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Review

Synapsins: From synapse to network hyperexcitability and epilepsy

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ABSTRACT

The synapsin family in mammals consists of at least 10 isoforms encoded by three distinct genes and composed by a mosaic of conserved and variable domains. Synapsins, although not essential for the basic development and functioning of neuronal networks, are extremely important for the fine-tuning of SV cycling and neuronal plasticity.

Single, double and triple synapsin knockout mice, with the notable exception of the synapsin III knockout mice, show a severe epileptic phenotype without gross alterations in brain morphology and connectivity. However, the molecular and physiological mechanisms underlying the pathogenesis of the epileptic phenotype observed in synapsin deficient mice are still far from being elucidated. In this review, we summarize the current knowledge about the role of synapsins in the regulation of network excitability and about the molecular mechanism leading to epileptic phenotype in mouse lines lacking one or more synapsin isoforms. The current evidences indicate that synapsins exert distinct roles in excitatory versus inhibitory synapses by differentially affecting crucial steps of presynaptic physiology and by this mean participate in the determination of network hyperexcitability.

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1. Introduction

The analysis of synapsin knock-out (KO) mouse lines has clearly shown that the synapsins (Syns) are involved in the regulation of the excitability of neuronal networks, and that impairment of Syn function can result in the onset of pathological conditions. In fact, SynI−/−, SynII−/− SynI,II−/− and SynI,II,III−/− but not SynIII−/− mice are all prone to epileptic seizures, which start to develop approximately at two months of age, and progressively aggravate with aging and with the number of Syn genes ablated [1,2].

The phenotype of the various Syn KO mice became even more interesting after the discovery of epileptogenic mutations of *SYN* genes in human. Genetic analyses in human populations have identified a nonsense mutation in the gene coding for SynI, likely to cause mRNA decay, as the cause of epilepsy in a family with history of epilepsy alone, or associated with aggressive behavior, learning disabilities or autism [3]. Very recently, a nonsense mutation in *SYN1* gene was identified in all affected individuals from a large French–Canadian family segregating epilepsy and autism spectrum disorders (ASDs) and additional missense mutations were found in 1.0% and 3.5% of French–Canadian individuals with ASDs and epilepsy, respectively [4]. In addition, genetic mapping analysis identified variations in the SYN2 gene as significantly contributing to epilepsy predisposition [5,6].

A recent study has analyzed and classified the types of seizures affecting SynI,II−/− mice, as well as the transitions between the

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different seizure elements. In this work, the authors describe three different clusters of epileptic activity: the truncus dominated cluster, the myoclonic cluster, and the running fit cluster. The first type of epileptic activity is characterized by a highly conserved sequence of elements, which likely reflects the sequential activation of specific neuronal populations. The two other clusters are instead much more variable, probably indicating a more random activation of neurons in multiple brain areas [7]. This study indicates how lack of Syn proteins can generate epileptic seizures by evoking a range of complex mechanisms, involving distinct neuronal populations in various brain areas.

Here we analyze and summarize various experimental evidences describing the neurophysiological mechanisms underlying the onset of seizures in Syn KO mouse models that will hopefully help the understanding of the molecular processes leading to the development of epilepsy.

2. Differential expression and localization of synapsins in excitatory and inhibitory synapses

In the central nervous system, the vast majority of nerve terminals express at least one Syn isoform and Syns are concentrated in presynaptic boutons and associated exclusively with small synaptic vesicles (SVs) [8,9]. Syn antibodies have been extensively used as reliable synaptic markers; however, already from the pioneer study on SynI and II distribution in the mammalian central nervous system, differential expression of Syn isoforms in subset of central synapses was observed. Greengard and coworkers, analysing rat brain frozen section by standard immunofluorescence, revealed that while hippocampal CA3 mossy fiber terminals expressed both SynI and II isoforms, in the deep cerebellar nuclei SynIIa was not detectable from Purkinje cell GABAergic terminals, and SynIa and IIb were expressed at lower level with respect to SynIb. In the nucleus of the trapezoid body of the brainstem, SynIb was equally present in all nerve terminals but SynIIb was present only in few synapses [9]. In the rat retina SynI and II are absent in all ribbon synapses and differentially distributed in the conventional synaptic terminals of amacrine cells [10,11]. In the olfactory bulb, whereas the core region expresses comparable level of SynI and II, the surface region had significantly higher levels of SynIIa comparing to SynI [12]. Axon terminals in the dorsal lateral geniculate nucleus of murine brain also revealed differences in term of SynI/II expression: glutamatergic cortical inputs express both isoforms, GABAergic synapses express only SynI while both isoforms were absent in the excitatory terminals arising from the retina [13]. Distribution of Syns I and II in glutamatergic and GABAergic murine terminals was also analyzed, revealing a complete colocalization of the two isoforms in VGlut1-positive terminals of the stratum lucidum as well as in VGlut2-positive terminals in the striatum. Also GABAergic VGAT-positive terminals in the striatum appeared to express both SynI and II, whereas VGAT-containing terminals in CA3 pyramidal cell layer and stratum lucidum of the hippocampus did contain neither SynI nor SynII [14]. In a subsequent study performed in the rat neocortex, 30% and 50% of VGAT positive terminals were also found to partially express SynI and SynII, respectively [15]. In the same paper it was shown that virtually all excitatory synapses positive for VGlut1 express both SynI and SynII, whereas 30% of VGlut2-positive puncta express SynI, another 30% express SynII and a significant portion of VGlut2 positive terminals are negative for either Syn isoform [15]. However, a recent study, in which the composition of glutamatergic and GABAergic synapses of mouse somato-sensory cortex has been analyzed by array tomography, showed that virtually all synapses are recognized by anti-SynI antibody, while antibodies to other synaptic proteins revealed the existence of several synaptic subtypes [16]. The SynI content, however, varied in intensity depending on the synapse type. In agreement with the work of Bragina, VGlut1-positive excitatory synapses and VGAT-positive inhibitory synapses expressed the highest and the lowest level of SynI, respectively. The presence of lower levels of expression in GABAergic terminals was previously revealed also for other synaptic proteins as SV2s, synaptotagmins, syntaxins and synaptophysins [15,17,18] and it is possibly in correlation with the different functional properties of the synapses.

The distribution of SynIII in the adult mouse forebrain was also examined and found to be significantly different from the distribution pattern seen for SynI and II [19]. The levels of SynIII in nerve terminals were much lower than those of Syns I and II. Moreover, differentially from SynI and II, SynIII was also highly expressed in the cell body and processes of immature neurons in neurogenic regions of the adult brain, such as the hippocampal dentate gyrus, the rostral migratory stream, and the olfactory bulb and was found to play a role in neural progenitor cell development in the adult hippocampus [20].

Considering that most of the functional studies on SV cycling and neurotransmission performed on Syn KO mice (see below) used hippocampal primary culture as an experimental model, we here analyzed the differential expression of Syn isoforms in excitatory and inhibitory synapses from cultured wild-type hippocampal neurons. As shown in Fig. 1, we revealed a significant lower level of colocalization for all Syn isoforms with inhibitory (VGAT-positive) synapses as compared to excitatory (VGlut1-positive) synapses and a lower level of expression of all three synapsins in GABAergic versus glutamatergic synapses. These data suggest that inhibitory synapses from hippocampal cultures alternatively express one Syn isoform or that a significant portion of GABAergic synapses is devoid of Syn, although we cannot exclude that the level of expression of the various Syn isoforms is below the detection threshold of our analysis.

The impact of the various Syn isoforms on synaptic physiology is also dependent on their developmental expression. The onset of Syns I and II expression coincides with the time of commitment from progenitor cells to differentiated neurons, and it is particularly high during synaptogenesis [8,21–25]. In cultured hippocampal neurons, the expression of Syns I and II progressively raises with time, whereas SynIII is highly expressed in the first week in culture during active process elongation, and is enriched in cell bodies, as well as in growth cones [26–28].

Taken together, immunolocalization data reveal distinct localization patterns among Syn isoforms. Particularly interesting for the goal of this review is the differential localization among excitatory and inhibitory synapses which suggest a different role of Syns in GABAergic versus glutamatergic synapses. The overall outcome of the papers investigating on Syn expression in central synapses, using different techniques and experimental models, suggests that synapsins are indeed less expressed in inhibitory versus excitatory terminals. The difference in expression has been revealed at the level of the cerebral cortex and the hippocampus and could possibly explain the hyperexcitability observed in neuronal networks lacking one or more *SYN* genes that lead to epileptic seizures in Syn KO adult animals.

3. Role of synapsins in SVs cycling assessed by live cell imaging techniques

Since the development of the optical technique to assay presynaptic function by quantitative fluorescence imaging of FM-dyes [29], studies on the role of Syns in the regulation of SV cycling by *in vivo* imaging have been performed. FM dyes, as well as the more recent genetically engineered probes of the synaptopHluorin family [30,31], are optimal tools for the study of Syn function as they allow an exquisite presynaptic analysis at the level of