An old dog learns a new trick: Synaptotagmin, a Ca^{2+} sensor for spontaneous release at central synapses.

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Abstract

Evoked and spontaneous neurotransmitter release mechanisms share similar components. Synaptotagmin-1 (Syt-1) plays a central role in evoked neurotransmitter release as a Ca^{2+} sensor. Syt-1 has also been implicated in the regulation of spontaneous release. A recent study perfomed on central synapses confirms the role of Syt-1 in spontaneous release, as a Ca^{2+} sensor, a fusion clamp and an inhibitor of a second, unknown Ca^{2+} sensor. We discuss the significance of these findings with regard to previous studies performed on *Drosophila* neuromuscular junction and mouse neurons. We also indicate the possibility of neuronal-context dependent regulation of Syt-1 function. In addition, we argue that the identification of additional Ca^{2+} sensors in neurotransmission and the analysis of their interplay with Syt-1 in evoked and spontaneous neurotransmission will contribute in the understanding of neurotransmission mechanisms as well as the elucidation of the biological role of spontaneous neurotransmitter release.

At neuronal chemical synapses, intercellular communication is mediated by the controlled release of neurotransmitters upon an evoked response. In the absence of neuronal stimulation, neurotransmitter release can still occur spontaneously, as first demonstrated by experiments at the neuromuscular junction (Fatt et al. 1952). The "miniature potentials" recorded in the absence of nerve impulses are known as "minis" and represent the fusion of single synaptic vesicles with the presynaptic membrane. Numerous proteins required for the evoked fusion of synaptic vesicles with the presynaptic membrane dramatically affect the frequency of single vesicle fusion events. These include proteins that are required for the priming of synaptic vesicles or are core components of the synaptic exocytic machinery, such as Munc-13, Munc-18, Vo-ATPase, complexin, synaptobrevin and syntaxin (Aravamudan et al. 1999; Augustin et al. 1999; Broadie et al. 1995; Deitcher et al. 1998; Hiesinger et al. 2005; Huntwork et al. 2007; Richmond et al. 1999; Schiavo et al. 2000; Schoch et al. 2001; Schulze et al. 1995; Verhage et al. 2000; Xue et al. 2008b). However, other proteins, such as CSP and HIP14, affect the evoked release of synaptic vesicles but do not affect either the frequency or amplitude of minis (Fernandez-Chacon et al. 2004; Ohyama et al. 2007; Washbourne et al. 2002; Zinsmaier et al. 1994). These data show that many, but clearly not all molecular components required for evoked synaptic transmission play a role in spontaneous release.

One of the proteins whose role in minis has been controversial in the past 16 years is a key player in synaptic transmission: Synaptotagmin (Syt). Synaptotagmin-1 (Syt-1), is a synaptic vesicle associated protein with two C2 domains that bind several Ca^{2+} ions, and is the primary Ca^{2+} sensor for evoked release (Chapman 2008; Koh et al. 2003). Upon Ca^{2+} entry, Syt-1 causes a dramatic acceleration of exocytosis of primed and

docked vesicles (Chapman 2008; Rizo et al. 2008). In the absence of Syt-1, evoked exocytosis is not abolished, but neurotransmitter release becomes asynchronous and is governed by the function of a second, unknown Ca^{2+} sensor (Geppert et al. 1994; Littleton et al. 1993; Yoshihara et al. 2002).

The role of Syt 1 in minis is much less clear than in evoked synaptic transmission. In the absence of stimulation, deletion of Syt-1 leads to a dramatic increase in mini frequency at *Drosophila* larval neuromuscular junctions (Littleton et al. 1993), and mass cultured mouse cortical neurons (Pang et al. 2006) and hippocampal neurons (Chicka et al. 2008). It was therefore proposed that Syt-1 functions as a fusion clamp, preventing the release of minis (Yoshihara et al. 2003). However, the frequency of minis was unaltered at *Drosophila* Syt-1 null embryonic neuromuscular junctions (Yoshihara & Littleton 2002) and upon acute photoinactivation of Syt-1 at *Drosophila* larval neuromuscular junctions (Marek et al. 2002), thus challenging the fusion clamp hypothesis. Moreover, in autaptic cultures of Syt-1 knockout mouse hippocampal neurons (Geppert et al. 1994), no increase in minis was observed, again questioning the role of Syt-1 as a fusion clamp. It is therefore interesting that Xu et al. (2009) now provide compelling evidence that Syt-1 regulates spontaneous release similarly to evoked release in mouse cortical neurons.

First, the authors confirm that in both excitatory and inhibitory synapses of cultured cortical neurons, spontaneous release depends on Ca^{2+}, as observed in many other neurons (Angleson et al. 2001; Glitsch 2008; Wasser et al. 2009). Pre-incubation of cortical neurons with membrane-permeable Ca^{2+} chelators (BAPTA-AM or EGTA-AM), almost abolish all spontaneous release (Xu et al. 2009). Xu et al. (2009) also

report that the frequency of spontaneous release in these neurons is augmented in the absence of Syt-1. Intriguingly, the observed increase in mini frequency is still dependent on Ca^{2+}, originating from Ca^{2+} influx or release from intracellular stores, as Ca^{2+} chelators also suppress the elevated mini frequency. However, these results are somewhat contradictory to previous results showing that EGTA-AM does not suppress the increased mini frequency in Syt-1 KO neurons (Pang et al. 2006). Second, point mutations in Syt-1 that affect the Ca^{2+} dependence of Syt-1 in evoked release, also affect the Ca^{2+} dependence of minis in a very similar fashion. These data provide strong evidence that Syt-1 has a dual function, as a sensor for evoked release as well as a sensor for spontaneous release. Furthermore, these data suggest, but do not prove, that both types of release are regulated by another, possibly common, Ca^{2+} sensor, which bears distinct biochemical properties than Syt-1.

Syt-1 senses Ca^{2+} through the C2 domains. The importance of these domains in the function of Syt-1 was assessed in evoked and spontaneous release. Although both domains are necessary for evoked and spontaneous release, the C2A domain is more important than C2B in spontaneous release. This is opposite to the requirements of evoked release, where C2B plays a more important role than C2A (Mackler et al. 2002; Nishiki et al. 2004; Xue et al. 2008a). In addition, increasing the distance of the C2A and C2B domains from the SV transmembrane domain of the protein, leads to a reduction of the amplitude of the evoked release, while the frequency of minis is similar to control neurons. This suggest that the distance of the Ca^{2+} binding domains from the SV membrane do not affect the clamping of the asynchronous Ca^{2+} sensor for the minis.

Hence, these data suggest that distinct properties of Syt-1 regulate spontaneous and evoked release.

Another recent study independently confirms that the mini frequency is unchanged at autapses. However, these authors observed that cultured neurons that form a network of synapses display an increase in mini frequency at interneuronal synapses in the absence of Syt-1. These data indicate that the neuronal context and/or developmental processes may regulate the Syt-1 clamping function for spontaneous release (Liu et al. 2009). The lack of change in mini frequency in fly embryos (Yoshihara & Littleton 2002) as well as upon acute photoinactivation of Syt-1 at *Drosophila* third instar larval neuromuscular junctions supports this possibility (Marek & Davis 2002). Hence, it will be important to establish if Syt-1 functions as a Ca^{2+} sensor for spontaneous release in many different neurons, and how this function is modulated during development. Furthermore, the identification of the asynchronous Ca^{2+} sensor will advance the analysis of the interaction between different Ca^{2+} sensors in evoked and spontaneous neurotransmission in different experimental contexts, e.g. in synapses of the calyx of Held, where the asynchronous and synchronous sensors compete with each other (Sun et al. 2007).

The identity of the high affinity, asynchronous Ca^{2+} sensor that is uncovered in the absence of Syt1 remains elusive. Members of the Syt family, as well as Syt-related molecules that exhibit similar properties to Syt, namely they are able to bind Ca^{2+} through C2 domains, are perhaps the best candidates for such a role. In *Drosophila*, Syt-4 and Syt-7 are the only other members of the Syt protein family, besides Syt-1, that are expressed in the nervous system. However, they have been excluded of playing a role as

asynchronous Ca^{2+} sensors at *Drosophila* NMJ (Saraswati et al. 2007). Alternatively, the asynchronous Ca^{2+} sensor might be a Syt-related molecule, that may belong to the families of double C2-like domain-containing (DOC2) proteins, Ferlins or Synaptotagmin-like proteins (SLPs) (Martens et al. 2008). In addition, other Ca^{2+} binding proteins, such as calmodulin and calmodulin like proteins, that regulate multiple aspects of synaptic properties (Zucker et al. 2002), might play a role in asynchronous release.

Understanding the mechanism of mini release may aid us to better understand the biological role of minis, which has remained unclear thus far. Recent evidence suggests that spontaneous neurotransmission may regulate local dendritic protein synthesis and the maturation and stability of synaptic networks (Chung et al. 2006; Wasser & Kavalali 2009). Understanding the mechanism of spontaneous release will not only shed light to unexplored aspects of regulation of neurotransmission, such as the function of unknown Ca^{2+} sensor (s), but it will also contribute to the comprehension of the meaning of minis in neuronal communication.

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