

HOW READY CAN A SYNAPTIC VESICLE BE? SV2 MAY HAVE THE ANSWER

SV2 Renders Primed Synaptic Vesicles Competent for Ca^{2+} -Induced Exocytosis. Chang WP, Südhof TC. *J Neurosci* 2009;29(4):883–897. Synaptic vesicle protein 2 (SV2), one of the first synaptic vesicle proteins identified, is characterized by multiple transmembrane regions that exhibit homology to sugar transporters, and by a highly glycosylated intravesicular sequence. Deletion of SV2 causes postnatal lethality in mice, primarily because of fulminant epilepsy. At the cellular level, deletion of SV2 impairs neurotransmitter release, but its function is unknown, and even the exact point at which release is affected in SV2-deleted synapses remains unclear. Using electrophysiological approaches, we now examine at what step in exocytosis the deletion of SV2 impairs release. Our data demonstrate that deletion of SV2 produces a decrease in evoked synaptic responses without causing changes in mini frequency, mini amplitude, the readily releasable pool of vesicles, or the apparent Ca^{2+} sensitivity of vesicle fusion. These findings indicate that a previously unidentified step may couple priming of synaptic vesicles to Ca^{2+} triggering of fusion, and that SV2 acts in this step to render primed synaptic vesicles fully Ca^{2+} responsive. To investigate the structural requirements for this function of SV2, we used rescue experiments. We demonstrate that conserved charged residues within the transmembrane regions and the intravesicular glycosylation of SV2 are required for its normal folding and trafficking. In contrast, the conserved putative synaptotagmin-binding sequence of SV2 is fully dispensable. Viewed together, these observations suggest that SV2 functions in a maturation step of primed vesicles that converts the vesicles into a Ca^{2+} - and synaptotagmin-responsive state.

COMMENTARY

There is no silver bullet for the medical treatment of epilepsy. Specifically, there is no single defect that can be targeted to correct the hyperexcitability and hypersynchrony that are the hallmarks of epileptic brain circuits. Vast numbers of proteins regulating neuronal excitability interact in an ex-

traordinarily complex manner to govern cellular and network behavior. Among the current antiepileptic therapies, only a very few proteins (e.g., voltage-gated sodium channels, GABA_A receptors) have been targeted, while many potential molecular candidates for antiepileptic drug development remain unexplored. For example, a large number of proteins govern the synaptic vesicle cycle and, consequently, the strength of synaptic transmission. Once an action potential reaches the axon terminal, rather than an all-or-nothing phenomenon, these proteins regulate the probability that a vesicle will fuse and release

its contents. Vesicles are filled with neurotransmitter, dock, are primed, and finally, fuse at the release site when Ca^{2+} enters the terminal. The rate at which vesicles cycle through these various stages helps to determine how synaptic responses change during repetitive activity. Thus, an ideal antiepileptic drug would not target a protein integral to the release process, which would disrupt normal neurotransmission, but rather would be directed at a modulatory protein that regulates the vesicle cycle during high activity (e.g., a seizure). The evolving story of a protein so intimately associated with synaptic vesicles that it is named synaptic vesicle protein 2 (SV2) and levetiracetam, the first agent in a new class of antiepileptic drugs with unknown mechanism of action, illustrates the promises and challenges faced in searching for antiepileptic medications with novel molecular targets.

SV2, discovered in 1985, is a large membrane-spanning transporter-like protein (although numerous attempts have failed to find a transporter function) found on all synaptic and other secretory vesicles (1). Three isoforms have been identified: SV2A is expressed ubiquitously in neurons and endocrine cells, while SV2B and SV2C are expressed in restricted patterns in the brain. Although SV2A binds to synaptotagmin—the Ca^{2+} sensor that couples Ca^{2+} entry to vesicle fusion—the function of SV2A has been difficult to determine. Crowder et al. showed that mice with knock out of one allele for SV2A have an elevated incidence of seizures, and mice with both copies knocked out have severe seizures after the first postnatal week and die by the third week (2). Action potential-independent release of vesicles from inhibitory neurons (i.e., spontaneous release of vesicles responsible for producing miniature IPSCs) is normal in hippocampal slices from SV2A-deficient mice, but action potential-dependent release (i.e., Ca^{2+} -triggered release) is markedly reduced. These findings suggested that SV2A is involved in the coupling of Ca^{2+} entry and vesicle release at inhibitory synapses.

Janz et al. studied excitatory neurotransmission in cultured neurons and observed changes in synaptic strength in SV2A/B knockout neurons during repetitive stimulation (3). Specifically, the short-term depression of synaptic responses at lower-frequency stimulation (2 Hz) was reduced, while depression at a higher frequency (20 Hz) was unchanged. This frequency-dependent effect suggested that SV2A plays a role in buffering the accumulating Ca^{2+} levels that mediate synaptic plasticity at low-frequency firing rates. Interestingly, similar changes were not observed at inhibitory synapses. Later studies by Custer et al. showed that the initial release of neurotransmitter (i.e., after one action potential) was reduced in SV2A/B deficient neurons (a finding that Janz et al. were not able to show), suggesting that the number of vesicles available to be released, or the readily releasable pool, was diminished (3,4). However, because the number of vesicles docked at active zones was unchanged in neurons from the knockout animals, the authors concluded that

SV2 plays a role in the priming of vesicles, that is, in the final step in which docked vesicles are readied for rapid release in response to Ca^{2+} influx. So, nearly 25 years after the discovery of SV2, its function remains controversial, particularly regarding differential regulation of initial versus repetitive release and at what point in the vesicle cycle it acts; Chang and Südhof aimed to elucidate this function.

Similar to Crowder et al., Chang and Südhof found that the initial Ca^{2+} -dependent release of vesicles from inhibitory neurons was impaired in neurons cultured from SV2A/B knockout mice, but in contrast to Custer et al., they did not find a corresponding decrease in the readily releasable pool of vesicles. Chang and Südhof were able to demonstrate differences in short-term plasticity such that repetitive stimuli at 10 Hz, which normally would result in synaptic depression, instead produced facilitation in knockout neurons. They hypothesized that in the absence of SV2, vesicles were less responsive to Ca^{2+} and that accumulating Ca^{2+} during repetitive stimuli enhanced the initially depressed response. The investigators supported this hypothesis with experiments showing that a Ca^{2+} chelator reduced initial release identically in knockout and control neurons, but it changed facilitation back to depression in knockout neurons. These results suggest that the amount of Ca^{2+} entering the terminal during the initial action potential is not altered by knockout of SV2, but the effects on short-term plasticity are due to a greater amount of Ca^{2+} required to trigger release of SV2 deficient vesicles—in other words, it is not the Ca^{2+} level but the Ca^{2+} sensitivity of vesicles (i.e., how ready they are for release) that is determined by SV2A.

Chang and Südhof continued to investigate the structure of SV2A, as it relates to its function. They first showed that the knockout phenotype is rescued acutely by viral-induced expression of the wild-type protein. Next, a series of mutant SV2 constructs was transduced into knockout neurons. Surprisingly, they found that the synaptotagmin-binding domain was not essential for SV2A function. In contrast, mutations of charged residues in one of the transmembrane domains (which would presumably affect any transporter function) or in the intracellular glycosylation sites severely disrupted the protein's trafficking to synapses and/or its overall expression level. Therefore, while the latter mutations reveal critical structural domains for proper processing of SV2A, the finding that synaptotagmin binding is not important for function raises additional questions about how SV2A regulates the Ca^{2+} sensitivity of vesicles.

Based largely on the finding that SV2A/B deletion reduced vesicle release during the initial action potential, Chang and Südhof came to a different conclusion than the Janz et al. theory that SV2 plays a role in the priming of vesicles. However, Chang and Südhof studied inhibitory synapses while Janz et al. studied excitatory responses—thus, is there a fundamental difference in the role of SV2A between excitatory and inhibitory

neurons? Resolving this question will be critically important to understanding the function of SV2 in more complex brain circuits and networks as well as in diseases, such as epilepsy. The need to understand whether SV2A plays different roles in excitatory and inhibitory neurons was underscored in 2004, when it was reported that SV2A is the binding site for levetiracetam, with correlation between binding affinity and antiseizure potency for a series of levetiracetam derivatives (5). However, despite exhaustive searches, no convincing effect of levetiracetam on synaptic transmission has ever been demonstrated.

A recent study may provide an explanation for this paradox. Rather than the typical acute application of levetiracetam to brain slices, Yang et al. applied levetiracetam for 3 h, which resulted in dramatic effects on excitatory synaptic transmission that reduced synaptic responses during trains of high-frequency stimuli (6). Their electrophysiological findings correlated with reduced vesicle cycling, as measured by imaging studies, and the results demonstrated that modulation of SV2A activity by levetiracetam alters vesicle release during repetitive stimulation. Levetiracetam's precise mechanism of action remains unknown, however, a model now exists that can be used to design studies and test hypotheses. It is intriguing to speculate that with the development of levetiracetam, investigators have serendipitously stumbled upon a new class of agents that could modulate synaptic vesicle release to block excessive excitatory drive and perhaps differentially affect excitatory and inhibitory neuro-

transmission. Elucidating the mechanism by which levetiracetam and SV2A interact to prevent seizures will undoubtedly open doors to the investigation of potential novel target proteins involved in the synaptic vesicle cycle.

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References

1. Buckley K, Kelly RB. Identification of a transmembrane glycoprotein specific for secretory vesicles of neural and endocrine cells. *J Cell Biol* 1985;100:1284–1294.
2. 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.
3. Janz R, Goda Y, Geppert M, Missler M, Sudhof TC. SV2A and SV2B function as redundant Ca^{2+} regulators in neurotransmitter release. *Neuron* 1999;24:1003–1016.
4. Custer KL, Austin NS, Sullivan JM, Bajjalieh SM. Synaptic vesicle protein 2 enhances release probability at quiescent synapses. *J Neurosci* 2006;26:1303–1313.
5. Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B. The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. *Proc Natl Acad Sci USA* 2004;101:9861–9866.
6. Yang XF, Weisenfeld A, Rothman SM. Prolonged exposure to levetiracetam reveals a presynaptic effect on neurotransmission. *Epilepsia* 2007;48:1861–1869.