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Synapsin I accelerates kinetics of neurotransmitter release in mouse motor terminals

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Synapsins are an abundant and highly conserved family of phosphoproteins associated with synaptic vesicles. In its dephosphorylated form, synapsin binds to synaptic vesicles and triggers actin polymerization (Greengard et al., 1993 for review; Han and Greengard, 1994), while synapsin phosphorylation causes its dissociation from vesicles and dispersion, thus allowing mobilization of synaptic vesicles for exocytosis (Hosaka et al., 1999; Murthy 2001; Chi et al., 2001,2003; Cousin et al., 2003). Mice lacking one or several synapsin genes (I, II, or III) have reduced vesicle density and modified short-term plasticity (Rosahl et al., 1995; Gitler et al., 2004; Samigullin et al., 2004). Injections of synapsin E-domain into squid giant axon (Hilfiker et al., 1998) slowed down the kinetics of release, while overexpression of this domain accelerated the release kinetics (Fassio et al., 2006), suggesting that synapsin may affect the final stages of exocytosis. In support of this hypothesis, our study presents the first evidence of altered release kinetics in the nerve terminals of synapsin gene deleted animals.

Mice homozygous for the *Syn1^{tm1Sud}* and *Syn2^{tm1Sud}* targeted mutation, synapsin I (-) and synapsin II (-), respectively, were purchased from The Jackson Laboratory (strains B6129S-*Syn1^{tm1Sud}/J* and B6129S-*Syn2^{tm1Sud}/J*). Strain B6129SF2/J (Jackson Laboratory) was used as a control (WT) to provide a genetic match to the synapsin I (-) and synapsin II (-) mice.

The diaphragms of adult mice were dissected and synaptic responses were recorded focally from visualized endplates as described elsewhere (Coleman et al., 2007, 2008). The diaphragm preparation has two major types of nerve terminals (Padykula and Gauthier, 1970; Prakash et al., 1996), and synapsin I and synapsin II are present in both terminal types (Coleman et al., 2008). To minimize the variability between preparations, we recorded focally the activity from type-identified terminals (Coleman et al., 2007) and analyzed separately Type I and Type II terminals. Synaptic latencies were determined as time elapsed between the negative peak of action potential invasion and the onset of synaptic response (Fig. 1A, 2 A).

Since it was demonstrated earlier (Samigullin et al., 2004; Coleman et al., 2008) that the effect of synapsin gene deletion on vesicle docking and quantal release is the most pronounced when extracellular Ca^{2+} concentration is reduced (from 2 to 0.5 mM), we first investigated the release kinetics at low Ca^{2+} conditions (0.5 mM Ca^{2+} in the extracellular media). An additional advantage of monitoring the release time-course at these conditions is that release is dramatically reduced, and the vast majority of synaptic responses represent single quantal events (Samigullin et al., 2004; Coleman et al., 2007, 2008), thus interpretation of the data is not complicated by the consideration of multiquantal responses.

We found that at low Ca^{2+} conditions the synaptic latency distribution was significantly shifted to longer latencies in synapsin I (-) terminals (Fig. 1), but it was unchanged in synapsin II (-) terminals. This result suggests that synapsin I accelerates the release kinetics during the last stages of exocytosis and that this function is specific to synapsin I isoform. This shift to longer latencies in synapsin I (-) terminals was observed in both type I terminals (Fig. 1) and type II terminals (data not shown). This lack of synapse specificity contrasts to synapsin I's role in regulating vesicle docking and quantal content, which was found to be restricted to Type I terminals (Coleman et al., 2008). Thus, our findings indicate that synapsin I performs a general post-docking role in determining the latency of release.

Next, we investigated the release kinetics at physiological Ca^{2+} conditions (2 mM Ca^{2+} in the extracellular media). We found that synaptic latencies were totally unaffected in either synapsin I (-) or synapsin II (-) terminals (Fig. 2 A,B). Since at these conditions all the synaptic responses are multiquantal and the synaptic latency only reflects the release kinetics of the first quantum, we compared the shape of the average synaptic response for WT, synapsin I (-) and synapsin II (-) terminals (Fig. 2 C). No difference in the shape of the synaptic response was detected among these three lines, indicating that release kinetics was unaffected by synapsin gene deletion.

Thus, we demonstrated that at low Ca^{2+} conditions (0.5 mM) synapsin I but not synapsin II accelerates the release kinetics. In contrast, at physiological Ca^{2+} conditions (2 mM) the release kinetics in synapsin I (-) terminals appeared to be normal. Interestingly, low Ca^{2+} conditions were also found to be revealing for the synapsin regulation of vesicle docking (Coleman et al., 2008). Together, these studies demonstrate that at low Ca^{2+} conditions synapsin has a prominent role in both regulating vesicle docking and postdocking final stages of exocytosis, which are manifested in the release time-course. At normal Ca^{2+} concentration, synapsin regulatory functions are not expressed either in vesicle docking or in final post-docking fusion steps. It is possible that at normal Ca^{2+} synapsin loses some of its regulatory functions. Alternatively, the effect of synapsin deficiency could be either masked or compensated for. The present study also reveals clear distinctions in synapsin isoform functioning, since it shows that only synapsin I but not synapsin II is capable of regulating the release kinetics. This result contrasts to a less general role of synapsin I in regulating vesicle docking, which is only limited to the type I terminal subtype, while the role of synapsin II in regulating vesicle docking is more general and not subtype-specific (Coleman et al., 2008). In summary, this study supports the synapsin role in the regulation of the last stages of synaptic vesicle fusion and demonstrates that this role is limited to the synapsin I isoform.

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Fig 1. Synapsin I but not synapsin II accelerates the kinetics of quantal release at reduced (0.5mM) extracellular Ca^{2+}

A. Representative synaptic responses and latency histograms from synapsin (+), synapsin I (-), and synapsin II (-) neuromuscular junctions. Each histogram represents the data collected from a single representative endplate. Arrows point to the modes of the latency distributions. The mode is shifted to the right in a synapsin I (-) synapse.

B. Synapsin I synapses have a significantly larger average mode of the latency distribution than WT and synapsin II (-) synapses. * indicates significant ($p < 0.05$) difference according to independent two-sided t-test.

C. Cumulative latency histograms of WT and synapsin I (-) synapses. The distributions are significantly ($p < 0.05$) different according to the Kolmogorov-Smirnov test. Latencies of 849 events from 9 WT endplates (3 animals), 3045 events from 18 synapsin I (-) endplates (6 animals), and 1316 events from 11 synapsin II endplates (7 animals) were analyzed.

Fig 2. Synapsin deletion does not alter the release kinetics release at physiological (2mM) Ca^{2+}

A. Representative synaptic responses and latency histograms at WT, synapsin I (-) and synapsin II (-) neuromuscular junctions.

B. Average modes of the latency distributions.

C. Average traces of normalized responses from WT, synapsin I (-), and synapsin II (-) endplates indicate that synapsin gene deletion did not alter the release kinetics. Latencies of 1500 events from collected from 15 animals were analyzed in each genotype.