

SV2A IN EPILEPSY: THE PLOT THICKENS

Decreased Expression of Synaptic Vesicle Protein 2A, the Binding Site for Levetiracetam, during Epileptogenesis and Chronic Epilepsy. van Vliet EA, Aronica E, Redeker S, Boer K, Gorter JA. *Epilepsia* 2009;50(3):422–433. **PURPOSE:** We previously showed that gene expression of synaptic vesicle protein 2A (SV2A), the binding site for the antiepileptic drug levetiracetam, is reduced during epileptogenesis in the rat. Since absence of SV2A has been associated with increased epileptogenicity, changes in expression of SV2A could have consequences for the progression of epilepsy. Therefore, we investigated hippocampal SV2A protein expression of temporal lobe epilepsy (TLE) patients and in rats during epileptogenesis and in the chronic epileptic phase. **METHODS:** SV2A immunocytochemistry and Western blot analysis were performed on the hippocampus of autopsy controls, patients that died from status epilepticus (SE), and pharmacoresistant TLE patients. In addition, in epileptic rats, SV2A expression was determined after SE during the acute, latent, and chronic epileptic phase. **RESULTS:** In control tissue, presynaptic SV2A was expressed in all hippocampal subfields, with strongest expression in mossy fiber terminals. SV2A positive puncta were distributed in a patchy pattern over the somata and dendrites of neurons. SV2A decreased throughout the hippocampus of TLE patients with hippocampal sclerosis (HS), compared to autopsy control, SE, and non-HS tissue. In most rats, SV2A was already decreased in the latent period especially in the inner molecular layer and stratum lucidum. Similarly as in humans, SV2A was also decreased throughout the hippocampus of chronic epileptic rats, specifically in rats with a progressive form of epilepsy. **DISCUSSION:** These data support previous findings that reduced expression of SV2A could contribute to the increased epileptogenicity. Whether this affects the effectiveness of levetiracetam needs to be further investigated.

COMMENTARY

It is unlikely that many people treating or researching epilepsy had ever heard of synaptic vesicle protein 2A (SV2A) prior to 2004. However, with the identification of SV2A as the binding site for levetiracetam in the brain, the term entered the consciousness of epilepsy experts and kick-started efforts to understand more about this protein and its role in the generation and perpetuation of seizures (1).

SV2A is a member of a family of membrane-bound glycoproteins found on synaptic vesicles of both neurons and endocrine cells. In mammals, this protein family is encoded by three different genes (*SV2A*, *SV2B*, *SV2C*), of which SV2A is the most abundant and widely expressed product (2). Knockout mouse studies have demonstrated that *Sv2a* deletion results in reduced action potential-dependent release of the inhibitory neurotransmitter GABA in the CA3 region of the hippocampus (3). In contrast, cultured hippocampal neurons from *Sv2a/2b* double knockouts show a sustained increase in calcium-dependent synaptic transmission, which is presumably excitatory in nature (4). These observations have given rise to the hypothesis that *Sv2a* dysfunction is associated with calcium accumulation during repeated action potential generation. The effect, in turn, leads to increased neurotransmitter

release and a destabilization of neuronal circuits, facilitated by excitatory transmission and a concurrent attenuation of inhibition. This hypothesis would explain why *Sv2a* and *Sv2a/2b* knockout mice have spontaneous seizures from birth and typically die within 3 weeks. How levetiracetam interacts with this system in order to prevent seizures is unknown. The efficacy of levetiracetam analogues against experimental seizures is directly related to their SV2A binding affinity, but the precise nature of the interaction between the drug and protein remains elusive (1,5).

A recent paper by van Vliet et al., reviewed here, provides further insight into the role of SV2A in epilepsy. The manuscript describes an investigation into the expression of SV2A protein, measured by both immunocytochemistry and Western blot analysis, in resected temporal lobe specimens from patients with refractory epilepsy, autopsy controls, and two patients who died during an acute episode of status epilepticus. Similar analyses were performed in the hippocampi of rats subjected to experimental epileptogenesis by tetanic stimulation of the angular bundle, with SV2A expression determined 1 day after the initial status epilepticus, 1 week later during the latent period, and 6–8 months following the appearance of spontaneous recurrent seizures.

In human control tissue, SV2A was robustly expressed on presynaptic terminals throughout the hippocampus. In contrast, SV2A expression was “patchy” (i.e., less prevalent and uniform) on postsynaptic cell bodies and dendrites in the hilus and

absent in the somata of granule and pyramidal cells. A similar pattern of expression was observed in the samples from two patients who died during acute status epilepticus as well as in those refractory epilepsy specimens that did not show histopathological evidence of hippocampal sclerosis. In contrast, SV2A immunoreactivity was reduced in all specimens from patients with confirmed hippocampal sclerosis, and Western blot analysis suggested a reduction in expression in the order of 32%.

In the rat hippocampus, control animals showed a remarkably similar pattern of Sv2a staining to the human control specimens. In the acute seizure phase (i.e., 1 day after status epilepticus), presynaptic Sv2a expression was reduced in the inner molecular layer and in hilar neurons of the dentate gyrus. It was further diminished across all hippocampal subfields during the latent period. This reduction in immunoreactivity at 1 week after status epilepticus could be partly, but not entirely, attributed to the degeneration of presynaptic terminals, as determined by synaptophysin labeling and Timm staining. In the chronic epileptic phase, 6–8 months after initial status epilepticus, Sv2a expression remained depressed throughout the hippocampus (approximately 28% less than in the controls) but only in those rats with a progressive form of epilepsy that were experiencing a multiple and increasing number of seizures per day. Rats with a nonprogressive epilepsy phenotype, averaging less than one seizure per day, showed a similar pattern and extent of expression as the control animals.

These findings are intriguing, not the least because of the striking similarity in the expression profile of the SV2A/Sv2a protein between human and rat hippocampus under control conditions but also because of the clear differences observed in subgroups of epileptic animals, with apparently progressive and nonprogressive forms of chronic seizures. The authors speculate that reduced expression of Sv2a does not cause immediate seizures but may contribute to a state of heightened epileptogenicity. This view is supported by the observation of widespread changes in Sv2a immunoreactivity in the latent period, during which time, by definition, seizures have yet to develop. It is also backed up by a recent study of Sv2a (+/–) heterozygous mice that showed an increased sensitivity to evoked seizures, although the animals were not overtly epileptic (6). In addition, Van Vliet et al. suggest that reduced SV2A expression might play a role in determining whether the resultant epileptic state is progressive in nature. This argument is less convincing, as it is partially based on the observation of ultimately fatal seizures in Sv2a knockouts and ignores the possibility of compensatory changes in knockout animals that may contribute to the overall phenotype. Their theory also fails to take into account potentially important differences in the consequences of the nonexistence of a protein compared with a modest 30% reduction in its expression.

As is often the case in experimental epilepsy studies, the distinction between cause and consequence in this investigation is difficult to pin down. It is entirely possible that the differences in Sv2a expression in animals with progressive and nonprogressive seizures may simply be a function of seizure frequency. Other issues also remain to be addressed, including an apparent discrepancy between the widespread and lasting changes in protein expression in some animals and the more modest, transient change in *Sv2a* gene expression reported in a previous microarray study (7). There is also the question of whether the progressive or nonprogressive seizure animals more closely reflect the clinical situation. Despite similarities in the Sv2a expression profile in rats with progressive seizures and SV2A among patients with temporal lobe epilepsy and hippocampal sclerosis, most human epilepsy syndromes are not progressive, at least not in terms of their seizure frequency.

How the findings of Van Vliet and colleagues sit with the proposed effect of levetiracetam on SV2A, its occasional efficacy in patients with previously refractory epilepsy (8), and the anecdotal reports of tolerance to the drug in both experimental and clinical studies (9,10) also remains to be resolved. Despite these deficiencies, studies such as this one have helped to advance understanding of SV2A in the 5 years since this protein first loomed large on the epilepsy radar. It is obvious that there is still much more to learn.

by Graeme J. Sills, PhD

References

1. Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B. The synaptic vesicle protein 2A is the binding site for the antiepileptic drug levetiracetam. *Proc Natl Acad Sci USA* 2004;101:9861–9866.
2. Janz R, Sudhof TC. SV2C is a synaptic vesicle protein with an unusually restricted localization: anatomy of a synaptic vesicle protein family. *Neuroscience* 1999;94:1279–1290.
3. Crowder KM, Gunther JM, Jones TA, Hale BD, Zhang HZ, Peterson MR, Scheller RH, Chavkin C, Bajjalieh SM. Abnormal neurotransmission in mice lacking synaptic vesicle protein 2A (SV2A). *Proc Natl Acad Sci USA* 1999;96:15268–15273.
4. Janz R, Goda Y, Geppert M, Missler M, Sudhof TC. SV2A and SV2B function as redundant Ca²⁺ regulators in neurotransmitter release. *Neuron* 1999;24:1003–1016.
5. Kaminski RM, Matagne A, Leclercq K, Gillard M, Michel P, Kenda B, Talaga P, Klitgaard H. SV2A protein is a broad-spectrum anticonvulsant target: functional correlation between protein binding and seizure protection in models of both partial and generalized epilepsy. *Neuropharmacology* 2008;54:715–720.
6. Kaminski RM, Gillard M, Leclercq K, Hanon E, Lorent E, Dassel D, Matagne A, Klitgaard H. Proepileptic phenotype of SV2A-deficient mice is associated with reduced anticonvulsant efficacy of levetiracetam. *Epilepsia* 2009;50:1729–1740.

7. Gorter JA, van Vliet EA, Aronica E, Breit T, Rauwerda H, Lopes da Silva FH, Wadman WJ. Potential new antiepileptogenic targets indicated by microarray analysis in a rat model for temporal lobe epilepsy. *J Neurosci* 2006;26:11083–11110.
8. Mohanraj R, Parker PG, Stephen LJ, Brodie MJ. Levetiracetam in refractory epilepsy: a prospective observational study. *Seizure* 2005;14:23–27.
9. van Vliet EA, Van Shaik R, Edelbrock PM, Lopes da Silva FH, Wadman WJ, Gorter JA. Development of tolerance to levetiracetam in rats with chronic epilepsy. *Epilepsia* 2008;49:1151–1159.
10. Löscher W, Schmidt D. Experimental and clinical evidence for loss of effect (tolerance) during prolonged treatment with antiepileptic drugs. *Epilepsia* 2006;47:1253–1284.