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Review

How synapsin I may cluster synaptic vesicles

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ABSTRACT

Synapsin I is the most abundant brain phosphoprotein present in conventional synapses of the CNS. Knockout and rescue experiments have demonstrated that synapsin is essential for clustering of synaptic vesicles (SVs) at active zones and the organization of the reserve pool of SVs. However, in spite of intense efforts it remains largely unknown how exactly synapsin I performs this function. It has been proposed that synapsin I in its dephosphorylated state may tether SVs to actin filaments within the cluster from where SVs are released in response to activity-induced synapsin phosphorylation. Recent studies, however, have failed to detect actin filaments inside the vesicle cluster at resting central synapses. Instead, proteins with established functional roles in SV recycling have been found within this presynaptic compartment. Here we discuss potential alternative mechanisms of synapsin I-dependent SV clustering in the reserve pool.

Contents

1. Introduction

Synapses in the central nervous system are predominantly established by long processes of nerve cells, axons, which form terminal boutons on the soma and on dendrites of the target neuron. These boutons are filled with small synaptic vesicles (SVs), which fuse with the plasma membrane and release neurotransmitters to a wide variety of stimuli. One of the unique properties of presynaptic terminals is their ability to concentrate SVs at sites of release, or active zones. This property allows synapses to sustain neurotransmitter release despite of variations in neuronal firing. How this property is achieved is unclear and a matter of debate.

The brain-specific protein synapsin I is one of the molecules, whose functional properties have been linked to the organization of SVs and the actin cytoskeleton in synapses in the central nervous system (CNS) [1]. Synapsin I belongs to the synapsin family of SV-associated phosphoproteins, which undergo association–dissociation cycles with SVs during neurotransmitter release. It has been proposed that synapsin serves as a linker between SVs and actin filaments to cluster SVs in a phosphorylation-dependent manner [1]. Deletion of the synapsin I gene in mice results in a decrease in SV packing density at active zones [2], strongly supporting a role of this molecule in SV clustering. However, a number of recent experiments indicate that this "classical" model has to be revised. In this review we summarize and analyze the data supporting alternative mechanisms.

2. Domain structure of synapsin I and post-translational modifications related to SV clustering

The synapsins were initially discovered as an abundant brain substrate for cAMP-dependent protein kinase (PKA) [3]. In fol-

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lowing studies it was found that synapsins are among the most abundant proteins on SVs in central synapses with about 8–9 synapsin molecules per SV [4]. Three distinct synapsin (SYN) genes are encoded in mammals. Synapsin I, one of the best studied members of the family, is a product of the SYN1 gene and is present in the CNS in two isoforms Ia (86 kDa) and Ib (80 kDa), which are generated by alternative splicing and have a similar distribution in the CNS. Each of these isoforms is composed of five genetically defined domains, among which the C domain is the largest (about 300 aa) and most conserved [5]. Domain C is structurally homologous to ATP synthases and known to bind ATP with high affinity [6], although it likely lacks enzymatic activity. The N-terminal A domain is also highly conserved across isoforms and between species. *In vitro* experiments have shown that both these domains interact with actin filaments, SV phospholipids, and are involved in homo- and heterodimerization of synapsin I. The other domains are more divergent. Domain B displays only weak similarity between different synapsins, and may function as a linker between the conserved domains A and C. Both synapsin I isoforms contain a proline-rich domain D, which is followed in synapsin Ia by domain E, which has been recently shown to mediate synapsin I oligomerization [7], and in synapsin Ib by domain F, whose functional properties have not yet been well studied.

The outlined functions of these domains are regulated by post-translational modifications of the synapsin I protein. Mass spectrometry analysis of synapsin I from hippocampal tissue revealed multiple types of modifications including phosphorylation, methylation, pyroglutamate tryptophan oxidation, N-acetylation, and deamidation [8]. So far, only phosphorylation–dephosphorylation of synapsin I has been linked to the regulation of SV clustering [9,10].

Nine and eight phosphorylation sites have been identified in synapsin Ia and Ib, respectively [11]. Site 1 (serine 9), or the PKA/Ca^{2+}-calmodulin-dependent protein kinase I (CaMKI)/CaMKIV site, is localized in domain A. This site regulates binding of synapsins to phospholipids. In the non-phosphorylated state, synapsins are bound to SVs as dimers, via a phospholipid-binding activity associated with the A-domain. Phosphorylation of the A domain inhibits phospholipid binding and causes dissociation of synapsin [12–14]. Sites 2, 3 correspond to serines 566 and 603 in domain D of synapsin I and are phosphorylated by Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII) [13]. Phosphorylation at these sites causes major conformational changes in the protein [15], reduces its affinity for SVs [16], and dramatically impairs its interaction with actin filaments [17,18]. Erk/MAP kinase (MAPK) phosphorylates four additional sites in the synapsin I molecule: sites 4, 5 (serines 62, 67) localized in domain B and sites 6, 7 (serines 549, 551). Site 6 may be also phosphorylated by cdk 1 and 5 [19,20]. Phosphorylation by MAPK and cdk1 decrease the ability of synapsin I to promote actin polymerization, but has no effect on SV binding *in vitro* [19]. Cdk5 also phosphorylates site 7, which does not have any clear effect on SV binding [20]. Phosphorylation of synapsin I at sites 2, 3 and 4–6 influence dispersion of synapsin in synapses of cultured neurons and it has been proposed that phosphorylation at these sites may modulate SV mobilization at different rates of neuronal firing [10]. The recently identified sites 8, localized in domain C (tyrosine 301), and 9, localized in domain E of synapsin Ia, are phosphorylated by Src [21] and by the ataxia-telangiectasia mutated kinase [22], respectively.

Synapsin I binds SVs in its dephosphorylated state. Thus, keeping synapsin I dephosphorylated appears to be critical for SV clustering. Two phosphatases, PP2A and PP2B/calcineurin, have been proposed to perform this function. Sites 1–3 are efficiently dephosphorylated by PP2A [23], while calcineurin dephosphorylates sites 4–7 [19].

3. Links between synapsin I and actin filaments during SV cycling

SVs at active zones do not form a homogenous population but are distinguished based on their release probability. Those closest to the active zone (AZ) are released first and are referred to as the readily releasable pool (RRP) [24]. A by far higher number of SVs is situated more distal to the release site and comprises the reserve pool (RP), which replenishes the RRP during its stimulation-induced depletion [25]. The RRP and the RP together constitute the recycling pool defined as those SVs that participate in exo/endocytic cycling during prolonged stimulation [24,26]. Since the recycling pool accounts for only about 5–20% of the total SVs in some synapses, the existence of a third, so-called resting pool has been suggested. This pool does not actively contribute to neurotransmission under normal conditions, but only upon sustained intense stimulation [27–29].

The ability of synapsin I to bind to SVs and to actin filaments in a phosphorylation-dependent manner *in vitro* led to the hypothesis that dephosphorylated synapsin I by linking SVs within the RP to actin filaments under resting conditions renders these vesicles unavailable for release. The phosphorylation state of synapsin I, thus, serves as a regulator of the availability of SVs for exocytosis. In this scenario, activation of CaMKII, results in the phosphorylation of synapsin I at sites 2, 3 and leads to the disruption of SV-synapsin-F-actin complexes. The liberated vesicles are then allowed to join the RRP. After fusion with the plasma membrane and subsequent endocytic retrieval, vesicles can be either recycled within the RRP or become sequestered within the RP. Reclustering of vesicles in the RP was hypothesized to occur due to dephosphorylation of synapsin. This in turn promotes synapsin rebinding to SVs and the nucleation of actin filaments, resulting in the reformation of SV–synapsin–F-actin complexes and the incorporation of SVs into the cytoskeletal meshwork composing the cluster [1,30].

This “classical” model [1] was initially postulated based on data indeed confirming that synapsin I plays a key role in SV clustering. Using immunocytochemistry, synapsin I has been localized to the SV cluster in central synapses [31–33] (Fig. 1A) and acute antibody-mediated perturbation of its function disrupts the RP of SVs in the lamprey giant reticulospinal synapse [33]. As mentioned above, structure–function analysis has shown that the binding sites for SVs and actin are located in distinct domains of synapsin I [34,35] allowing for simultaneous binding of both effectors. Several observations seem to support the “classical” model of synapsin function. Binding of dephospho-synapsin I to actin filaments promotes the formation of actin bundles while phosphorylation of synapsin I by CaM kinase II *in vitro* reduces actin binding and abolishes its actin-bundling activity [18]. Moreover, introduction of CaMKII into nerve terminals increases neurotransmitter release [36,37]. Furthermore, high K^+-induced depolarization and neuroexocytosis was accompanied by increased synapsin I phosphorylation and its concomitant dissociation from SV membranes [38]. Real-time imaging in hippocampal cultures has confirmed that synapsin disperses from the SV cluster during stimulation [9]. This dispersion occurs faster than the dispersion of integral SV proteins or SVs labeled with the fluorescent dye FM4-64. In support of a role of phosphorylation (EGFP)-synapsin Ia carrying mutations in three CaMK sites disperses more slowly upon stimulation [9,10,39]. Vesicle exocytosis also slowed, presumably because of the reduced motility of vesicles [9]. Hence, these data seemed to argue in favor of a phosphoregulated synapsin-actin switch regulating SV clustering.

Several facts however, could not be explained within the frame of this hypothesis. One of the major contradictions is that vesicle clusters are not destroyed when actin-directed compounds are used to disrupt actin filaments in synapses at rest [40–42].