Forum Minireview

New Aspects of Neurotransmitter Release and Exocytosis: Dynamic and Differential Regulation of Synapsin I Phosphorylation by Acute Neuronal Excitation In Vivo

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Abstract. Synapsin I is a synaptic vesicle-associated protein that is phosphorylated at multiple sites by various protein kinases. It has been proposed to play an important role in the regulation of neurotransmitter release and the organization of cytoskeletal architecture in the presynaptic terminal. In the present minireview, I describe the dynamic changes in synapsin I phosphorylation induced by acute neuronal excitation in vivo, and discuss its regulation by protein kinases and phosphatases and its functional significance in vivo. When acute neuronal excitation was induced by electroconvulsive treatment (ECT) in rats, phosphorylation of synapsin I at multiple sites was decreased during brief seizure activity in hippocampal and parieto-cortical homogenates. After termination of the seizure activity, phosphorylation at mitogen-activated protein kinase-dependent sites was increased dramatically. Phosphorylation at a Ca^{2+} /calmodulin-dependent protein kinase II-dependent site was also increased moderately afterwards. The dynamic and differential changes in synapsin I phosphorylation induced by acute neuronal excitation may be involved in plastic changes induced by ECT and may have some role in its effectiveness for the treatment of psychiatric diseases in humans.

Keywords: synapsin I, phosphorylation, dephosphorylation, mitogen-activated protein kinase, Ca^{2+} /calmodulin-dependent protein kinase II

Introduction

Synapsin I is a representative synaptic vesicle-associated phosphoprotein and localizes exclusively in the presynaptic terminal. Its phosphorylation state controls its association to synaptic vesicles and actin filaments and has been considered to play an important role in the regulation of neurotransmitter release and presynaptic actin network organization. Synapsin I is phosphorylated at multiple sites by various protein kinases (Fig. 1). Cyclic AMP-dependent protein kinase (PKA) and Ca^{2+} /calmodulin-dependent protein kinase I (CaMKI) phosphorylate Ser-9 (site 1); Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) phosphorylates Ser-566 and Ser-603 (sites 2 and 3); mitogen-activated protein kinase (MAPK) phosphorylates Ser-62, Ser-67, and Ser-549

(sites 4, 5 and 6); and cyclin-dependent protein kinases (cdks) phosphorylate Ser-549 and Ser-551 (sites 6 and 7) (1–5). Dephosphorylation of synapsin I was also studied recently; phospho-sites 1, 2, and 3 were shown to be preferentially dephosphorylated by protein phosphatase (PrP) 2A, and phospho-sites 4/5 and 6 were dephosphorylated by PrP2B (calcineurin, Ca^{2+} /calmodulin-dependent protein phosphatase) (6). Synapsins II and III, other structurally-related, different gene products of the synapsin family, have homologous N-terminal portions (domains A, B, and C). Phospho-site 1 is conserved among all synapsins, but other sites are unique to synapsin I (7–9).

In vitro studies using purified synapsin I showed that the dephospho-form of synapsin I binds synaptic vesicles, binds and bundles F-actin, and promotes G-actin polymerization. These functions are modified by phosphorylation of synapsin I as summarized in Fig. 2

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Fig. 1. Phosphorylation sites of synapsin I by various protein kinases. The domain structure of synapsin I (A through F) is schematically shown. The numbering of amino acids is based on the rat synapsin I sequence. Most effective protein phosphatases for dephosphorylation at each site are also shown. PKA, cyclic AMP-dependent protein kinase; CaMKI, Ca^{2+} /calmodulin-dependent protein kinase I; CaMKII, Ca^{2+} /calmodulin-dependent protein kinase II; MAPK, mitogen-activated protein kinase; cdk5, cyclin-dependent protein kinase 5; PrP2A, protein phosphatase 2A; PrP2B, protein phosphatase 2B (calcineurin, Ca^{2+} /calmodulin-dependent protein phosphatase).

synapsin I phosphorylated by				
dephospho-synapsin I function	CaMKII	PKA/CaMKI	MAPK	cdk5
	sites 2 & 3	site 1	sites 4, 5 & 6	site 6 & 7
(1) G-actin nucleating activity (promoting G-actin polymerization)	↓	↓	↓	↓
(2) bundling actin filaments	↓↓	↓	↓	↓ or →
(3) binding to synaptic vesicles	↓↓	↓	→	→
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facilitatory role in neurotransmitter release	+	n.d.	+	n.d.

Fig. 2. Summary of the effects of phosphorylation on synapsin I function. n.d., not determined.

(3, 4, 10–15). Phosphorylation of synapsin I at either site generally reduces G-actin nucleating activity and F-actin-bundling activity, which seems to be related to the flexibility of the presynaptic actin network organization. Phosphorylation of synapsin I at sites 2 and 3 and at site 1 reduces its binding to synaptic vesicles, which seems to be related to synaptic vesicle mobilization for neurotransmitter release in the presynaptic terminal. Experiments using synaptosomes indicated the importance of CaMKII-dependent phosphorylation and MAPK-dependent phosphorylation of synapsin I for the regulation of neurotransmitter release (2, 16, 17).

Regulation of synapsin I phosphorylation by protein kinases and phosphatases has been studied by using in vitro preparations such as synaptosomes, brain slices,

and cultured cells; and phospho-sites 2 and 3 were shown to be controlled by CaMKII and phospho-sites 4 and 5, by MAPK and calcineurin in these preparations (3, 6, 17–19). On the other hand, studies on the regulation of synapsin I phosphorylation in vivo in whole animals are still limited (20–22).

In this minireview, I describe the in vivo regulation of synapsin I phosphorylation based on the study of an animal model of acute, reversible neuronal excitation induced by electroconvulsive treatment (ECT) in rats (21, 22). Some of the results are parallel to the ones obtained with in vitro preparations, but not others. I would like to emphasize the importance of the analyses of whole animals, which would become more informative when combined with the characterization of geneti-

Fig. 3.

cally engineered animals, in understanding the physiological importance of synapsin I function in vivo.

Changes in synapsin I phosphorylation after ECT

ECT stimulation delivered through ear clips (60 Hz, sine wave, 100 volts, 2 s) induced maximal seizure activity, starting with tonic convulsion succeeded by clonic convulsion, and the generalized seizure activity ceased in 90 s at the latest (21, 22). The induced seizure was completely reversible (23). In appropriate periods of time after the electrical stimulation, hippocampal and parieto-cortical homogenates were prepared and subjected to quantitative immunoblot analyses and protein kinase assays (21, 22). Among the time points examined, the earliest point (30 s after ECT) was during generalized seizure activity and other time points were all in the post-seizure period.

Phospho-synapsin I levels in ECT-animals were determined by using phospho-site specific antibodies (Fig. 3) (22). The levels of phospho-sites 3, 4/5, and 6 were all decreased during seizure activity in both the hippocampus and parietal cortex. The extent of the decrease in phospho-sites 3 and 4/5 was larger than that in phospho-site 6. Phospho-site 1 also showed an initial decrease during seizure activity (data not shown). Phospho-site 4/5 showed a subsequent rapid large increase peaking at 5 min (Fig. 3B). Phospho-site 6 showed a slower moderate increase peaking at 20 min (Fig. 3C). Recovery of phospho-site 3 was much slower and a moderate increase was observed at later time points (Fig. 3A). All these changes were reversible and no longer significant at 60 min, although the levels tended to be higher in the parietal cortex. The total synapsin I level remained relatively unchanged.

Changes in protein kinase activities after ECT

The Ca^{2+} /calmodulin-independent autonomous activity of CaMKII was decreased during seizure activity and then showed a small increase in parietal cortex at later time points (Fig. 4) (21). The change in autophosphorylation of CaMKII at Thr-286 (α)/287 (β), which is essential for autonomous activity, paralleled that in

autonomous activity (data not shown) (21). The pattern of changes over time in the phospho-site 3 was similar to that of CaMKII activity, and the extent of the increase or decrease was larger in the phospho-site 3 than in CaMKII activity. MAPK activity showed a large increase during and soon after ECT without any initial decrease, peaked at 2.5 min, and returned to the control level in 20 min (data not shown) (22). Cdk5 activity remained relatively unchanged (data not shown) (22).

Effect of SL327, a MEK inhibitor, on the increase in synapsin I phosphorylation

When rats were pretreated with SL327, a MAPK kinase (MEK) inhibitor that crosses the blood-brain-barrier (24, 25) prior to ECT, significant suppression was observed not only in the increase in MAPK activity, but also in that in phospho-site 4/5 level (Fig. 5) (22). Thus, the increase in phospho-site 4/5 level after ECT was attributable to increased MAPK activity induced by ECT.

Regulation of synapsin I phosphorylation by protein kinases and phosphatases in rats in vivo

In vitro studies showed that the phospho-site 3 of synapsin I was a good substrate for PrP2A (6), and autophosphorylated CaMKII was dephosphorylated by PrPs 1, 2A, and 2C and CaMKII phosphatase (26–31). Both of them are poor substrates for calcineurin (6, 32, 33). Since both the level of the phospho-site 3 and the autonomous activity of CaMKII were decreased during seizure activity induced by ECT, it is most probable that the activation of PrP2A should be responsible for their dephosphorylation. The fact that the level of the phospho-site 1, another good substrate for PrP2A, was similarly decreased during seizure activity also supports this assumption. The mechanism of PrP2A activation during seizure activity is not clear at this moment.

On the other hand, a decrease in phospho-sites 4/5 and 6 during seizure activity seems to be caused by the activation of calcineurin, because these sites were shown to be good substrates for calcineurin in vitro, and high K^{+} -depolarization of synaptosomes in the presence

Fig. 3. Changes in the levels of phosphorylation of synapsin I at sites 3 (A), 4/5 (B), and 6 (C) after ECT in homogenates from the hippocampus and parietal cortex. Phospho-synapsin I levels at each site and total synapsin I level were determined by quantitative immunoblot analyses using phospho-site specific and anti-synapsin I antibodies, respectively. The ordinate expresses the values obtained from ECT animals as percent of controls. The abscissa expresses the time after the electrical stimulation. An arrow with "E" at time zero indicates ECT stimulation and a horizontal bar in the left lower corner indicates the approximate duration of seizure activity. Data are shown as means $\pm$ S.E.M. (bars) ($n = 5 - 10$) except for that at time 0, which indicates the control value (100%). Significantly different values from the control (100%) are indicated: * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$ (Bonferroni-Holm test). Modified from Yamagata et al. (Ref. 22).

Fig. 4. Changes in the Ca^{2+} /calmodulin-independent autonomous activity and the total activity of CaMKII after ECT in homogenates from the hippocampus and parietal cortex. Activities were measured by using a specific peptide substrate. Other details are as in the legend of Fig. 3. Modified from Yamagata and Obata (Ref. 21).

of extracellular Ca^{2+} also induced a decrease in phosphorylation at these sites (6). Other proteins such as high-molecular-weight MAPs (MAP2a and MAP2b) were also reported to be dephosphorylated soon after ECT (34). Such dephosphorylation of a variety of proteins may be a common phenomenon induced by seizure activity. In fact, in another study, an even larger decrease was observed in phospho-sites 1, 3, 4/5, and 6 in the status epilepticus induced by kainic acid in rats (35). The initial decrease in phospho-site 4/5 after ECT was soon overcome by a subsequent large increase, which was attributable to MAPK activation. The more delayed smaller increase in phospho-site 6 was not shown to be attributable to MAPK activation, and another unknown protein kinase may be involved.

Physiological implication of dynamic changes in synapsin I phosphorylation by ECT in vivo

ECT is an effective treatment for many psychiatric diseases such as major depression and schizophrenia in humans, especially in pharmacotherapy-resistant cases, but its mode of action still remains unknown (36). Studies of animal models have shown increased neurogenesis, mossy fiber sprouting, and an enhanced synap-

tic response following repeated ECT (37–40). Dynamic changes in synapsin I phosphorylation induced by ECT may be involved in such plastic changes in the presynaptic terminal (Fig. 6). The initial rapid dephosphorylation at all sites of synapsin I implies a more rigid organization of actin-cytoskeleton and sequestration of remaining synaptic vesicles to reserve pool during seizure activity. The former may be important for preserving the presynaptic morphological structure from epileptic discharges, and the latter may be beneficial to prevent further neurotransmitter release, because massive release should have already occurred by electrical stimulation itself. The following increase in phosphorylation largely at site 4/5 after termination of the seizure activity may lead to loosening of the presynaptic actin-network organization and enhanced availability of synaptic vesicles for release, which may be related to possible plastic changes such as synaptic reorganization and enhanced synaptic response observed after repeated ECT. More studies on the regulation of synapsin I phosphorylation in vivo will shed light on the normal physiology and pathophysiology of neuronal excitation, as well as on the therapeutic mechanisms of ECT in humans.

Fig. 5. Effects of SL327 on the increase in MAPK activity and the phospho-synapsin I levels after ECT. ECT samples were obtained at 5 min after ECT and the values are expressed as percent of controls. Vehicle-treated and SL327-treated groups are compared. Data are shown as means $\pm$ S.E.M. (bars) ($n = 3$). Significantly lower values in SL327-treated samples than those in vehicle-treated samples are indicated: * $P < 0.05$, *** $P < 0.001$ (one-tailed paired t -test). Modified from Yamagata et al. (Ref. 22).

Fig. 6. Schematic illustration of changes in synapsin I phosphorylation induced by ECT and its physiological implications.

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