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SYNAPSINS AND NEUROEXOCYTOSIS: RECENT VIEWS FROM FUNCTIONAL STUDIES ON SYNAPSIN NULL MUTANT MICE

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INTRODUCTION

The high dynamism, which moulds postsynaptic response as presynaptic activity evolves, is a crucial determinant of the pattern of activity generated by neuronal networks. This prominent characteristic of chemical synaptic transmission is critically defined by the numerous proteins that are localized within the presynaptic terminal and participate in synapse formation, maintenance and function. Among many presynaptic actors, the most abundant phosphoproteins are the synapsins, a highly conserved multigene family of neuron-specific phosphoproteins. Synapsins exist in all organisms with a nervous system and in mammalian species are encoded by three distinct genes, SYNI, SYNII and SYNIII located in chromosome X, 3 and 22, respectively and are composed of a mosaic of conserved and individual domains (Fig. 1) (15, 18, 22). Synapsins I and II are stably expressed at synapses of mature neurons, where they associate with the cytoplasmic surface of small synaptic vesicles (SV), whereas the expression of synapsin III is developmentally controlled and not strictly confined to synaptic terminals (Fig. 2). Synapsins are excellent substrates for a multiplicity of brain protein kinases including protein kinase A, Ca^{2+}/calmodulin-dependent protein kinases (CaMK) I, II and IV, MAP kinase and cyclin-dependent kinase-1 that phosphorylate them on distinct serine residues. Synapsins interact *in vitro* with lipid and protein components of SV, as well as with various cytoskeletal proteins including actin, and control multiple aspects of synapse structure and function, from synaptogenesis to regulation of SV clustering and trafficking and modulation of short-term synaptic plasticity. This review will be focused on the functional studies which have outlined the role played by synapsins in regulation of neurotransmitter (NT) release. In the first part, we will focus on those studies that have led to a proposed model based on a pre-docking mechanism of action of the synapsins. Then, we will critically summarize the recently growing body of evidence suggesting that synapsins, in addition to their pre-docking action, directly control

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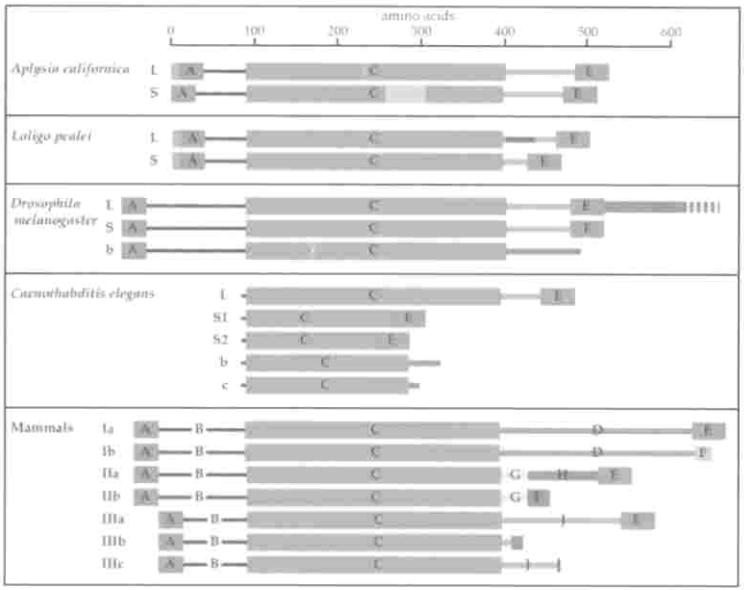

Fig. 1. - *Evolutionary conservation of the synapsins.*
Synapsins have been cloned from a variety of species, from invertebrates to man. Synapsins are composed of a mosaic of conserved and individual domains that are schematically represented in blocked form and indicated by letters A-J. The length of the polypeptide chains is shown at the top in number of amino acid residues. Different shades depicted within domains represent different sequences (e.g. within domain C of *Aplysia* synapsin). In the figure, highly conserved domains are shown as thick boxes. Domains A, C, and E are defined by significant homology to their mammalian counterparts. The models for mammalian synapsins are based on homology between the synapsin genes and alternative splicing observed for synapsin III. While in mammals, synapsins are coded by three distinct genes, in lower vertebrates and invertebrates one single gene gives rise to multiple synapsin isoforms.

the efficiency of synaptic transmission and the rate of NT release by acting at a post-docking level. For further details concerning structure, biochemistry, genetics, cellular and molecular biology and developmental role of the synapsins, the reader is referred to more extensive reviews (15, 3, 22, 18, 10).

THE SYNAPSINS AND THE RESERVE POOL OF SYNAPTIC VESICLES

A large body of experimental evidence obtained in reconstituted nerve terminals has proposed that the synapsins reversibly cross-link SV to each other and to the actin-based cytoskeletal meshwork. This action is believed to be important for the formation and maintenance of a reserve pool of SV (SV located > 150 nm from the active zone) as well as in the fine regulation of the balance between the reserve pool and a pool of SV ready to undergo exocytosis (readily releasable pool; SV located < 150 nm from the active zone) in an activity- and phosphorylation-dependent manner.

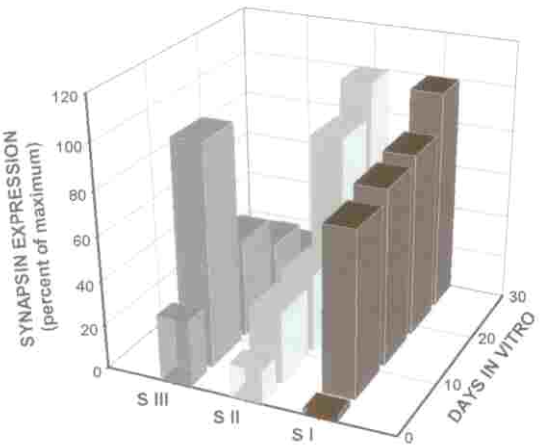

Fig. 2. - *Temporal pattern of expression of synapsin I (S I), synapsin II (S II) and synapsin III (S III) in primary hippocampal neurons as a function of the days in vitro (DIV).* Expression at various times is shown in percentage of the maximal level of expression over the analyzed time window. The three synapsin isoforms have a clearly different expression pattern, with synapsin III peaking at 7 DIV and decreasing thereafter and synapsins I and II increasing rapidly (synapsin I) or more slowly (synapsin II) during the time in vitro to reach the maximal level of expression after completion of synaptic maturation (21-28 DIV).

In order to perturb synapsin function at the nerve terminal and define its functional role in neuroexocytosis, two main experimental approaches have been used: (i) microinjection of exogenous synapsin, antibodies to synapsin or peptides derived from evolutionary conserved synapsin sequences into presynaptic terminals of invertebrate neurons; (ii) deletion of one or more of the synapsin genes in mice by gene knockout (KO) technology. Both techniques have advantages and limitations.

Microinjection studies are potentially the best method to acutely study functional changes induced by perturbation of intraterminal synapsin levels. It allows to interfere directly with synapsin function and follow the effects generated by the injected agent in real-time. However, injection of proteins, peptides or antibodies could have non-specific effects for the relative high concentrations that are often required and for the possibility that the injected agent undergoes a non-physiological targeting within the neurons. Genetic approaches are probably the best approach to give an answer to the ultimate function of a given protein, i.e. the function that cannot be compensated during development by other genes, but again they have some drawbacks. First of all, very often a change in a single protein promotes a series of homeostatic responses in downstream processes that allow neuronal systems to respond to the initial manipulation with secondary changes, making the interpretation of the phenotype difficult. Moreover, the effects of specific gene deletions are often attenuated by the presence of homologue gene products with redundant functions. This is particularly true in the case of the synapsins that are encoded by three genes further spliced to give rise to multiple isoforms. Despite these limitations, the knocking out of synapsin genes represents a fundamental technique to study *in vivo* the action of synapsin on development, synaptogenesis, maintenance and function of synapses in a long-term time scale.

Pioneer experiments testing the effect of exogenous synapsin I in squid giant synapses showed that the injection of dephosphorylated synapsin I decreased the amplitude and rate of rise of postsynaptic potentials, whereas the injection of either phosphorylated synapsin I or heat-inactivated dephosphorylated synapsin I were

ineffective. Conversely, injection of CaMKII increased the rate of rise and the amplitude of postsynaptic potentials (24, 25). Analysis of synaptic noise in the same system revealed that dephosphorylated synapsin I reduced the rate of spontaneous and evoked quantal release, whereas the injection of CaMKII increased evoked release without affecting the frequency of spontaneous miniature events (26). Further data obtained in vertebrate goldfish neurons showed that the presynaptic injection of dephosphorylated synapsin I reduced both spontaneous and evoked synaptic transmission (16). Internalization of dephosphorylated synapsin, phosphorylated synapsin or activated CaMKII into rat brain synaptosomes using freeze-thaw permeabilization confirmed the results obtained by in vivo injections (27, 28).

These data suggested an initial model in which dephosphorylated synapsin I inhibits synaptic transmission without interfering directly with the release process, but recruiting SV to the reserve pool and inhibiting SV mobilization to the readily releasable pool, a process that can be reverted upon phosphorylation. Studies on the physical distribution of the protein in response to a depolarising stimulus conducted in frog nerve muscle preparation showed that synapsin I dissociation from the SV membrane is not a prerequisite for fusion and that under high frequency electrical stimulation synapsin I partially dissociates from SV during exocytosis and reassociates with the SV membrane following endocytosis (39, 40). In agreement with the latter data, phosphorylation of synapsin I in rat brain synaptosomes treated with depolarising agents is associated with a rapid translocation of the protein from the membrane fraction to the synaptosol (35). These data have been recently confirmed in living hippocampal neurons, in which synapsin was found to disperse in the presynaptic terminal and preterminal axon during depolarization and to recluster at SV sites following return to the resting state (5). In these studies it was also found that the rates of dispersion and reclustering are indeed controlled by synapsin phosphorylation and dephosphorylation, respectively, and that CaMK-mediated phosphorylation controls SV mobilization at low frequency of stimulation, whereas MAP kinase phosphorylation is recruited at both low and high frequencies of stimulation (5, 6).

Ultrastructural studies were consistent with early functional studies. In living lamprey reticulospinal axons forming *en passant* synapses, presynaptic injection of an anti-synapsin antibody, specifically recognizing sequence of the synapsin domain E (Fig. 1), caused the disappearance of SV distal to synaptic cleft (reserve pool), leaving unaffected the SV docked at the active zone. The depletion of the reserve pool was in turn associated with a markedly enhanced depression following high, but not low, frequency stimulation (31). Using a similar approach, the presynaptic injection of a highly conserved peptide fragment of the synapsin domain E into the squid giant synapse greatly reduced the number of SV far from the active zone and increased the rate and extent of synaptic depression, indicating that domain E, present in both synapsin isoforms expressed in squid (Fig. 1), is essential for the synapsin-mediated maintenance and regulation of the SV reserve pool (17). A later study showed that injection of anti-synapsin antibodies into cholinergic synapses of *Aplysia californica* markedly increased the rate and extent of synaptic depression in response to repe-

titive stimulation (21). Interestingly, the antibody injection did not affect the plateau level reached by the postsynaptic current upon 15 minutes of stimulation, providing the first electrophysiological demonstration that synapsin neutralization does not affect the SV recycling capacity, as predicted from the unchanged endocytosis and repriming rates previously observed in synapsin KO mice using enhanced imaging techniques with FM dyes (34).

A fundamental contribution to the study of the role of synapsin in NT release derives from genetic experiments in mice in which synapsin genes have been inactivated to generate single, double and triple KO animals (32, 33, 23, 7, 37, 34, 38, 8, 14). All strains of KO mice were viable and fertile. Despite the absence of gross defects in brain morphology or behaviour, synapsin I and synapsin II (but not synapsin III) KO mice as well as double synapsin I/II and triple synapsin I/II/III KO mice exhibited early onset spontaneous and sensory stimuli-evoked (audiogenic) epileptic seizures. Attacks consisted of partial, secondarily generalized "grand mal" attacks followed by post-seizure grooming (33). Electroencephalogram analysis showed that subconvulsive electrical stimulation in the amygdala was able to induce seizures when applied to synapsin mutant mice (23). Typically, seizures develop after 2 months of age and mice become more susceptible with age. The incidence of seizures is higher in synapsin II than in synapsin I KO mice and

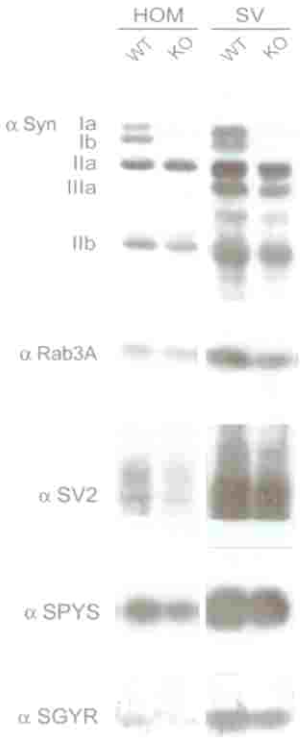

Fig. 3. - *The specific decrease in synaptic vesicle density in central synapses is reflected by a decrease in the expression of the major synaptic vesicle proteins.*
Homogenate (HOM) and purified synaptic vesicle (SV) fractions obtained from wild-type (WT) and synapsin I KO mice were analyzed by immunoblotting for their content in synapsin isoforms, Rab 3A and the integral SV proteins SV2, synaptophysin (SPYS) and synaptogyrin (SGYR). Note the high enrichment of SV markers in purified SV and the similar and homogeneous decrease in the levels of virtually all SV markers (including the products of the synapsin I and II genes), except for Rab3A levels whose marked decrease suggests an additional function of synapsin in Rab3 targeting to SV.

is proportional to the number of inactivated synapsin genes. While the synapsin II and I/II KO mice have been reported to have impaired contextual conditioning and triple KO mice exhibited impaired motor coordination and defective spatial learning (14), a detailed analysis of the behavioural phenotype of the synapsin KO mice is still lacking. Ultrastructural and physiological abnormalities observed in adult synapsin mutant mice largely confirmed and validated the data obtained by injection studies. Synapsin I, II and I/II KO mice showed a selective decrease in the total number of SV as demonstrated by a decrease in the levels of most SV markers (Fig. 3) (33, 13) and by electron microscopy of central synapses (23, 37). Similarly to what observed with the injection studies (31, 18), the nerve terminal ultrastructure showed a dramatic decrease and disassembly of SV in the reserve pool, while SV docked at active zones were only poorly affected (23, 37). In synapsin I KO mice, SV depletion was accompanied by a strong impairment in glutamate release from cortical synaptosomes and by a greater delay in the recovery of synaptic transmission after NT depletion by high frequency stimulation observed (23). The study of SV recycling at individual synaptic boutons using FM dyes showed that the number of exocytosed SV during brief action potential trains and the total recycling SV pool are significantly reduced in synapsin I KO mice, while the kinetics of endocytosis and SV repriming appear normal (34). The results were similar to those obtained in a different strain of synapsin KO mice by an independent laboratory (33), except that (i) the SV depletion was not restricted to the reserve pool, but affected also the readily releasable pool of SV to the same extent in double KO mice and (ii) there was no detectable increase in synaptic depression induced by 30 s of repetitive stimulation at 10 Hz in synapsin I KO mice. However, depression was increased in synapsin II KO mice and further enhanced in I/II double KO mice, suggesting a participation also of synapsin I in the build-up of depression.

The causal link between synapsin deficiency and the epileptic phenotype observed is still far from being elucidated. It has been hypothesized that synaptic depression during repetitive stimulation may contribute to seizure development by causing an imbalance between excitatory and inhibitory systems attributable to the fact that inhibitory GABAergic interneurons experience high frequency firing that may make GABA release particularly sensitive to the depletion of reserve SV induced by synapsin deletion. Terada and coworkers (38) investigated the impairment of inhibitory transmission in synapsin I KO mice and demonstrated that, in cultured hippocampal synapses from mutant mice, inhibitory, but not excitatory, synapses become easily fatigued and recovered slowly from depression upon repeated application of hypertonic sucrose. Stimulated terminals showed a decrease in the number of SV in the reserve pool, but not in the readily releasable pool, that was slightly more intense in GABAergic terminals than in glutamatergic ones. However, the young age of the hippocampal neurons used in this study (8 DIV), in which synaptogenesis is in progress and the formed synapses are still immature, suggests that the observed effects could be ascribed, at least in part, to a defect in synaptogenesis rather than to a change in the mature exocytosis machinery.

Taken together, the effects observed in synapsin I, II, and I/II KO mice are in general agreement with the data obtained by injection studies and strongly support the predocking model in which synapsins I and II participate in the formation and maintenance of the reserve pool of SV (Fig. 4). This pool provides a strategically localized SV reserve buffering the depletion of the readily released pool when sustained and repetitive release overrides the tonic SV recycling capacity of the terminal through the direct (kiss & stay/kiss & run) or clathrin-mediated endocytosis (15, 18).

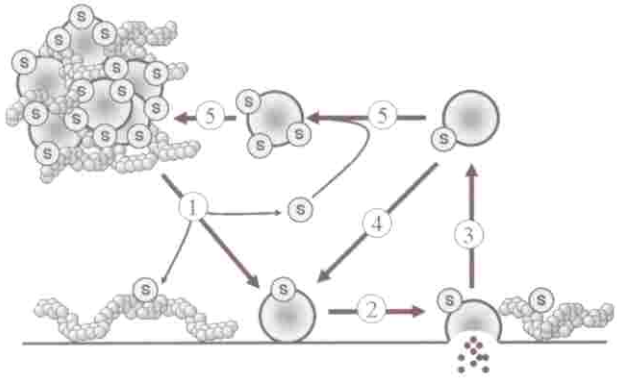

Fig. 4. - *Schematic model of the exo-endocytosis process and of the putative physiological role of the synapsins.*
Evoked neurotransmitter release is a multi-step process in which SV, after being released from the reserve pool where they are bound to the actin cytoskeleton (step 1), dock to presynaptic membrane and undergo the sequential steps of priming and Ca^{2+}-triggered fusion (step 2). After fusion, SV are retrieved through a process of endocytosis (step 3) and either become competent for a new round of exocytosis (step 4) or are recaptured in the reserve pool (step 5). Synapsins (S) modulate this cycle by acting at various levels, namely: (i) synapsins partially dissociate from SV and actin upon activity-dependent phosphorylation (step 1), making SV availabile for exocytosis; (ii) upon dissociation, phosphorylated synapsins diffuse within the nerve terminal and the preterminal regions of the axon (step 1); (iii) by acting at the active zone level, synapsins increase the rate of the post-docking events of priming and/or fusion (step 2), possibly by interacting with the dynamic submembrane actin cytoskeleton meshwork or by removing the inhibitory action of Rab3 on the fusion process; and (iv) upon dephosphorylation, synapsins reassociate with SV after endocytosis and promote the recruitment of SV to the reserve pool (step 5).

Synapsin III, the most recently identified member of the synapsin family, plays a role in synaptic function and NT release that appears completely distinct from that of synapsin I or II. First of all, synapsin III is expressed early during neuronal development and its expression is downregulated in mature neurons (9), while the other two synapsins have an opposite pattern of expression (Fig. 2). Mice lacking synapsin III exhibited a marked delay in neurite outgrowth, no change in SV density, an increase in the size of the recycling pool of SV and a significant decrease in synaptic depression (8), in sharp contrast with what observed in synapsins I and II KO mice (23, 34, Ryan unpublished observations). These data indicate a unique non redundant role for synapsin III in the regulation of NT release. One of the most intriguing functions of synapsin III is its ability to limit the size of the recycling pool of SV that allows more SV to be recruited for NT release during repetitive stimulation in synapsin III KO mice. It is possible that synapsin III, highly expressed in early stages of synaptogenesis, may serve to tether SV to the cytoskeleton and keep them from recycling during synaptic activity as previously suggested for synapsins I and II (1, 29). However, the marked decrease in the SV

population observed in synapsin I and II KO mice indicates that, while synapsins I and II have profound effects on SV clustering and stability (2, 30), synapsin III may be devoid of this activity. Although no physiological evidence for a role of ATP binding to synapsins has been provided thus far, the differential effect of Ca^{2+} on ATP binding to synapsins (Ca^{2+} inhibits ATP binding to synapsin III and stimulates ATP binding to synapsin I) (20) suggests another potential molecular difference between synapsins I and III.

Notwithstanding the absence of an overt or latent epileptic phenotype, synapsin III KO mice also showed an impairment of GABAergic transmission, while excitatory transmission was unaffected. These results leave open the possibility that the function of synapsin III in inhibitory terminals may differ from that at excitatory synapses. A recent study on synapsin I/II/III triple KO mice investigated this possibility in detail. Excitatory and inhibitory synaptic transmission was differentially altered in these mice: excitatory synapses exhibited normal basal transmission, but decreased number of SV in the reserve pool and marked depression, whereas inhibitory synapses exhibited impaired basal transmission, mild changes in the number of SV and no changes in depression. Although these observations leave completely open the physiological basis of the increased seizure propensity of synapsin I, II, I/II and I/II/III KO mice, but not of synapsin III KO mice, they demonstrate that the synapsins have a critical role in maintaining the balance between excitatory and inhibitory synapses in brain networks (14).

THE SYNAPSINS AND SHORT-TERM SYNAPTIC PLASTICITY

There are only relatively few data concerning the role played by synapsin on short-term synaptic plasticity and their interpretation is still unclear and debated. Field EPSPs recorded in hippocampal slices of synapsin I KO mice exhibited increased paired-pulse facilitation (PPF) (32, 33), but no effect was observed on post-tetanic potentation (PTP) (33). On the other hand, synapsin II and I/II KO mice showed no changes in PPF, but a dramatic decrease of PTP (33). Cultured hippocampal neurons (7-14 DIV) obtained from synapsin III or I/II/III KO mice showed no changes in PPF (8, 14). In cholinergic synapses of *Aplysia californica*, synapsin functional ablation by antibody injection produced a virtual disappearance of PTP that was, substituted by an intense post-tetanic depression. In the same study, basal synaptic transmission was not altered, but the PPF was significantly decreased at physiological Ca^{2+} concentrations. However, decreasing release probability by lowering the Ca^{2+}/Mg^{2+} ratio to remove synaptic depression revealed that PPF was not affected by synapsin neutralization (21). Finally, presynaptic injection of the peptide fragment of domain E in squid giant synapses dramatically decreased postsynaptic potential in response to a single action potential but did not affect PPF (17). Thus, most of the available data indicate that PPF is not a primary target of synapsin action in excitatory terminals, although the function of synapsin on short-term plasticity of inhibitory synapses remains completely unexplored.

Moreover, PPF has an intrinsic kinetics of tens of milliseconds, a time-range much faster than the time necessary for the Ca^{2+}-dependent mobilization of SV from the reserve to the readily releasable pool to occur (41). Thus, the possibility exists that synapsin affects PPF either through a post-docking mechanism (see below) or by altering the baseline level of transmission and indirectly influencing the magnitude of short-term plasticity, as suggested by recent data (34, 21, 18, 8, 38). Indeed, at most synapses, an increase in the initial probability of transmitter release decreases the magnitude of synaptic enhancement (lower PPF), and, conversely, a decrease in the probability of release results in larger synaptic enhancement or smaller synaptic depression (higher PPF) (41).

At variance with PPF, synapsins appear to have a definite role in the presynaptic expression of PTP, as both synapsin II and I/II KO mice (33) and invertebrate synapses after synapsin neutralization (21) exhibit a marked impairment in this form of plasticity. Although synapsin I KO mice showed no detectable changes in PTP, the almost double effect on PTP observed in synapsin I/II double KO mice as compared to the single synapsin II KO mice strongly indicates that also synapsin I plays a role in regulating PTP and that the absence of changes in PTP observed in synapsin I KO mice is attributable to the compensatory effect of synapsin II. PTP is characterized by a time-course in the order of seconds in mammals, a time certainly long enough to involve SV mobilization from the reserve pool. Thus, the action of synapsins on PTP are consistent with the pre-docking mechanism model described above, in which Ca^{2+} accumulation induced by tetanic stimulation activates a Ca^{2+}-dependent phosphorylation of synapsin liberating SV from the actin cytoskeleton and increasing their availability for exocytosis.

The reasons for the partial disagreement among some of the observed effects of synapsin lack on short-term plasticity could be several-fold: (i) except for some of the most recent studies (8, 14), a parallel analysis at inhibitory and excitatory synapses was not carried out; (ii) the data were obtained using different neuronal preparations, i.e. either primary cultures of hippocampal neurons or acute slices; (iii) primary cultures were used at different stages of maturation and, in most studies, before a complete synaptic maturation had occurred; and (iv) it is experimentally very difficult to measure the baseline level of synaptic transmission, especially in slices. For instance, studies of PPF were performed in brain slices of synapsin KO mice through extracellular stimulation evoking a response that was detected with extracellular electrodes (field EPSPs) (32, 33). Under these conditions, the amplitude of the evoked response does not provide a measure of baseline transmission because it reflects the activation of many presynaptic fibers and depends upon several factors (i.e., positioning of the electrodes, intensity of stimulation, slice viability). As it is very difficult to quantify the initial magnitude of release with extracellular methods, previous studies did not determine whether the observed changes in plasticity were direct effects on facilitation or depression or they were secondary to changes in some quantal parameter characterizing the efficiency of synaptic transmission, such as initial release probability and rate of release. Single cell patch-clamp recordings represent a more adequate experimental approach, although this technique has to be used

with caution. In particular, using neuronal cultures obtained by KO mice, a change in the amplitude of evoked postsynaptic currents can be due to impaired synaptogenesis and/or neurite elongation that dramatically decrease the number of functioning synapses and consequently the number of SV released in response to presynaptic stimulation. Only a detailed quantal analysis of miniature currents and a noise analysis of evoked postsynaptic currents will provide the quantal parameter of neurotransmission necessary to interpret the effects on short-term plasticity.

THE SYNAPSINS AND RELEASE PROBABILITY

According to the general model of synapsins tethering SV to the actin cytoskeleton at a distance from the active zones and releasing them upon activity through phosphorylation-dependent dissociation, the SV recruited to the readily releasable pool should be depleted of synapsins. Although this general picture is still valid and accounts for most of the physiological data, it has become clear that synapsins also have a function at the membrane stages of release after SV have docked to the active zones. Several observations support the latter view: (i) SV in the readily releasable pool are only partially desaturated of synapsins, and about 20-35% of the synapsin molecules associated with SV in the reserve pool remain associated with actively recycling SV during high frequency stimulation (39); (ii) while in resting synapses, synapsins were preferentially confined to the reserve pool, during synaptic activity, synapsins were detected in SV of the readily releasable pool and on uncoated recycled SV (4); (iii) synapsins colocalize with actin in the dynamic filamentous cytomatrix present in sites of intense SV recycling (4).

The hypothesis that synapsin could play some role in the post-docking stages of neurexocytosis, initially suggested by the uncertain effects on PPF, was recently demonstrated by growing evidence showing that synapsins can directly affect the probability and the rate of NT release. The first functional evidence suggesting a possible post-docking effect of synapsin was obtained by studying SV dynamics with FM dyes. In hippocampal neurons from synapsin I KO mice, the reduction in the total functional recycling SV pool size was found to be associated with a decrease in the total number of SV which undergo exocytosis during brief trains of action potentials (20 impulses) at individual synaptic boutons (97). While the former observation was in agreement with the decrease in the reserve pool of SV (23, 37), the latter result was rather unexpected, since stimuli in this range would be expected to draw solely upon the readily releasable pool of SV which appears relatively intact in synapsin I KO mice, and suggests a decrease in release probability. This finding offers the possibility to reinterpret the puzzling increase in PPF in hippocampal synapses in the absence of effects on synaptic depression found in synapsin I KO mice (32, 33): while the increase in PPF could reflect a decrease of initial release probability, the lack of effect of synapsin I deletion on the rate of synaptic depressionduring repetitive stimulation could be attributable to a simultaneous decrease in release probability and in the size of the functional SV pool.

More recently, the presynaptic injection of a peptide corresponding to a highly conserved region of domain E of squid synapsins into the squid giant synapse completely inhibited NT release in the absence of appreciable changes in the number of docked SV (17). Interestingly, this effect was accompanied by an increase in the rise and decay times of postsynaptic currents. The kinetics of release was also profoundly altered in cholinergic synapses of *Aplysia californica* injected with a specific antibody to snail synapsins (21). In this study, the rise time of the evoked postsynaptic current was significantly slowed in the absence of any changes in decay time and mean amplitude of postsynaptic response.

A post-docking action of synapsins is likely to be involved also in the decrease of evoked inhibitory postsynaptic currents (eIPSCs) observed in CA3 pyramidal neurons from hippocampal slices (P10-14) of synapsin I KO mice (38). Mutant mice showed a decrease in the amplitude and an increase in the coefficient of variation of eIPSCs, while the amplitude of miniature IPSCs was not affected, suggesting that synapsin I deficiency reduces the efficiency of inhibitory synaptic transmission by decreasing the number of SV released by a single action potential. The decrease of eIPSCs observed in cultured hippocampal autaptic neurons (7-9 DIV) from synapsin III KO mice (8) could be also attributable to a decreased release probability, although a more detailed electrophysiological analysis is necessary to exclude other possibilities.

Taken together, the data summarized here strongly suggest that the synapsins are also involved in the post-docking steps of release, by directly or indirectly regulating priming and/or fusion reactions thus playing a role in determining the rate and the amount of docked SV released in response to the action potential (Fig. 4). This post-docking action could be accounted for interactions of the synapsins with the dynamic actin cytoskeleton present at the active and periactive zones and/or with presynaptic proteins involved in the priming/fusion steps. On one hand, it has been recently shown that the synapsin domain E peptide has the ability to inhibit the binding of endogenous synapsins to actin, an effect that is shared by other synapsin peptides (i.e. peptides encompassing regions of the conserved C domain) that also alter the kinetics of release (19), suggesting that an interaction with actin at the active zone may play a role in the post-docking effects of synapsin. On the other hand, synapsins have been recently shown to interact with the SV-associated G protein Rab 3A and to modulate Rab3 cycling and GTPase activity in nerve terminals (12, 13). As Rab 3A has been proposed to function to limit the amount of NT released in response to the Ca^{2+} signal in a late step that follows docking and priming (11), the post-docking effect of synapsins can be achieved by the removal of the Rab3-mediated inhibitory constraint on quantal release.

CONCLUSIONS

In this review we have attempted to summarize and compose into a unifying frame the numerous physiological observations and hypotheses on synapsin func-

tions that have been put forward over the last 15 years in a large array of experimental systems, from reconstituted or isolated nerve terminals to mice genetically altered for single and multiple isoforms, generated in distinct laboratories. The emerging picture (summarized in Figure 4) is complex, as expected from a complex family of proteins that includes several isoforms with partly redundant functions and distinct developmental and regional patterns of expression and that are targets of partly distinct signal transduction pathways. Notwithstanding this complexity, the extremely high evolutionary conservation and the overt deficits in synaptic function and neural circuit activity observed in their absence, strongly support a central role of the synapsins in the regulation of information transfer among neurons.

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