

SV2A: MORE THAN JUST A NEW TARGET FOR AEDS

The Synaptic Vesicle Protein SV2A Is the Binding Site for the Antiepileptic Drug Levetiracetam

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Here, we show that the synaptic vesicle protein SV2A is the brain binding site of levetiracetam (LEV), a new antiepileptic drug with a unique activity profile in animal models of seizure and epilepsy. The LEV-binding site is enriched in synaptic vesicles, and photoaffinity labeling of purified synaptic vesicles confirms that it has an apparent molecular mass of ≈ 90 kDa. Brain membranes and purified synaptic vesicles from mice lacking SV2A do not bind a tritiated LEV derivative, indicating that SV2A is necessary for LEV binding. LEV and related compounds bind to SV2A expressed in fibroblasts, indicating that SV2A is sufficient for LEV binding. No binding was observed to the related isoforms SV2B and SV2C. Furthermore, there is a high degree

of correlation between binding affinities of a series of LEV derivatives to SV2A in fibroblasts and to the LEV-binding site in brain. Finally, there is a strong correlation between the affinity of a compound for SV2A and its ability to protect against seizures in an audiogenic mouse animal model of epilepsy. These experimental results suggest that SV2A is the binding site of LEV in the brain and that LEV acts by modulating the function of SV2A, supporting previous indications that LEV possesses a mechanism of action distinct from that of other antiepileptic drugs. Further, these results indicate that proteins involved in vesicle exocytosis, and SV2 in particular, are promising targets for the development of new CNS drug therapies.

COMMENTARY

Identification of a synaptic vesicle protein SV2A as a binding site for levetiracetam (LEV) and the association of SV2A with antiepileptic efficacy support the speculation that LEV is unique among the currently marketed antiepileptic drugs (AEDs). This view was based on earlier observations showing that, unlike most other AEDs, LEV did not demonstrate any significant anticonvulsant efficacy in seizure models classically used to screen novel AEDs, including maximal electroshock or pentylenetetrazol-induced seizures (1). Furthermore, research on the mechanisms of action of LEV did not reveal any similarities with those of conventional AEDs, such as facilitation of γ -aminobutyric acid (GABA)-mediated neurotransmission, modulation of sodium channels, or modulation of low-voltage-activated calcium currents. Two studies have provided evidence that LEV, unlike any other AED, may have modulatory effects on activity-dependent plasticity and its behavioral consequences. First, Löscher and colleagues showed that administration of LEV during induction of kindling resulted in long-lasting reduction in afterdischarge duration, even after discontinuation of treatment (2). Recently Sasa et al. investigated a strain of rats that develop spontaneous seizures as adults (3). They administered LEV over the long term to these rats before the appearance of seizures. Even though seizures continued to develop, a significant decrease in the frequency and duration of both tonic and absence seizures was noted compared with

untreated animals. These data suggest that LEV has a different spectrum of action, as compared with other AEDs, which could relate to its novel mechanism of action.

Research on the LEV binding site began in the mid-1990s when Noyer and coworkers showed that LEV binds to synaptic plasma membranes of the rat hippocampus, cortex, cerebellum, and striatum in a reversible, saturable, and stereoselective manner (4). Further, only one binding site appeared for LEV. Other AEDs, including carbamazepine, phenytoin, valproate, phenobarbital, and clonazepam, had no affinity for the LEV binding site. Interestingly, ethosuximide, pentobarbital, and convulsant pentylenetetrazol competed with binding site at concentrations active in vivo. Eight years later Fuks and coworkers studied photoaffinity labeling of LEV analogue by autoradiography and found that its distribution did not match with any of the classic receptors (5). The highest binding density was found in the dentate gyrus, superior colliculus, several thalamic nuclei, and molecular layer of the cerebellum. Subcellular analysis revealed that binding sites were located in synaptic vesicles. The present study of Lynch and collaborators shows that the binding site in synaptic vesicles is the SV2A protein and that binding to SV2A is both necessary and sufficient for its antiepileptic action.

SV2A proteins are specific to neurons and endocrine cells. Three isoforms of this 90-kDa protein exist: SV2A, SV2B, and

SV2C, of which, SV2A is the most widely distributed. Much of the data regarding the function of this protein comes from studies using SV2A and SV2A/B knockout mice. Interestingly, these mice express spontaneous seizures and die within 3 weeks of birth. Crowder et al. showed that SV2A gene disruption leads to reduction in the action potential–dependent release of GABA in the CA3 subfield of the hippocampus, whereas action potential–independent neurotransmission remains normal (6). If, however, two or more action potentials were fired in succession in cultured hippocampal neurons from SV2A-deficient mice, a sustained increase in calcium-dependent synaptic transmission occurred (7). Based on these observations, Janz and colleagues hypothesized that disruption of SV2A results in calcium accumulation during repetitive action potentials and leads to increased neurotransmitter release and synaptic circuitry destabilization, which could explain the seizures in these animal. Alterations in synaptic transmission happened without any change in synapse or synaptic vesicle density or morphology, suggesting that SV2A modulates exocytosis at the level that precedes the final event of vesicle fusion (8). It also was suggested that SV2A proteins are required to maintain a pool of vesicles available for Ca-stimulated exocytosis (8).

Questions arise: how does the binding of LEV to SV2A protein lead to the coordinated actions required for the prevention or suppression of various types of seizures, which can begin in different locations of the brain? What are the molecular events underlying long-lasting shortening of afterdischarge duration when LEV is administered during the development of kindling? One explanation for this phenomenon may relate to the distribution of the LEV binding site within the brain. Unfortunately, details about LEV binding in the brain (particularly in the medial temporal lobe, including various subfields of the hippocampus, amygdaloid complex, and the surrounding parahippocampal cortex) are not available. These data would be of interest regarding the contribution of various regions to the onset and spread of the types of seizures that are documented to respond to LEV treatment. A more detailed description about the location of SV2A protein in different neuronal cell types also is needed. For example, is the binding of LEV to synaptic vesicles similar in excitatory and inhibitory neurons, and is the transmitter release affected to the same extent?

Molecular profiling of gene expression induced by LEV administration, using large-scale arrays, potentially could shed light on downstream events after LEV binding to SV2A. Interestingly, Gu and colleagues reported the effects of LEV on gene expression, 5 months before the present article was published (9). Work for both articles was partly done in the same research centers. Therefore one would expect to find links between these two articles that would give, at least, a clue as to how LEV binding on SV2A protein affects gene expression. The information

provided on gene expression was a great disappointment. The molecular profiling was performed by using Affymetrix Rat Genome U4 set, which measures ~26,000 full-length genes and expressed sequence tags (ESTs). The data obtained by Gu and colleagues generated a “master gene list,” which contained genes that were modulated by kindling or genes modulated by kindling but normalized by LEV treatment administered during kindling development. Altogether the list included 1,109 genes that could be annotated to 15 protein pathways or functional classes. One of the 15 classes was “synaptic vesicle/transmission” and included 16 genes. The authors specified that only one of these genes, α -N-ethylmaleimide-sensitive factor-attachment protein (α -NAPA), showed decreased expression in kindled brain. NAPA binds to the soluble *N*-ethylmaleimide-sensitive fusion (NSF) attachment protein receptor (SNARE) complex that forms transmembrane complexes, which participate in vesicle docking and are required for membrane fusion and, thus, function in exocytosis and neurotransmitter release like SV2A. Interestingly, however, LEV treatment during kindling development did not normalize the downregulation of NAPA gene.

Unfortunately, the study by Gu (9) provided no data about the SV2A mRNA expression in kindled brain tissue or whether LEV treatment administered during kindling had any modulatory effects on it. In addition, no data were provided about the changes in expression of protein pathways associated with SV2A. The investigators, however, noted that LEV treatment had little effect on overall gene expression in controls. Thus one can assume that LEV did not affect SV2A expression in normal brain. Interestingly, as in the case of NAPA, a link exists between the SV2A and SNARE—the loss of SV2A is associated with fewer SDS (sodium dodecyl sulphate)-resistant SNARE complexes (8). Otherwise, the number of SNARE complexes is increased in the hippocampus after entorhinal cortex kindling and remains elevated for ≥ 1 month (10). Even though information about the effect of epileptogenesis and seizures on SV2A (and associated proteins in the membranes of synaptic vesicles) is fragmentary, these data provide evidence that synaptic vesicles may have a more remarkable role in neurobiology of epilepsy than previously anticipated.

As outlined earlier, several questions must be explored regarding the consequences of LEV binding on neuronal function during epileptogenesis and epilepsy. Identification of the synaptic vesicle protein, SV2A, as a binding site for LEV, however, can be seen as a new era in the search for novel molecular targets in the treatment of seizures. It also shows that data collected at the systems level, such as observations of the unusual anticonvulsant profile of LEV, provide valuable clues to facilitate the exploration of novel molecular mechanisms of AED effects. The study also generates a great deal of hope. It shows that molecules that were not previously associated with epilepsy may provide novel targets for drug development in the treatment of

epilepsy patients. Data provided by Lynch and colleagues offer new avenues for those who wish to explore molecular mechanisms of activity-dependent plasticity, which may be one of the critical issues underlying epileptogenesis and its treatment in the future.

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