Review

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Activity-controlled proteolytic cleavage at the synapse

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Activity-controlled enzymatic cleavage of proteins on the surface of synaptic membranes or in the synaptic or perisynaptic interstitial compartment represents a direct way to regulate synaptic structure, function, and number. Extracellular proteolysis at synapses was initially understood to be plasticity enabling by freeing synapses from the constraints provided by the extracellular matrix. However, recent observations indicate that at least part of the extracellular protein cleavage results in activation of previously cryptic functions that regulate adaptive changes of synapses and neuronal circuits. Here, we focus on peptidases with distinct localization and function at synapses combined with regulation by neuronal and synaptic activity, and evaluate their function in the context of developmental and/or adult synaptic plasticity.

Extracellular proteolysis awakes dormant synapse-regulating signals

The capacity of the brain to encode and retain memories is based on experience-driven modification of synaptic strength and structural remodeling of neural circuits, with long-term potentiation (LTP; see Glossary) and synapse formation and/or elimination, respectively, as the most extensively studied and most widely accepted underlying processes [1,2]. Both are elicited by the combination of neuronal activity with electrical activity-dependent chemical signal transmission at individual synapses. The resulting synaptic changes are achieved by diverse biochemical processes, including the regulated proteolytic cleavage of both intracellular and extracellular components. Whereas all known proteolytic cleavage within the pre- and postsynaptic compartments is degradative and thought to either serve protein turnover or support the reorganization of the synaptic proteome [3], at least part of the extracellular protein cleavage at synapses is productive, giving rise to new forms of proteins with altered functions [4,5]. Extracellular proteolytic cleavage at synapses is implemented by a relatively small number of peptidases, each with a distinct, selective proteolytic mechanism and a limited set of target proteins. Proteolytic cleavage may cause dislocation of the fragments, masking or unmasking of functional sites, or conformational changes, which in turn may activate or inactivate functions of the target protein [5,6]. Thus, extracellular peptidases with activity-controlled function at synapses are important regulators of both the strength of existing synapses and the structure of neuronal circuits in the context of developmental and/or adult synaptic plasticity. Here, we focus on activity-regulated peptidases with distinct localization at synapses, their regulation by neuronal and synaptic activity, their extracellular synaptic target proteins, and the synaptic effects resulting from target protein cleavage. For systemic effects resulting from synaptic proteolysis by these peptidases, the reader is referred to specialized reports.

Tissue plasminogen activator and plasmin

Tissue plasminogen activator (tPA) was originally identified as a multidomain serine peptidase of the fibrinolytic

Glossary

Dense-core granule (DCG): a special type of secretory vesicle, usually storing peptides or proteins; morphologically distinct from synaptic vesicles storing small molecule neurotransmitters.
Early- and late-phase LTP: in the hippocampus, two distinct phases of LTP, termed 'E-LTP' and 'L-LTP' have been defined. E-LTP is elicited by a weak stimulus, is independent of protein synthesis, and normally fades within 90 min. By contrast, L-LTP is elicited by a strong stimulus, lasts longer than 180 min, and requires gene transcription and protein synthesis in the postsynaptic cell. A conceptual model termed 'synaptic tagging and capture hypothesis' aims to explain how the newly synthesized synaptic proteins or mRNAs required for L-LTP are specifically targeted to active synapses (Box 2).
Hebbian learning: according to the postulate of Donald O. Hebb, synaptic connections with correlated activity will be strengthened, whereas those with uncorrelated activity are weakened and eventually disassembled [120]. In many forms of Hebbian learning, correlated activity of the pre- and postsynaptic neuron is thought to be detected by the coincidence detector function of NMDA-Rs.
Long-term potentiation (LTP): a long-lasting enhancement in synaptic signal transmission between two neurons that results from stimulating them synchronously. LTP is the most extensively described phenomenon underlying the ability of CNS synapses to change their strength and is widely considered to be one of the major cellular mechanisms of learning and memory.
Neural plasticity: the ability of a neural cell or circuit to undergo biochemical, structural, or physiological change.
NMDA receptor (NMDA-R): a subtype of ionotropic glutamate receptors. NMDA is a selective agonist that binds to NMDA-Rs, but not to other glutamate receptors. Calcium flux through NMDA-Rs is thought to be a critical trigger of synaptic plasticity. NMDA-Rs function as 'molecular coincidence detectors' for coincident activation of the pre- and postsynaptic cell. At resting membrane potential, the ion channel of NMDA-Rs is blocked by extracellular Mg^{2+}. Its activation requires both glutamate, released from the presynaptic bouton, and depolarization of the postsynaptic membrane, which removes the Mg^{2+} block of the channel.
Peptidase: an enzyme that cleaves peptide bonds; older terms are protease and proteinase.

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Box 1. Zymogens and zymogen activation: a reminder

A zymogen, also called a proenzyme, is an inactive protein precursor of an enzyme that is activated by proteolytic cleavage. Synthesis as a zymogen and subsequent activation via limited proteolysis allows for robust regulation of critical biological processes by preventing ectopic and premature protein cleavage [106]. Extracellularly active peptidases, including serine peptidases and metallo-peptidases, represent a prototypical group of enzymes that are regulated by limited proteolysis. Their zymogens, except those of the chymotrypsin-like serine peptidase family, exhibit a preformed active site cleft that is sterically obstructed by a so-called 'activation segment' or 'activation peptide', which needs to be excised for activation [107].

In the zymogenic precursors of the members of the MMP family, an amino-terminal propeptide of approximately 90 amino acids, containing the so-called 'cysteine switch' consensus sequence –Pro–Arg–Cys–X–X–Pro–Asp–, is positioned on top of the zinc-containing catalytic site [60,108]. The cysteine in this sequence interacts with the Zn^{2+} of the zymogen, thus preventing a H_2O molecule essential for catalysis from binding.

The zymogens of the chymotrypsin-like serine peptidases exhibit a 'distorted', nonfunctional active site [107,109]. Their propeptide has no steric obstruction function, but contains a masked activation motif that becomes active upon proteolytic cleavage. Binding of the amino group of the new amino-terminal amino acid with an aspartic acid carboxylate group located next to the catalytic serine residue restructures the active site region and activates the enzyme.

system. It initiates the lysis of the fibrin clot by conversion of the inactive zymogen plasminogen into the active plasmin. However, tPA and plasminogen are also expressed in other tissues, including regions of the central nervous system (CNS), such as the hippocampus, cerebellum, and amygdala [7–9]. In contrast to the zymogens of other serine peptidases (Box 1), the single-chain pro-enzyme of tPA has considerable activity, because peculiarities in the active site region promote an active conformation before proteolytic activation cleavage [10,11]. In the CNS, both indirect actions of tPA via the activation of plasminogen and direct proteolytic substrate cleavage have been found.

Synaptic localization, activity-regulated functions, and effects on synaptic structure and function

The synthesis of tPA-encoding mRNA in the hippocampus is enhanced by neuronal activity and NMDA receptor (NMDA-R) activation [12]. The local synthesis of tPA protein in dendrites is stimulated by glutamate acting via metabotropic glutamate receptors [13]. In hippocampal neurons, tPA and plasminogen are contained within dense-core granules (DCGs) in both dendritic spines and presynaptic boutons [14–16]. A fraction of these DCGs contains both tPA and plasminogen, suggesting intracellular conversion into plasmin [15]. The exocytosis of tPA from DCGs is induced by cell depolarization [14,17].

tPA and plasmin are involved in the expression of the late phase of LTP (L-LTP), because L-LTP is impaired in tPA-deficient mice [18] and enhanced in transgenic mice overexpressing tPA [19]. In addition, a single-tetanus stimulation, which typically induces the early phase of LTP (E-LTP), was found to result in L-LTP when combined with tPA application [20].

Selected synaptic target proteins of tPA and plasmin

NMDA-Rs. Direct cleavage of the NR1 subunit by tPA was reported to result in increased Ca^{2+} influx [21,22]. However, a follow-up study concluded that the NR1 subunit is cleaved by plasmin, rather than by tPA [23]. Plasmin was also identified as the relevant peptidase for cleaving of the NR2A subunit [24]. By contrast, tPA is responsible for cleavage of the NR2B subunit, resulting in altered pharmacological properties of NR2B-containing receptors [25].

Pro-BDNF. Cleavage of the inactive precursor pro-brain-derived neurotrophic factor (pro-BDNF) by tPA-plasmin can generate mature BDNF [26]. Given that BDNF activates the tyrosine receptor kinase B (TrkB) receptor, whereas pro-BDNF preferably activates the p75 neurotrophin receptor, this finding suggested a differential effect of pro-BDNF and BDNF, with the extracellular cleavage of pro-BDNF by tPA-plasmin as the critical regulatory mechanism [27]. However, doubts on the validity of this mechanism were raised by studies demonstrating that the conversion of pro-BDNF into mature BDNF (mBDNF) occurs intracellularly and, therefore, only mature BDNF is secreted [28]. This issue may be resolved by findings suggesting that some pro-BDNF is colocalized with tPA and plasmin in DCGs of both presynaptic boutons [16,29] and dendritic spines [15]. Therefore, it is possible that tPA and plasmin have a role in the intracellular conversion of pro-BDNF to mBDNF.

Nonenzymatic actions of tPA. tPA binding to lipoprotein receptor-related protein 1 (LRP-1) was reported to trigger NMDA-R activation indirectly, via signals downstream of LRP-1 [30]. The functional relevance of the tPA–LRP-1 interaction was demonstrated by findings that an antagonist for the binding of tPA to LRP impairs L-LTP [31].

Matrix metallopeptidase-9

Matrix metallopeptidase-9 (MMP-9) is a member of the large family of Zn^{2+}-dependent endopeptidases. MMP-9 is expressed in many tissues and organs, including the brain. It cleaves numerous substrates and is a crucial effector in many developmental and adult processes [32]. The zymogenic precursor, pro-MMP-9, contains an amino-terminal propeptide of 87 amino acids that occludes access to the catalytic site. Several peptidases can activate MMP-9, including several MMPs, plasmin, cathepsin G, and tissue kallikrein [33]. MMP-9 acts on several synaptic extracellular matrix (ECM) components, pro-forms of growth factors, and membrane proteins [34,35] (Table 1).

Synaptic localization, activity-regulated functions, and effects on synaptic structure and function

MMP-9 expression is enhanced by the neuronal activity of seizures, spatial learning in the Morris water maze [36], inhibitory avoidance learning [37], contextual fear conditioning [38], and by stimulation of the Schaffer collaterals of the hippocampus [39,40]. LTP-inducing stimuli increase synaptic MMP-9 and the proportion of active MMP-9 in an NMDA-R-dependent manner [39,40]. High-resolution *in situ* hybridization indicated that MMP-9 mRNA is located in dendrites and dendritic spines of excitatory synapses [41]. Transport of MMP-9 mRNA to dendrites, and local dendritic polyadenylation and translation, secretion, and activation are enhanced by neuronal activity [42].