for recycling to support ongoing neurotransmission.

SNARE complexes were measured in adult rats (sex unknown) at various stages up to 30 days after kindling of the right entorhinal cortex was completed (defined as a stage 5 seizure, which occurred on 2 successive days). As predicted, an asymmetric accumulation of 7S complexes occurred in the hippocampus ipsilateral to the stimulus site, which appears early, peaks at stage 5, and then plateaus. As noted in the abstract, this event did not occur in control animals, although apparently none of the experimental rats was ever kindled from the left entorhinal cortex. Nonetheless, of primary importance is the finding of accumulation of 7S complexes in the ipsilateral hippocampus—a result that appears to be persistent, lasting at least 1 month after cessation of stimulation. This finding is noteworthy because, at present, it is the singular finding of a focal molecular defect in the kindling model of epilepsy.

Matveeva et al. make clear that their method does not distinguish *cis* from *trans* configurations. However, they reason that increased synaptic activity would cause more *cis* complexes and that heightened levels of primed synaptic vesicles in a pre-fusion state (i.e., a readily releasable pool) would cause an increase in *trans* complexes, both of which are consistent with SNARE complexes and increased glutamate release. The investigators also consider particular mechanisms that might account

COMMENTARY

Development of rat entorhinal kindling includes both electrographic and behavioral stages, which correlates well with human complex partial seizures in stages 1 to 2 and secondarily generalized motor seizures in stages 3 to 5. Entorhinal cortex kindling is known to be associated with a permanent enhancement of glutamate release in the ipsilateral hippocampus.

for the accumulation of the 7S SNARE complexes and would, likewise, promote transmitter release, such as increased presynaptic calcium-induced activation of such regulatory molecules as synaptotagmins, or calmodulin. This mechanism seems plausible, given that alterations in synaptosomal calcium have been seen in kindled epilepsy.

The data suggest that a focal molecular signature is associated with neurons within the epileptogenic focus but not

with analogous contralateral tissue that is secondarily involved via seizure spread and generalization. Therefore, it is tempting to say that the increase in glutamate release, via increased SNARE complexes, underlies epileptogenesis. It is probably one strong presynaptic mechanism that leads to the development of epilepsy, but it is likely that postsynaptic molecular alterations are as important, if not more important, to epileptogenesis.

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