

Synaptic Vesicle Phosphoproteins and Regulation of Synaptic Function

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Complex brain functions, such as learning and memory, are believed to involve changes in the efficiency of communication between nerve cells. Therefore, the elucidation of the molecular mechanisms that regulate synaptic transmission, the process of intercellular communication, is an essential step toward understanding nervous system function. Several proteins associated with synaptic vesicles, the organelles that store neurotransmitters, are targets for protein phosphorylation and dephosphorylation. One of these phosphoproteins, synapsin I, by means of changes in its state of phosphorylation, appears to control the fraction of synaptic vesicles available for release and thereby to regulate the efficiency of neurotransmitter release. This article describes current understanding of the mechanism by which synapsin I modulates communication between nerve cells and reviews the properties and putative functions of other phosphoproteins associated with synaptic vesicles.

Neurotransmitter release from nerve terminals is quantal, that is, it involves the release of transmitter in packets containing equal numbers of molecules. Small synaptic vesicles, organelles with a diameter of approximately 50 nm (Fig. 1A), provide the anatomical basis of quantal release. Each quantum of neurotransmitter is contained within one of these vesicles and is released by exocytosis, a process involving the fusion of the vesicle with the plasma membrane (1).

The arrival of the action potential at the nerve terminal permits Ca^{2+} influx through voltage-gated Ca^{2+} channels, triggering the fusion of synaptic vesicles with the plasma membrane and the release of neurotransmitter into the synaptic cleft. The rapidity of fusion of synaptic vesicles, as compared with exocytotic processes in non-neuronal cells, may be accounted for by the existence of a stable complex of the vesicles with the presynaptic membrane before exocytosis. It remains to be determined whether exocytosis involves only the formation of a proteinaceous pore or whether it involves fusion of the lipid bilayers of the vesicle and plasma membranes. After releasing its contents, the fused synaptic vesicle is retrieved from the plasma membrane by endocytosis, refilled with locally synthesized neurotransmitter, and utilized in subsequent exocytosis (1, 2).

Two functional pools of small synaptic vesicles appear to be present within the nerve terminal: (i) a releasable pool of vesicles, some of which fuse with the plasma membrane upon arrival of a nerve impulse, and (ii) a reserve pool of vesicles, which are tethered to the cytoskeleton (3, 4) and are recruited to the releasable pool in response to the physiological requirements of the cell. Usually the number of

vesicles in the releasable pool represents a small proportion of the total number of vesicles, and the number of vesicles that fuse in response to an action potential represents a small proportion of the number of vesicles in the releasable pool. Regulation of the relative number of vesicles in the releasable and reserve pools represents an important mechanism by which nerve cells regulate the amount of neurotransmitter released, and, therefore, the efficiency of synaptic transmission (3).

Among the proteins associated with synaptic vesicles, potential candidates for mediating these processes have been identified. Several of these candidates are phosphoproteins (Fig. 2), which suggests that their functional role is modified by phosphorylation. Modulation of neurotransmitter release is brought about by changes in the levels of intracellular second messengers that activate protein kinases (5). The resultant phosphorylation of synaptic vesicle-associated proteins appears to play a key role in regulating exocytosis from nerve cells.

The Synapsins

The synapsins are a family of phosphoproteins that are specifically associated with the cytoplasmic surface of the synaptic vesicle membrane (6, 7; Fig. 1B). They comprise four homologous proteins, synapsins Ia and Ib (collectively referred to as synapsin I) and synapsins IIa and IIb (collectively referred to as synapsin II), which together account for approximately 9% of total vesicle protein (8, 9). Single genes encode synapsins I and II; the a and b isoforms arise from differential splicing of the transcripts.

For both synapsin I and synapsin II, the structural difference between the a and b isoforms is restricted to the COOH-terminal part of the molecule. A high degree of similarity between the amino acid sequences of synapsins I and II extends from the NH_2 -terminus and covers more than half of each protein, with conservation among mammalian species (9). Virtually all nerve terminals contain synapsins. However, there are some terminals in specific brain regions in which one or more of the four synapsin isoforms are not detected (9, 10).

The phosphorylation state of the synapsins is increased under all conditions that promote Ca^{2+} -dependent release of neurotransmitter. These conditions include electrical stimulation of nerve cells, veratridine- or K^+ -induced depolarization, and activation of some classes of presynaptic receptors (5). Synapsins I and II are physiological substrates both for cyclic adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase and for Ca^{2+} -calmodulin-dependent protein kinase I, which phosphorylate them on the same serine residue near the NH_2 -terminus. In addition, synapsin I, but not synapsin II, is a physiological substrate for Ca^{2+} -calmodulin-dependent protein kinase II (CaM kinase II), which catalyzes the incorporation of phosphate into two serine residues in the COOH-terminal region (11).

The phosphorylation of synapsin I by CaM kinase II occurs rapidly and to high stoichiometry during physiological activity of neurons. Phosphorylation by CaM kinase II causes a conformational change in the synapsin I molecule (12) and is associated with major changes in its biological properties. The introduction of the dephosphorylated form of synapsin I into the squid giant axon (Fig. 3A) (13), the goldfish Mauthner neuron (14), or nerve terminals isolated from mammalian brain (15) inhibits neurotransmitter release. In contrast, introduction of synapsin I after its phosphorylation by CaM kinase II has no effect on release (Fig. 3B) (13, 15). These results indicate that the dephosphorylated form provides an inhibitory constraint for synaptic vesicle exocytosis and that this constraint is relieved upon phosphorylation by CaM kinase II. As predicted, introduction of CaM kinase II into the nerve terminal increases neurotransmitter release (Fig. 3C) (13, 16).

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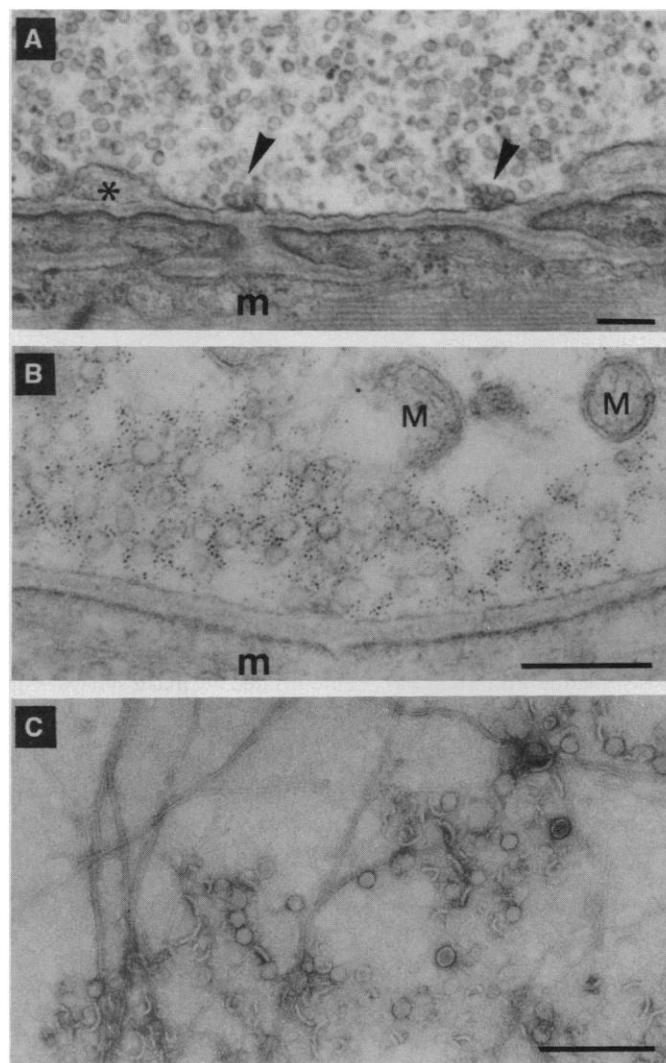
The cell biological basis for the regulation of neurotransmitter release by synapsin I has been partially elucidated. Synapsin I reversibly binds to synaptic vesicles with high affinity and saturability. The binding affinity is decreased upon phosphorylation of synapsin I by CaM kinase II (7, 17). In resting nerve terminals, 96 to 97% of synapsin I and of synapsin I-binding sites on synaptic vesicles exist in complexed form (17, 18), which indicates that they are present in a molar ratio close to unity. Structure-function analysis has demonstrated that the hydrophobic NH₂-terminal region of synapsin I interacts with vesicle phospholipids and partially penetrates the hydrophobic core of the membrane. The COOH-terminal region, which is elongated, basic, and hydrophilic, binds to a protein component of the vesicles, and this binding is virtually abolished upon phosphorylation by CaM kinase II (19). Photo-labeling experiments have demonstrated that a major vesicle-binding protein for the COOH-terminal region of synapsin I is the α subunit of CaM kinase II (20).

The cell cytoskeleton has important structural and dynamic functions. It is an internal scaffold for the cell, defining shape, and is also important for the guidance of organelles toward, and their accumulation within, functional compartments, which is especially critical in highly polarized cells such as neurons (21). Synapsin I is able to interact in vitro with various cytoskeletal proteins (22, 23), including actin. This and other evidence indicate that synapsin I reversibly tethers synaptic vesicles to the actin-based cytoskeleton of the nerve terminal and that this linkage is disrupted when synapsin I is phosphorylated by CaM kinase II. The interaction of synapsin I with actin is likely to be physiologically relevant to the accumulation of synaptic vesicles within the nerve terminal and to the regulation of their availability for exocytosis.

The binding of synapsin I to actin filaments leads to the formation of actin bundles in vitro. Phosphorylation of synapsin I by CaM kinase II reduces actin binding and abolishes actin-bundling activity (23). Structure-function analysis has shown that the binding sites for synaptic vesicles and for actin are located in distinct domains of the synapsin I molecule (19, 24).

Synapsin I also affects the dynamics of actin filament assembly. Dephosphorylated synapsin I triggers the condensation of actin monomers into nuclei, thereby accelerating actin polymerization and promoting the formation of new filaments. The actin-nucleating activity is prevented by phosphorylation of synapsin I by CaM kinase II (25, 26). The effect of dephosphorylated synapsin I is maintained when the protein is bound to synaptic vesicles (25), with the

Fig. 1. (A) Ultrastructure of a nerve terminal. The electron micrograph shows a portion of a neuromuscular junction from *Rana pipiens* cutaneous pectoris muscle. The axonal ending contains numerous synaptic vesicles. Some of the vesicles are clustered (arrowheads) close to plasma membrane specializations (active zones) where fusion preferentially occurs. Opposite the active zones are junctional folds of the muscle fiber (m). Schwann cell invagination (*). **(B)** Distribution of synapsin I in nerve terminals. The electron micrograph shows a portion of a neuromuscular junction with synapsin I marked by the immunoferritin procedure. Ferritin particles are concentrated virtually exclusively around synaptic vesicles. The mitochondria (M) and plasma membrane are not labeled with particles. Muscle fiber (m). [Reprinted from (62) with permission © Pergamon Press, Ltd.] **(C)** Interaction of synaptic vesicles with actin filaments. The electron micrograph shows a negatively stained sample of actin polymerized in the presence of synaptic vesicles purified from rat forebrain. Most of the synaptic vesicles appear to be associated with actin filaments. Scale bars = 0.25 μ m. [Reprinted from (25) with permission © Cell Press]



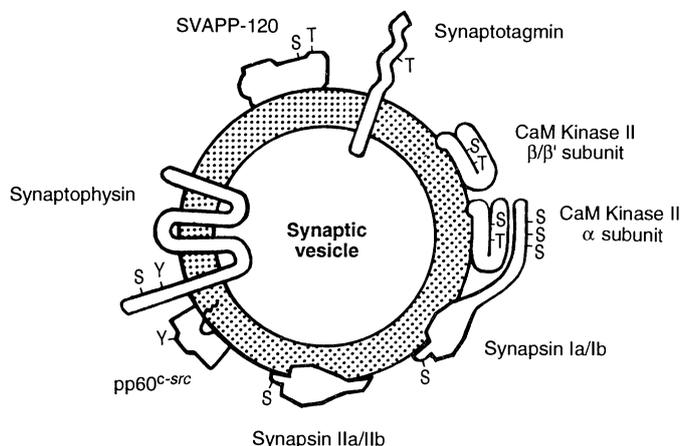
result that the vesicles are embedded in the cytoskeletal meshwork (Fig. 1C). Therefore, synapsin I phosphorylation not only potentiates the release of synaptic vesicles from the actin cytoskeleton but also prevents their recapture within the actin meshwork (8). This interpretation is supported by observations with video microscopy that synaptic vesicles are able to reorganize the actin filament network in a manner dependent on the phosphorylation state of synapsin I (27).

The effect of phosphorylation of synapsin I on the binding of synaptic vesicles to actin filaments has been modeled by computer simulation with the use of estimated concentrations of synapsin I, actin, and synaptic vesicles in nerve terminals and experimentally determined binding constants for synapsin I interactions with actin and synaptic vesicles (18). The analysis indicates that under resting conditions, in

which synapsin I is in the dephosphorylated state, most of the synaptic vesicles are anchored to the cytoskeleton. After neuronal activity and the associated phosphorylation of synapsin I by CaM kinase II, the ternary complex composed of synaptic vesicles, synapsin I, and actin dissociates, resulting in an increase in the fraction of synaptic vesicles that are liberated from the cytoskeleton and in the amount of synapsin I free in the cytosol. Thus, phosphorylation of synapsin I may regulate the transition of synaptic vesicles between reserve and releasable pools (Fig. 4).

Additional experimental evidence supporting this model has been obtained with rat brain synaptosomes. In this preparation, the release of neurotransmitter, evoked by K⁺-induced depolarization, is accompanied by an increase in the phosphorylation of synapsin I and the dissociation of synapsin I from the vesicle membrane (28). In addi-

Fig. 2. Schematic representation of phosphoproteins associated with synaptic vesicles. The topology of each phosphoprotein is depicted, with putative transmembrane domains shown traversing the lipid bilayer of the vesicle membrane (hatched area). Serine (S), threonine (T), and tyrosine (Y) indicate that at least one residue of the specified type is phosphorylated on the indicated protein. In most cases, the number and exact positions of the phosphorylated residue(s) and the protein kinase(s) responsible for the phosphorylation have been identified (9, 11, 33–36, 48, 49, 54, 56, 60). The location of four identified phosphorylation sites is shown for synapsin I.



tion, immuno-electron microscopy of frog neuromuscular junctions stimulated to release neurotransmitter has demonstrated that synapsin I dissociates from (and reassociates with) the synaptic vesicle membrane during the exocytotic (and endocytotic) cycle. The extent of dissociation seems to depend on the rate of secretion: a significant decrease in the amount of membrane-bound synapsin I has been observed at high rates of secretion (in response to electrical stimulation of the nerve) but not at low rates (in response to α -latrotoxin) (29).

In summary, physiological and cell biological results support a model in which the state of phosphorylation of synapsin I regulates the availability of synaptic vesicles for exocytosis. Many of the changes in the functional properties of the nerve terminal can be explained by this molecular mechanism (Fig. 5). As in the case of synapsin I, synapsin II binds to synaptic vesicles and is able to bind and bundle actin filaments (30). The common properties of synapsins I and II suggest that synapsin II may also mediate interactions of synaptic vesicles with the cytoskeleton. The CaM kinase II phosphorylation sites in synapsin I may provide an additional level of regulation of the interaction of this protein with synaptic vesicles and actin.

The Synapsins and the Formation of Synaptic Terminals

In addition to regulating neurotransmitter release from mature nerve terminals, recent evidence suggests that the synapsins may also regulate the formation of new nerve terminals. Transfection of a neuroblastoma-glioma cell line with the cDNA for synapsin IIb resulted in the formation of more nerve terminals in each cell and

more synaptic vesicles in each nerve terminal. In addition, these cells appeared to make synapses upon other neuroblastoma-glioma cells, in contrast to nontransfected or mock-transfected cells. Consistent with these morphological observations, transfection with synapsin IIb cDNA caused a severalfold increase in the amounts of other synaptic vesicle proteins with no detectable effect on proteins not specifically associated with the nerve terminal (31).

Results implicating the synapsins in the maturation of synaptic contacts were also obtained in experiments in which embryonic *Xenopus* spinal neurons were loaded with synapsin I. In this system, the injected synapsin I promoted the functional maturation of neurons, accelerating the development of quantal-secretion mechanisms (32). As in the case of regulation of neuro-

transmitter release from adult nerve terminals, the synapsins may act on synaptogenesis, at least in part, by altering the actin cytoskeleton.

Synaptophysin, Synaptoporin, and p29

Synaptophysin (previously referred to as p38) is a major integral protein of the synaptic vesicle membrane (33). It is a substrate for endogenous protein kinases both in intact synaptosomes and in purified synaptic vesicles (34, 35). The kinases responsible for the phosphorylation of synaptophysin have been identified as CaM kinase II, which phosphorylates synaptophysin on serine residues (35), and the proto-oncogene product pp60^{c-src}, which phosphorylates synaptophysin on tyrosine residues (36). Both kinases are present on the synaptic vesicle membrane (20, 37). The tyrosine phosphorylation reaction occurs on individual synaptic vesicles; that is, it does not require an interaction either between vesicles or between a vesicle and another cellular structure (34).

The presence of synaptophysin is required for vesicle fusion and release of neurotransmitter to occur. Antibodies against synaptophysin, as well as antisense oligonucleotides to synaptophysin mRNA, were able to inhibit Ca²⁺-dependent neurotransmitter release that had been reconstituted in *Xenopus* oocytes by injection of total rat cerebellum mRNA. In addition, loading of embryonic *Xenopus* spinal neurons with antibodies against synaptophysin inhibited both spontaneous and evoked neurotransmitter release (38).

Synaptophysin oligomers have been reported to form channels when reconstituted in lipid bilayers (39). This observa-

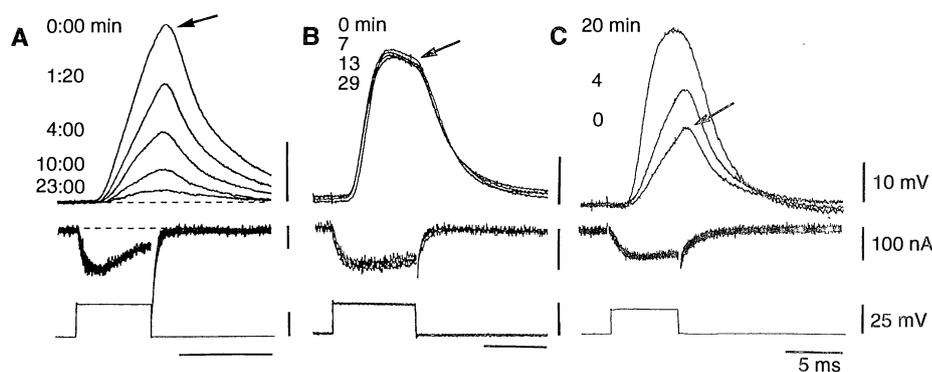
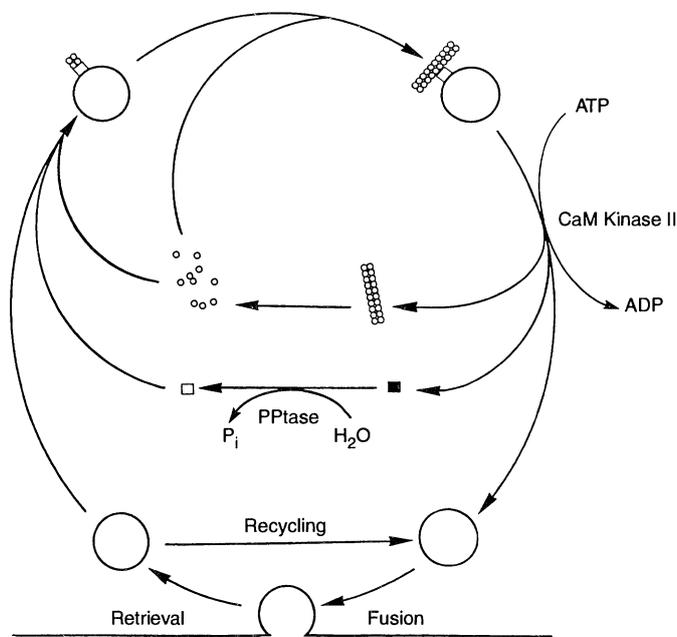


Fig. 3. Effects of (A) dephosphorylated synapsin I, (B) phosphorylated synapsin I, and (C) CaM kinase II on neurotransmitter release (13). The indicated proteins were injected into the presynaptic terminal digit of the squid giant synapse. The postsynaptic potentials (upper tracings) and the inward presynaptic Ca²⁺ currents (middle tracings) were generated by constant presynaptic depolarizing steps (lower tracings) delivered before injection (0 min, arrow) and at the indicated times after injection. The changes in the postsynaptic potentials observed after the injection of dephosphorylated synapsin I or CaM kinase II, in the absence of any effect on the presynaptic Ca²⁺ current, are indicative of a change in the efficiency of intraterminal free Ca²⁺ in promoting the release process.

Fig. 4. Model illustrating the postulated mechanism by which the state of phosphorylation of synapsin I regulates the availability of synaptic vesicles for exocytosis. Under resting conditions, dephosphorylated synapsin I (\square) cross-links synaptic vesicles (\circ) to actin filaments ($\circ\circ\circ\circ$), making them unavailable for release (reserve pool). Activation of CaM kinase II results in phosphorylation of synapsin I (\blacksquare) and disruption of the complexes. The liberated vesicles can now join the releasable pool. After fusion with the plasma membrane and subsequent endocytotic retrieval, the vesicles can be either recycled within



the releasable pool or resequenced within the reserve pool. Resequencing occurs because dephosphorylation of synapsin I promotes its rebinding to the vesicles and nucleation of filaments from actin monomers (\circ), resulting in the reformation of complexes and the embedding of vesicles in the cytoskeletal meshwork. Ppase, protein phosphatase; P_i , inorganic phosphate.

tion—together with limited structural similarities between synaptophysin and gap junction proteins, the connexins (40)—has led to the suggestion (39, 40) that synaptophysin participates in the formation of an exocytotic fusion pore, which leads to the release of neurotransmitter. However, the formation and opening of a fusion pore would require the interaction of synaptophysin with a protein located on the plasma membrane. Synaptophysin has been reported to interact with a plasma membrane protein, physophilin (41), making physophilin a candidate for such a fusion pore component. Like synaptophysin, the connexins are phosphorylated on tyrosine and serine residues, and their phosphorylation ap-

pears to modulate gap junction permeability (42). It is therefore conceivable that the phosphorylation of synaptophysin modulates the formation or the conductance of the putative exocytotic fusion pore.

Two synaptic vesicle proteins homologous to synaptophysin have been identified: synaptoporin and p29. Synaptoporin was identified by low-stringency hybridization with synaptophysin cDNA probes (43). Two serine residues in synaptoporin are located within a consensus sequence for phosphorylation by CaM kinase II. An integral membrane protein of synaptic vesicles, p29, appears to have some degree of similarity with synaptophysin, as assessed by antibody cross-reactivity (44). This pro-

tein is one of the major synaptic vesicle-associated substrates for tyrosine kinases (34, 44).

Synaptotagmin

Synaptotagmin (also referred to as p65) was the first integral membrane protein of synaptic vesicles to be identified (45). It has a single transmembrane domain, a small intravesicular domain, and a large cytoplasmic tail that contains two domains homologous to the regulatory Ca^{2+} - and phospholipid-binding region of protein kinase C (46). Synaptotagmin is responsible for the hemagglutinating activity present on synaptic vesicles, binds acidic phospholipids and Ca^{2+} , and has been reported to bind calmodulin (46, 47). In vitro, synaptotagmin is phosphorylated on an identified threonine residue by casein kinase II (48, 49).

Synaptotagmin interacts with the α -lactotoxin receptor (49), a protein specifically localized to the presynaptic plasma membrane (50), and with a novel neuronal protein, syntaxin, which is concentrated at synaptic sites (51). Syntaxin appears to mediate the interaction of synaptotagmin with the N-type Ca^{2+} channel, which is thought to be localized at active zones (52).

Taken together, these data suggest that synaptotagmin is involved in mediating the interaction of synaptic vesicles with the plasma membrane, possibly in the docking of synaptic vesicles at the release sites or in their subsequent fusion with the plasma membrane. That synaptotagmin is essential to the secretory process was recently questioned by experiments with PC-12 cells (rat adrenal pheochromocytoma) in which the presence of synaptotagmin was not obligatory for catecholamine release (53); however, the organelles responsible for the release of catecholamines were not identified in that study.

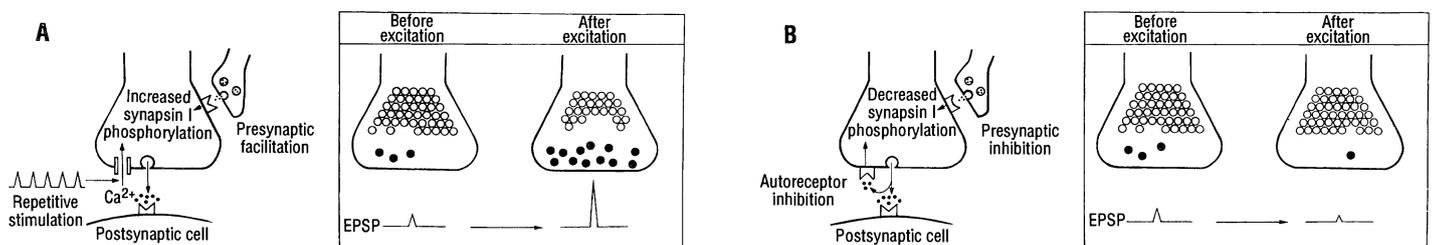


Fig. 5. Proposed functional role for synapsin I in regulation of the efficiency of transmitter release from nerve terminals. **(A)** Presynaptic excitation increases the efficiency of neurotransmitter release. (Left) Excitatory signals, such as repetitive nerve stimulation or activation of facilitatory presynaptic receptors, raise free Ca^{2+} in the nerve terminal and result in an increase in the state of phosphorylation of synapsin I. (Right) Increased phosphorylation of synapsin I increases the transition of vesicles from the reserve (\circ) to the releasable (\bullet) pool. This causes enhanced release of neurotransmitter in response to a constant stimulus,

as represented by the larger excitatory postsynaptic potential (EPSP). **(B)** Presynaptic inhibition decreases the efficiency of neurotransmitter release. (Left) Inhibitory signals, mediated through the activation of inhibitory autoreceptors or of other presynaptic inhibitory receptors, lower free Ca^{2+} in the nerve terminal and result in a decrease in the state of phosphorylation of synapsin I. (Right) Decreased phosphorylation of synapsin I decreases the transition of vesicles from the reserve to the releasable pool. This causes diminished release of neurotransmitter in response to a constant stimulus, as represented by the smaller EPSP.

SVAPP-120

Synaptic vesicle-associated phosphoprotein (SVAPP-120) (120 kD) is a peripheral membrane protein of synaptic vesicles (54). It is phosphorylated on serine and threonine residues, both in intact nerve terminals and in vitro, by multiple protein kinases including cAMP-dependent protein kinase, protein kinase C, and an endogenous Ca²⁺-calmodulin-dependent protein kinase. Its low abundance relative to other synaptic vesicle-associated proteins suggests that SVAPP-120 may be associated with only a subpopulation of synaptic vesicles.

Synaptic Vesicle-Associated Protein Kinases

The product of the cellular homolog of the viral *src* oncogene, pp60^{c-src}, is highly conserved phylogenetically and appears to be present on the plasma membrane of virtually all cells, although its presence is not required for cell viability (55). A variant form known as pp60^{c-src+} is found specifically in neural tissue (56). In secretory cells, pp60^{c-src} has also been found in association with secretory granules (57). The enzyme is expressed in large amounts in neural tissues (58), is four to five times more concentrated in purified synaptic vesicles than in brain homogenate, and accounts for 70% of the tyrosine kinase activity of these organelles (36, 37). Unlike receptor tyrosine kinases, pp60^{c-src} lacks transmembrane domains. Its association with membranes involves myristylation of the NH₂-terminus of the protein and may also involve interactions with membrane proteins that could account for the specific targeting of the kinase (59). The activity of this kinase is known to be negatively modulated by autophosphorylation on tyrosine (56). The role of pp60^{c-src} in synaptic vesicle function has not been determined, and signaling pathways that regulate the activity of the enzyme within nerve terminals have not been identified.

The CaM kinase II is a major component of synaptic vesicles, representing approximately 2% of total vesicle protein, and serves as a binding protein for synapsin I (20). Although both the α and β/β' subunits of CaM kinase II are present on synaptic vesicles, only the α subunit is specifically involved in binding. The COOH-terminal region of synapsin I interacts with the autoregulatory domain of the α subunit of the kinase. As with pp60^{c-src}, the association of CaM kinase II with the synaptic vesicle membrane is tight, requiring the use of detergents to achieve solubilization and suggesting the presence of a posttranslational modification of the kinase. Autophosphorylation of CaM kinase

II occurs on multiple serine and threonine residues. This causes its activity to become Ca²⁺-independent, allowing phosphorylation of substrates after termination of the Ca²⁺ signal (60).

The complex between synapsin I and the α subunit of CaM kinase II on the synaptic vesicle membrane may be part of a regulatory system for the rapid modulation of the efficiency of neurotransmitter release. Mutant mice lacking the α subunit of CaM kinase II exhibit impairment both of spatial learning and of long-term potentiation (61). The extent to which the lack of the synaptic vesicle-associated form of the α subunit of CaM kinase II contributes to these phenomena, through an impairment of the binding of synapsin I to synaptic vesicles, remains to be determined.

Conclusions

Changes in the efficiency of neurotransmitter release are believed to play a major role in synaptic plasticity. These changes are achieved, in part, through regulation of the number of vesicles available for exocytosis. Synapsin I, a phosphoprotein specifically localized to synaptic vesicles, appears to regulate the number of vesicles available for release by reversibly tethering them to the actin cytoskeleton of the nerve terminal. Through this mechanism, synapsin I and the proteins with which it interacts provide a molecular machinery by which the flow of information impinging on a given nerve terminal regulates the release of neurotransmitter. Thus, nerve impulses, as well as the activation of presynaptic receptors by signals coming from other cells, affect the state of phosphorylation of synapsin I, thereby determining the distribution of vesicles in the reserve and releasable pools. This protein phosphorylation pathway is a vital component of the mechanisms involved in synaptic plasticity and may contribute to the cellular basis of learning and memory.

REFERENCES AND NOTES

1. J. del Castillo and B. Katz, *Prog. Biophys. Biophys. Chem.* **6**, 121 (1956); B. Ceccarelli and W. P. Hurlbut, *Physiol. Rev.* **60**, 396 (1980). In addition to the small synaptic vesicles, which contain neurotransmitter and represent the large majority of secretory vesicles in the brain, neurons also possess a population of large, dense-core vesicles, which store and release neuropeptides. These latter vesicles are not specific to neurons, and a discussion of their properties is beyond the scope of this article.
2. R. B. Kelly, *Neuron* **1**, 431 (1988); P. De Camilli and R. Jahn, *Annu. Rev. Physiol.* **52**, 625 (1990); W. Almers, *ibid.*, p. 607.
3. F. Valtorta *et al.*, *Neuroscience* **35**, 477 (1990); M. Bahler, F. Benfenati, F. Valtorta, P. Greengard, *BioEssays* **12**, 259 (1990).
4. D. M. D. Landis, A. K. Hall, L. A. Weinstein, T. S. Reese, *Neuron* **1**, 201 (1988); N. Hirokawa, K. Sobue, K. Kanda, A. Harada, H. Yorifuji, *J. Cell*

- Biol.* **108**, 111 (1989); T. Gotow, K. Miyaguchi, P. H. Hashimoto, *Neuroscience* **40**, 587 (1991).
5. E. J. Nestler and P. Greengard, *Protein Phosphorylation in the Nervous System* (Wiley, New York, 1984).
6. P. De Camilli, R. Cameron, P. Greengard, *J. Cell Biol.* **96**, 1337 (1983); P. De Camilli, S. M. Harris, W. B. Huttner, P. Greengard, *ibid.*, p. 1355.
7. W. B. Huttner, W. Schiebler, P. Greengard, P. De Camilli, *ibid.*, p. 1374.
8. P. De Camilli, F. Benfenati, F. Valtorta, P. Greengard, *Annu. Rev. Cell Biol.* **6**, 433 (1990); F. Valtorta, F. Benfenati, P. Greengard, *J. Biol. Chem.* **267**, 7195 (1992).
9. T. C. Sudhof *et al.*, *Science* **245**, 1474 (1989).
10. J. W. Mandell *et al.*, *Neuron* **5**, 19 (1990); J. W. Mandell, A. J. Czernik, P. De Camilli, P. Greengard, E. Townes-Anderson, *J. Neurosci.* **12**, 1736 (1992); M. R. Dino, E. Mugnaini, A. J. Czernik, P. Greengard, *Soc. Neurosci. Abstr.* **17**, 637 (1991).
11. W. B. Huttner and P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5402 (1979); W. B. Huttner, L. V. DeGennaro, P. Greengard, *J. Biol. Chem.* **256**, 1482 (1981); M. B. Kennedy, T. McGuinness, P. Greengard, *J. Neurosci.* **3**, 818 (1983); A. C. Nairn and P. Greengard, *J. Biol. Chem.* **262**, 7273 (1987); A. J. Czernik, D. T. Pang, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7518 (1987). Synapsin I has also been reported to be phosphorylated in vitro by protein kinase C [S. E. Severin, E. L. Moskvitina, E. V. Bykova, S. V. Lutzenko, V. I. Shvets, *FEBS Lett.* **258**, 223 (1989)], by a proline-directed protein kinase [F. L. Hall, J. P. Mitchell, P. R. Vulliet, *J. Biol. Chem.* **265**, 6944 (1990)], and by Ca²⁺-calmodulin-dependent protein kinase α [C.-A. Ohmsted, K. F. Jensen, N. E. Sahyoun, *ibid.* **264**, 5866 (1989)].
12. F. Benfenati, P. Neyroz, M. Bahler, L. Masotti, P. Greengard, *J. Biol. Chem.* **265**, 12584 (1990).
13. R. Llinás, T. L. McGuinness, C. S. Leonard, M. Sugimori, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3035 (1985); R. Llinás, J. A. Gruner, M. Sugimori, T. McGuinness, P. Greengard, *J. Physiol. (London)* **436**, 257 (1991); J.-W. Lin, M. Sugimori, R. Llinás, T. L. McGuinness, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8257 (1990). The possible role of synapsin II in the regulation of neurotransmitter release has not been investigated.
14. J. T. Hackett *et al.*, *J. Neurophysiol.* **63**, 701 (1990).
15. R. A. Nichols, T. J. Chilcote, A. J. Czernik, P. Greengard, *J. Neurochem.* **58**, 783 (1992).
16. R. A. Nichols, T. S. Sihra, A. J. Czernik, A. C. Nairn, P. Greengard, *Nature* **343**, 647 (1990).
17. W. Schiebler, R. Jahn, J.-P. Doucet, J. Rothlein, P. Greengard, *J. Biol. Chem.* **261**, 8383 (1986).
18. F. Benfenati, F. Valtorta, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 575 (1991).
19. F. Benfenati, M. Bahler, R. Jahn, P. Greengard, *J. Cell Biol.* **108**, 1863 (1989); F. Benfenati, P. Greengard, J. Brunner, M. Bahler, *ibid.*, p. 1851.
20. F. Benfenati *et al.*, *Nature* **359**, 417 (1992).
21. R. D. Burgoyne, Ed., *The Neuronal Cytoskeleton* (Wiley-Liss, New York, 1991).
22. A. J. Baines and V. Bennett, *Nature* **315**, 410 (1985); *ibid.* **319**, 145 (1986); J. R. Goldenring *et al.*, *J. Biol. Chem.* **261**, 8495 (1986).
23. M. Bahler and P. Greengard, *Nature* **326**, 704 (1987); T. C. Petrucci and J. S. Morrow, *J. Cell Biol.* **105**, 1355 (1987).
24. M. Bahler, F. Benfenati, F. Valtorta, A. J. Czernik, P. Greengard, *J. Cell Biol.* **108**, 1841 (1989).
25. F. Benfenati, F. Valtorta, E. Chiergatti, P. Greengard, *Neuron* **8**, 377 (1992).
26. F. Valtorta, P. Greengard, R. Fesce, E. Chiergatti, F. Benfenati, *J. Biol. Chem.* **267**, 11281 (1992); R. Fesce, F. Benfenati, P. Greengard, F. Valtorta, *ibid.*, p. 11289.
27. P. E. Ceccaldi, *et al.*, in preparation.
28. T. S. Sihra, J. K. T. Wang, F. S. Gorelick, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8108 (1989).
29. F. Torri Tarelli *et al.*, *J. Cell Biol.* **110**, 449 (1990); F. Torri Tarelli, M. Bossi, R. Fesce, P. Greengard, F. Valtorta, *Neuron* **9**, 1143 (1992).
30. G. Thiel, P. Greengard, T. C. Sudhof, *Proc. Natl.*

- Acad. Sci. U.S.A.* **88**, 3431 (1991); C. Siow, T. Chilcote, F. Benfenati, P. Greengard, G. Thiel, *Biochemistry* **31**, 4268 (1992), T. Chilcote, C. Siow, G. Thiel, P. Greengard, in preparation.
31. H.-Q. Han, R. A. Nichols, M. R. Rubin, M. Bahler, P. Greengard, *Nature* **349**, 697 (1991).
 32. B. Lu, P. Greengard, M.-m. Poo, *Neuron* **8**, 521 (1992).
 33. R. Jahn, W. Schiebler, C. Ouimet, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4137 (1985); B. Wiedenmann and W. Franke, *Cell* **41**, 1017 (1985).
 34. D. T. Pang, J. K. T. Wang, F. Valtorta, F. Benfenati, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 762 (1988).
 35. J. R. Rubenstein, P. Greengard, A. J. Czernik, *Synapse* **13**, 161 (1993).
 36. A. Barnekow, R. Jahn, M. Scharlt, *Oncogene* **5**, 1019 (1990).
 37. D. T. Pang *et al.*, *Soc. Neurosci. Abstr.* **14**, 106 (1988).
 38. J. Alder, B. Lu, F. Valtorta, P. Greengard, M.-m. Poo, *Science* **257**, 657 (1992); J. Alder, Z.-P. Xie, F. Valtorta, P. Greengard, M.-m. Poo, *Neuron* **9**, 759 (1992).
 39. L. Thomas *et al.*, *Science* **242**, 1050 (1988).
 40. R. E. Leube *et al.*, *EMBO J.* **6**, 3261 (1987); T. C. Sudhof, F. Lottspeich, P. Greengard, E. Mehl, R. Jahn, *Science* **238**, 1142 (1987).
 41. L. Thomas and H. Betz, *J. Cell Biol.* **111**, 2041 (1990).
 42. J. C. Sáez *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2473 (1986), L. S. Musil, E. C. Beyer, D. A. Goodenough, *J. Membr. Biol.* **116**, 163 (1990).
 43. P. Knaus, B. Marqueze-Pouey, H. Scherer, H. Betz, *Neuron* **5**, 453 (1990).
 44. M. Baumert *et al.*, *J. Cell Biol.* **110**, 1285 (1990).
 45. W. D. Matthew, L. Tsavaler, L. F. Reichardt, *ibid.* **91**, 257 (1981).
 46. M. S. Perin, V. A. Fried, G. A. Mignery, R. Jahn, T. C. Sudhof, *Nature* **345**, 260 (1990).
 47. M. Popoli and A. Mengano, *Neurochem. Res.* **13**, 63 (1988); N. Brose, A. G. Petrenko, T. C. Sudhof, R. Jahn, *Science* **256**, 1021 (1992); J. M. Trifaró, S. Fournier, M. L. Novas, *Neuroscience* **29**, 1 (1989).
 48. M. K. Bennett, N. Calakos, T. Kreiner, R. H. Scheller, *J. Cell Biol.* **115**, 304a (1991); M. K. Bennett, K. G. Miller, R. H. Scheller, *J. Neurosci.*, in press.
 49. A. G. Petrenko *et al.*, *Nature* **353**, 65 (1991).
 50. F. Valtorta, L. Madeddu, J. Meldolesi, B. Ceccarelli, *J. Cell Biol.* **99**, 124 (1984).
 51. M. K. Bennett, N. Calakos, R. H. Scheller, *Science* **257**, 255 (1992).
 52. R. Robitaille, E. M. Adler, M. P. Charlton, *Neuron* **5**, 773 (1990); F. Torri Tarelli, M. Passafaro, F. Clementi, E. Sher, *Brain Res.* **547**, 331 (1991).
 53. Y. Shoji-Kasai *et al.*, *Science* **256**, 1820 (1992).
 54. M. Bahler, R. L. Klein, J. K. T. Wang, F. Benfenati, P. Greengard, *J. Neurochem.* **57**, 423 (1991).
 55. P. Soriano, C. Montgomery, R. Geske, A. Bradley, *Cell* **64**, 693 (1991).
 56. J. A. Cooper, in *Peptides and Protein Phosphorylation*, B. E. Kemp, Ed. (CRC Press, Boca Raton, FL, 1990), pp. 85–113.
 57. S. J. Parsons and C. E. Creutz, *Biochem. Biophys. Res. Commun.* **134**, 736 (1986); C. Grandori and H. Hanafusa, *J. Cell Biol.* **107**, 2125 (1988); J. E. Ferrell, J. A. Noble, G. S. Martin, Y. V. Jacques, B. F. Bainton, *Oncogene* **5**, 1033 (1990); A. D. Linstedt and R. B. Kelly, *J. Cell Biol.* **117**, 1077 (1992).
 58. P. C. Cotton and J. S. Brugge, *Mol. Cell. Biol.* **3**, 1157 (1983); J. S. Brugge *et al.*, *Nature* **316**, 554 (1985); A. A. Hirano, P. Greengard, R. L. Huganir, *J. Neurochem.* **50**, 1447 (1988); S. I. Walaas, A. Lustig, P. Greengard, J. S. Brugge, *Mol. Brain Res.* **3**, 215 (1988).
 59. M. D. Resh, *Cell* **58**, 281 (1989).
 60. P. I. Hanson and H. Schulman, *Annu. Rev. Biochem.* **61**, 559 (1992).
 61. A. J. Silva, C. F. Stevens, S. Tonegawa, Y. Wang, *Science* **257**, 201 (1992); A. J. Silva, R. Paylor, J. M. Wehner, S. Tonegawa, *ibid.*, p. 206.
 62. F. Valtorta *et al.*, *Neuroscience* **24**, 593 (1988).
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