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Mosaic serine proteases in the mammalian central nervous system

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1. ABSTRACT

We review the structure and function of three kinds of mosaic serine proteases expressed in the mammalian central nervous system (CNS). Mosaic serine proteases have several domains in the proenzyme fragment, which modulate proteolytic function, and a protease domain at the C-terminus. Spinesin/TMPRSS5 is a transmembrane serine protease whose presynaptic distribution on motor neurons in the spinal cord suggests that it is significant for neuronal plasticity. Cell type-specific alternative splicing gives this protease diverse functions by modulating its intracellular localization. Motopsin/PRSS12 is a mosaic protease, and loss of its function causes mental retardation. Recent reports indicate the significance of this protease for cognitive function. We mention the fibrinolytic protease, tissue plasminogen activator (tPA), which has physiological and pathological functions in the CNS.

2. INTRODUCTION

Recent reports show that serine proteases have various physiological and pathological functions in the mammalian CNS (reviewed in 1, 2). Over the past several years, we have isolated and studied cDNAs encoding serine proteases expressed in the CNS to understand the roles of proteolytic events in neuronal functions (3 - 13). Spinesin/TMPRSS5 and motopsin/PRSS12 have unique mosaic structures (Figure 1), although most of the identified proteases have simple structures comprising a signal peptide, a short proenzyme region, and a protease domain. Here we focus on the structure and function of three mosaic serine proteases, including spinesin, one of the transmembrane serine proteases in the CNS, and motopsin/PRSS12. We mention the functions in the CNS of another mosaic protease, tissue plasminogen activator (EC3.4.21.68), which was originally identified as a fibrinolytic enzyme.

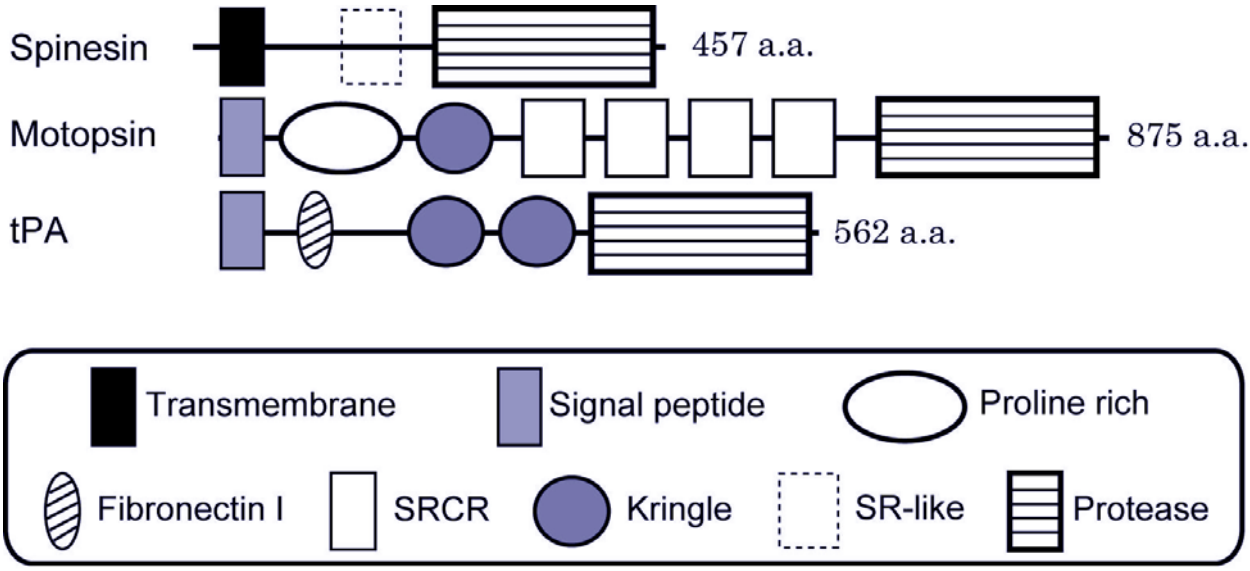

Figure 1. Schematic structures of human mosaic serine proteases in the CNS. Spinesin is only one transmembrane serine protease in the CNS. It has a short cytoplasmic domain at the N-terminus and a scavenger receptor (SR)-like domain followed by a serine protease domain within the extracellular region. Motopsin is a multidomain protease with a proline-rich domain, a kringle domain, four SRCR domains, and a protease domain. tPA also has a multidomain structure, comprising a fibronectin type I domain, kringle domains, and protease domain. The protease domains of these proteases are linked with the proenzyme domain through a disulfide bond.

3. SPINESIN/TMPRSS5

3.1. Structure of spinesin and variant forms

Spinesin comprises a short cytoplasmic domain, a transmembrane domain, a scavenger receptor (SR)-like domain, and a protease domain (Figure 1). The domain organization of spinesin is similar to that of hepsin, TMPRSS2, TMPRSS3, and TMPRSS4, although spinesin and these TMPRSSs show poor homology in their amino acid sequences (33%–39% identical). Human hepsin comprises a transmembrane domain and a protease domain, which is predicted to associate with the proenzyme region through a single disulfide bond (Cys^{153}–Cys^{277}). Analysis of the crystal structure of hepsin shows that the tertiary structure of the SR domain of Mac-2 binding protein is similar to the corresponding region of human hepsin regardless of the low sequence similarity to the SR domain of other proteins (14). In addition, this analysis suggests that SR and protease domains lie essentially flat against the plasma membrane, with the two domains splayed out on either side of the transmembrane strand. The similarity of domain organization between spinesin and hepsin allows us to speculate on the hepsin-like tertiary structure of spinesin. When expressed in COS cells, spinesin–enhanced green fluorescent protein (EGFP) fusion protein is localized around the plasma membrane (15). In addition to a potential disulfide bond site at Cys^{209}–Cys^{328}, the putative cleavage site is conserved at Arg^{217}–Ile^{218} in spinesin, suggesting an association between the proenzyme and the protease domains such as hepsin.

Splicing variants of spinesin having different domain organization have been isolated from mouse and rat (15, 16), but such variants have not been reported in human tissues. In the mouse CNS, at least four splicing variants of spinesin, termed types 1–4, are expressed. Type 1–4 variants encode polypeptides of 273, 311, 445, and 455 residues, respectively. Type 1 and type 2 variants lack transmembrane and SR domains, whereas type 3 and type 4 variants contain these domains. Differences in domain organization influence the subcellular localization of variant polypeptides. Expression of the EGFP fusion protein of each variant in COS cells causes type 3 and type 4 variants to localize to the ER, Golgi apparatus, and plasma membrane, whereas type 1 and type 2 variants are found in the cytoplasm. These variants appear to be derived from exon shuffling of the primary transcript (Figure 2). The human spinesin gene is located on chromosome 11q23.3 and contains 13 exons. The mouse spinesin gene, comprising 13 exons, is mapped to the syntenic region on chromosome 9. The type 1 variant is generated by deletion of exons 1–4 and the 3′-expansion of exon 6. The type 2 variant is derived by joining exon 4 to an alternative acceptor site in exon 5. A potential initiation codon for the type 2 variant is positioned in exon 5 with a long 5′-noncoding region, because such alternative splicing causes a frameshift. Similarly, the type 3 variant is produced by using an alternative acceptor site in intron 1 and an initiation codon in exon 2.

The distribution of splicing variants in the CNS can be analyzed by real-time PCR using primers specific to distinctive exon regions of each variant. Both type 1 and type 4 variants are expressed predominantly in the spinal cord, but the type 4 variant is expressed only in the cerebrum and cerebellum. Neuroblastoma and astrocytic cell lines have different expression patterns of these variant

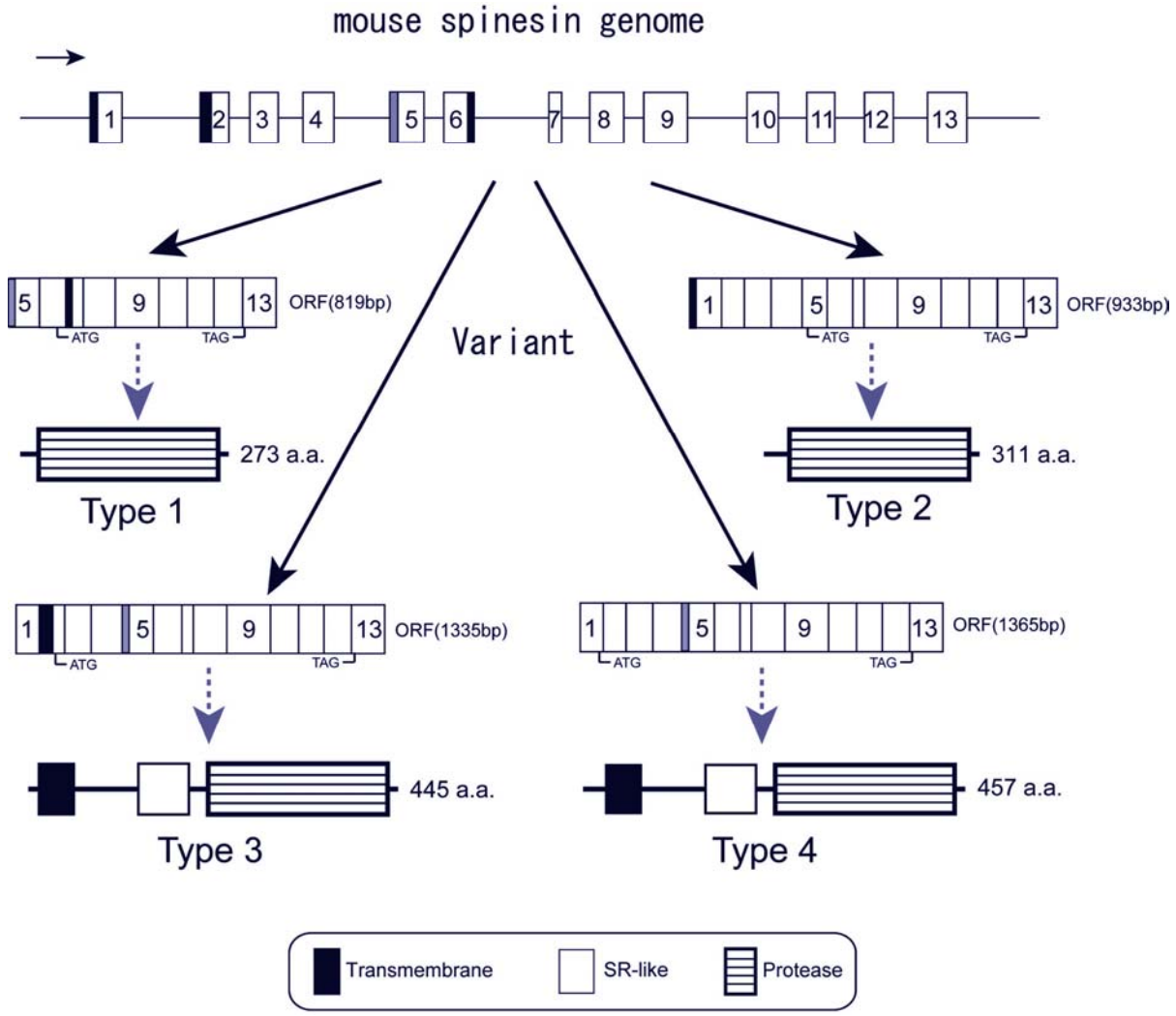

Figure 2. The structure of mouse spinesin variants. Four kinds of variant mRNAs, type 1–4, are generated by alternative splicing. Type 1 and type 2 variants lack a transmembrane domain and a scavenger-receptor-like domain. Black boxes in the gene and mRNAs mean the expanded region of the original exons that were identified by comparing to the human spinesin gene sequence. The gray region in exon 5 indicates the deleted region in type 2 variant. Schematic structures of spinesin variant proteins are indicated below their mRNAs.

(17). In mouse neuroblastoma cell lines, only type 4 spinesin is detected, whereas both type 1 and type 4 are expressed in astrocytic cell lines. Thus, by modulating domain organization, alternative splicing defines the intracellular localization appropriate for the physiological substrates.

3.2. Distribution of spinesin

Spinesin and TMPRSS9 are expressed in the CNS, although other TMPRSSs are observed in non-CNS tissues (18). A recent report shows that many serine proteases play important roles in the CNS, and some researchers have speculated that spinesin, along with motopsin and tPA, is involved in neuronal functions (described in section 4). In the CNS, the expression level of mouse spinesin is more than 6 times higher in the spinal cord than in the cerebrum. This expression pattern is unique, because other serine proteases in the CNS are expressed predominantly in the cerebrum. Immunohistochemical analysis has demonstrated an interesting distribution of spinesin (19). Immunoreactivity against human spinesin is detected in axons of projection neurons and oligodendrocytes, including the myelin sheath in the white matter of the spinal cord. In the anterior horn, spinesin localizes to the synapses on motor neurons. It seems likely that human spinesin produced in the cytoplasm of projection neurons in the brain is transported within axons to synaptic termini on motor neurons at the anterior horn of the spinal cord, and there proteolyses a protein or proteins in the presynaptic region. We have shown recently that mouse spinesin is expressed in astrocytes, as well as in neurons and oligodendrocytes (17).

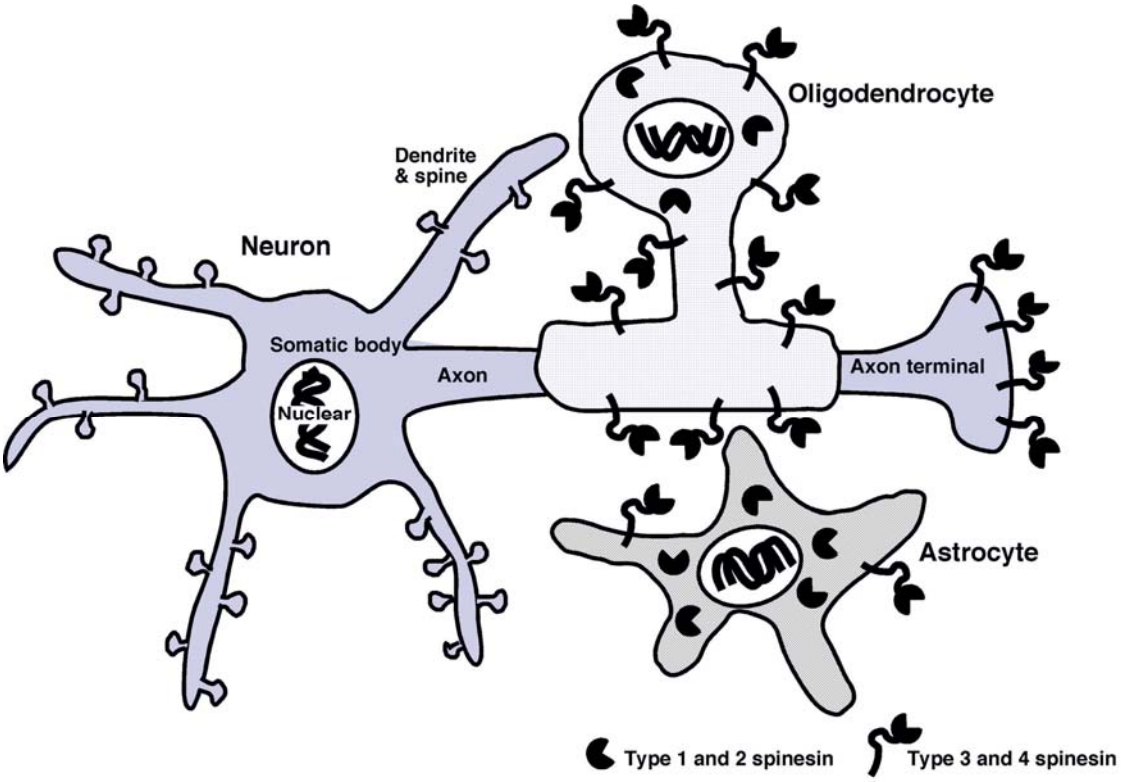

Figure 3. Different localization of mouse spinesin variants in neuron and glial cells. Type 3 and 4 variants are localized predominantly in the axon terminal and myelin sheath of oligodendrocytes. In contrast, type 1 spinesin is distributed in the cytoplasm of astrocytes and to a lesser extent as a transmembrane variant. The type 1 variant may degrade an astrocyte-specific substrate in the cytoplasm.

In situ hybridization and immunohistochemical analyses show that spinesin is expressed in astrocytes along blood vessels and axons in the white matter of the spinal cord. Interestingly, gray matter astrocytes express a low level of spinesin. In addition, the different expression of variant forms has been observed in neuronal and astrocytic cell lines derived from the mouse CNS. Type 4 spinesin is detected in mouse neuroblastoma and astrocyte cell lines, whereas the type 1 variant, a cytoplasmic spinesin, is expressed only in astrocyte cell lines (17). These observations suggest that spinesin plays multiple functions in neurons, oligodendrocytes, and astrocytes, and that the cytoplasmic-type spinesin may have an astrocyte-specific function (Figure 3).

3.3. Regulation of spinesin gene expression

In the CNS, splicing variants of some serine proteases are generated by cell type-specific use of multiple promoters (20, 21). However, analysis of 5′-rapid amplification of the cDNA ends of mouse spinesin indicates a single promoter, despite the observation that mouse spinesin has four splicing variants. The 5'-flanking region of spinesin gene lacked a TATA box and a CAAT box. The fluorescent promoter assay shows that an essential promoter region of the spinesin gene is located at between −224 bp and −188 bp (17). This region contains the recognition sites of GATA-1 and Sp1 transcription factors, and is conserved between mouse and human spinesin genes. We have isolated two kinds of proteins that act as binding proteins to this region, using oligonucleotide affinity chromatography, and have identified them as TLS/FUS and PTB-associated splicing factor (PSF) using mass spectrometry (unpublished data). Both TLS/FUS and PSF are multifunctional factors involved in DNA repair, homologous recombination, transcriptional regulation, and pre-mRNA processing (22). Proto-oncoprotein TLS/FUS was identified originally through its fusion to CHOP transcription factor in human myxoid liposarcoma with the t(12;16) chromosomal translocation (23). TLS/FUS is associated with a subpopulation of TFIID complex, and it enhances NF-kappa B-mediated transcription by interacting with the p65 subunit of NF-kappa B (24, 25). Based on these reports, TLS/FUS and PSF may act as cofactors in the regulation of spinesin gene expression.

As mentioned above, both neurons and astrocytes express spinesin, although their expression appears to be regulated differently in different cells. Treatment with the cell-permeable cAMP, dibutyryl cAMP (DBcAMP), increases spinesin mRNA levels by up to three times in astrocytic cells without any morphological change, but has no influence on its expression in neuroblastoma cells (17). Both type 1 and type 4 variants are upregulated by DBcAMP, supporting the concept of a single promoter of

the mouse spinesin gene. The signaling pathway involved in cAMP-dependent regulation of spinesin expression in astrocytes has not been identified yet. PKA/PKC inhibitors such as H-7 and KT5720 do not affect the cAMP-dependent expression of spinesin. 8CPT-cAMP, an activator of an exchange protein activated directly by cAMP (Epac), which mediates a PKA-independent pathway (26), also has no effect on spinesin expression. Further study is needed to identify the regulatory mechanism underlying spinesin expression and to understand its distinctive functions in neurons and astrocytes.

3.4. Potential spinesin function

In this review, we speculate on the multiple roles of spinesin in the CNS. Spinesin is expressed in neurons, oligodendrocytes, and astrocytes. In neurons, spinesin is localized presynaptically at the synapses of motor neurons. As discussed in section 4, some serine proteases are secreted from presynaptic neurons and modulate neuronal plasticity, so spinesin may modulate synaptic function. Astrocytes have protease-activated receptors (PARs) on the cell surface. PARs are G-protein-coupled receptors that are activated by serine proteases, including thrombin (27). Spinesin may contribute to PAR-dependent functions by activating zymogen proteases, which activate PARs. The type 1 variant is distributed in the cytoplasm of astrocytes. Astrocytes along blood vessels express spinesin strongly, suggesting that it functions in maintaining the blood–brain barrier. Spinesin detected in oligodendrocytes may contribute to myelin sheath formation. A serine protease, neurosin, appears to be involved in the expression of myelin basic protein and proteolipid protein, components of the myelin membrane (28). Spinesin may also be involved in diseases and injury of the spinal cord, because spinesin is expressed predominantly in the spinal cord. Many reports show the functions of proteases such as metalloprotease in spinal cord injury (e.g. 29, 30). Thus, spinesin may be a newly identified component of a complex protease cascade involved in the maintenance of spinal cord functions, although the substrates of spinesin in neurons and astrocytes must first be identified to elucidate spinesin's physiological functions.

4. OTHER MOSAIC SERINE PROTEASES

4.1. Motopsin/PRSS12

The cDNA encoding motopsin/PRSS12 was isolated from mouse brain cDNA libraries independently and simultaneously by two groups (31, 32). The HUGO Gene Nomenclature Committee defined this protease as protease, serine 12 (neurotrypsin, motopsin) and approved the gene symbol *PRSS12*. Mouse motopsin has a unique structure with a signal peptide at the N-terminal end followed by a proline-rich domain, a kringle domain, and three scavenger receptor cysteine-rich (SRCR) domains, as well as a protease domain at the C-terminus. The elongated proenzyme domain suggests that motopsin interacts with other molecules. We recently found that seizure-related protein-6 (Sez-6), which is a transmembrane protein expressed in neuronal cells, binds to motopsin through the proline-rich and kringle domains (unpublished data). Like spinesin/TMPRSS5, motopsin may anchor on the cell surface by binding to a cell surface protein (Figure 4). The amino acid sequence of mouse motopsin shows 82.5% identity to human motopsin (33). Human motopsin has four SRCR domains, of which the first domain is lacking in mouse motopsin. The human motopsin gene is mapped on chromosome 4q25 and contains 13 exons spanning a length of 71 kb (34). Conservation of the coding sequence of 12 primate motopsin genes suggests an essential role of motopsin in cognitive function in primates (35).

The expression of motopsin mRNA in the CNS is detected at embryonic day (E) 11.5, is most abundant at postnatal day (P) 10, and decreases gradually, but continues for at least seven months. In the CNS of the adult mouse, motopsin mRNA is detected in neuronal cells in cerebral cortical layers II/III, V, and VIb, the endopiriform cortex, and hippocampal CA1 region (32, 36–38). In addition, a moderate level of motopsin mRNA expression occurs in neurons in the motor nuclei of cranial nerve nuclei, including the oculomotor nucleus, trochlear nucleus, mesencephalic and motor nuclei of the trigeminal nerve, abducens nucleus, facial nucleus, nucleus of raphe pontis, dorsal motor nucleus of the vagus nerve, hypoglossal nucleus, and ambiguus nucleus. Such complex expression patterns suggest that motopsin has multiple functions in the nervous system.

The significant physiological function of motopsin in the brain has been identified by genome-wide screening against sibs of mentally retarded children. In an Algerian family, four of eight children show severe cognitive impairment with an intelligence quotient below 50. Analysis of microsatellite markers and detailed sequence analysis revealed a 4 bp deletion in exon 7 of the *PRSS12* gene, which causes a truncation in the middle of the third SRCR domain (39). It is unknown whether this truncated protein is synthesized by neuronal cells. It should be noted that MRI analysis reveals no gross anatomical abnormality and a normal ratio between gray and white matter in the patients (40). This suggests that motopsin is essential for acquisition or maintenance of cognitive function rather than for the development of the CNS. This report provides the first evidence of the link between a cognitive disease and deficit of extracellular proteolysis. The highest expression of motopsin mRNA is detected in the cortical plate, hippocampus, and tegmental nuclei of the brainstem of the 15-week human fetus. In the adult human cerebral cortex, the most prominent immunoreactivity to motopsin is detected at the presynaptic membrane. However, intracellular localization of motopsin protein appears to depend on the expression level. Both human and mouse motopsin is transported to axons when it is overexpressed in cultured neurons, whereas a low level of expression distributes motopsin protein around the somatic body and proximal part of dendrites (36). Regardless of the localization pattern, motopsin plays an essential role in cognitive function even in the fruit fly *Drosophila melanogaster,* which has a motopsin ortholog, termed tequila (41). The fly is capable of associative learning between electric shock and odorant stimuli. The expression of tequila is induced in Kenyon cells of the mushroom

body, part of the fly brain essential for long-term memory, only after conditioning for long-term memory training.

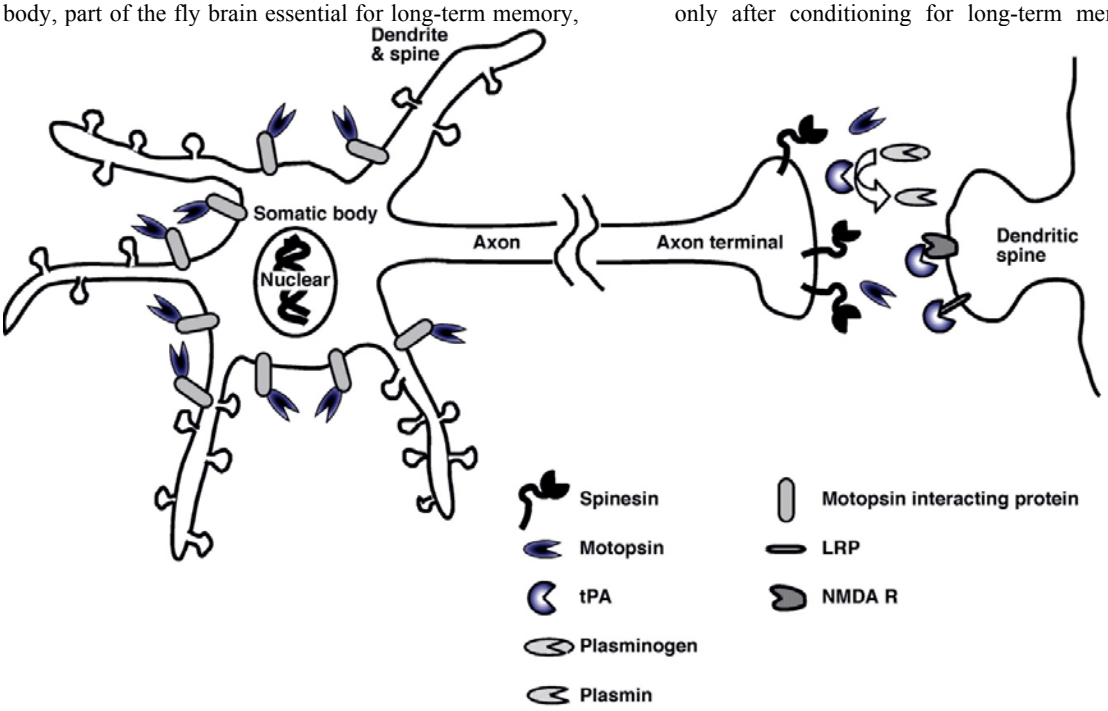

Figure 4. Mosaic serine proteases on a neuronal cell. The transmembrane serine protease, spinesin, is localized at the presynaptic membrane. tPA and plasmin are secreted into the synaptic cleft and digest various kinds of proteins, including extracellular matrix protein and neurotrophic factors. In contrast, motopsin anchors around the somatic body and proximal part of dendrites. The distribution of these proteases suggests that extracellular proteolysis induced by diverse serine proteases is essential for neuronal function.

The downregulation of tequila impairs long-term memory, but does not affect short-term memory or the morphological change of the mushroom body. Interestingly, the deficit of long-term memory is recovered by the restoration of tequila expression. These observations suggest that the cognitive deficit caused by the loss of motopsin function results from physiological dysfunction rather than unusual development of the nervous system. Our previous report supports this idea. Injury to the facial nerve by axotomy transiently decreases motopsin mRNA, and recovery of mRNA expression is involved in the reinnervation of facial nerves to the arrector pili muscle of vibrissae (42). However, we cannot rule out the possibility that a motopsin deficit causes morphological changes at the ultrastructural level in the developing CNS.

Further investigations are needed to understand the molecular mechanism of mental retardation related to mutation of the motopsin gene, especially to elucidate the physiological substrates for this protease and the effect of motopsin on the electrophysiological properties of neuronal cell.

4.2. Tissue plasminogen activator (tPA)

tPA is a fibrinolytic enzyme that activates zymogen plasminogen to plasmin. tPA has multiple domains comprising a fibronectin 1 domain, two kringle domains, and a protease domain at the C-terminus. Numerous studies have identified the physiological and pathological functions of tPA in the CNS. During development, tPA facilitates cell migration. The granule neurons of tPA knockout mice migrate at one-half the rate observed in wild-type neurons (43). In contrast, rat neuronal pheochromocytoma PC12 cells overexpressing tPA can migrate through the extracellular matrix faster than control cells can (44). tPA is also involved in the experience-dependent pruning of dendritic spines in the postnatal visual cortex by digesting extracellular matrix protein and adhesion molecules (45). In adulthood, tPA expression is induced during LTP (46, 47), which reflects long-term changes in synaptic plasticity and is a well-studied model of learning and memory. Mice lacking tPA show deficits in the late phase of LTP (L-LTP) and impairment in long-term memory (48). Transgenic mice overexpressing tPA in postnatal neurons show enhanced LTP and improved performance in a spatial memory test (49). tPA-deficient mouse shows that tPA is also involved in cerebellum-dependent motor learning (50). tPA may also affect emotional behavior. Acute restraint stress increases anxiety behavior after the induction of tPA activity in the amygdala, but tPA knockout mice show a normal level of anxiety behavior after the stress (51). The induction of tPA activity in the amygdala is mediated by a major stress neuromodulator, corticotrophin-releasing factor (52).

The pathological functions of tPA have also been reported. Occlusion of the middle cerebral artery causes

ischemic damage to neuronal cells, whereas tPA knockout mice have reduced infarct volume and less neuronal damage after vascular occlusion (53). Intravenous administration of tPA increases infarct volume even in the tPA knockout mouse brain. Mice lacking tPA or plasminogen are resistant to excitotoxin-induced neuronal cell death (54). Hippocampal injection of excitotoxin such as kainic acid (KA) induces the accumulation of tPA mRNA and protein, and upregulates tPA activity in the hippocampal formation (55). The induced tPA activates plasminogen to plasmin, which degrades the extracellular matrix protein laminin, and sensitizes neuronal cells to cell death (56). In contrast to its neurotoxicity, tPA may also act to protect against ischemic damage. For example, intracerebroventricular administration of tPA attenuates KA-induced neuronal cell death (57, 58).

As described above, neuronal cells secrete tPA in an activity-dependent manner, and activated microglia also secrete tPA in ischemic stroke. tPA from these sources appears to have different roles (59, 60). Siao et al. introduced the tPA gene under the control of a neuron-specific or microglia-specific promoter into tPA knockout mouse (61). Compared with wild-type mice or microglial tPA-expressing mice, mice expressing tPA only in neurons exhibited accelerated microglial activation but milder neuronal cell death against neurotoxic injury. In contrast, microglial tPA-expressing mice showed more serious neuronal degeneration than did the wild-type or neuronal tPA-expressing mice. A recent report shows that plasmin processes monocyte chemoattractant protein-1 to its active form and that the active form causes microglial chemotaxis to injured sites (62). There are a few reports of tPA-associated pathology besides excitotoxin-induced neuronal cell death. The tPA–plasmin system is involved in drug addiction through its regulation of dopamine release (63). It has also been reported that the tPA–plasmin system may also promote neuronal regeneration (64).

Several mechanisms have been proposed to explain the functions of tPA, as well as the degradation of extracellular matrix proteins by the tPA–plasmin system (56, 65). First, the tPA–plasmin system processes nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) precursors to their mature forms (66, 67). Both factors are secreted in an inactive precursor form from neuronal cells with an activity-dependent manner. Another mechanism is the modulation of the NMDA receptor, a key molecule for neuronal plasticity. tPA cleaves the NR1 subunit of the NMDA receptor at Arg^{260} in the amino-terminal domain of this subunit (68). Such interaction potentiates NMDA-evoked Ca^{2+} influx (69). Another group has reported the non-proteolytic interaction between tPA and NR2B subunit, which causes activation of ERK1/2 (70, 71). This signaling cascade, together with *de novo* protein synthesis, results in the hyperphosphorylation of tau protein, which leads to neuronal cell death, and may be involved in Alzheimer's disease. tPA has another receptor on the cell surface of hippocampal neurons: tPA binds to low-density lipoprotein receptor-related protein on hippocampal neurons, and this interaction enhances L-LTP by activating cyclic AMP-dependent protein kinase (72). The cross talk or relationship among tPA-evoked signaling systems should be resolved in future studies.

5. PERSPECTIVE

Recent research analyzing the phenotypes of knockout and transgenic mice reveals the significance of extracellular proteolysis for neuronal functions. In addition to the reports we have mentioned here, the significance of other secreted-type serine proteases such as kallikreins and thrombin has been reported. However, the substrates for many of these proteases are still unknown. Proteomic analysis of mutant and wild-type mice may identify the physiological substrates. Like tPA, extracellular proteases may affect intracellular signal cascade. Transmembrane protease, spinesin, and motopsin anchored by a transmembrane protein are candidates for substances that affect the intracellular signal system in neuronal cells (Figure 4). Identifying the physiological substrates and signal cascades will give us further insights into role of extracellular proteolysis in the regulation of neuronal function and will help in the development of novel drugs to improve neuronal functions.

6. ACKNOWLEDGEMENT

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Abbreviations: 8CPT: 8-chlorophenylthio, AD: Alzheimer disease, BDNF: brain derived neurotrophic factor, CNS: central nervous system, DBcAMP: dibutyryl cAMP, E: embryonic day, EGFP: enhanced green fluorescent protein, Epac: exchange protein activated directly by cAMP; ER: endoplasmic reticulum, ERK: extracellular regulated kinase, KA: kainic acid, LTP: long term potentiation, MRI: magnetic resonance imaging, NF: nuclear factor, NGF: nerve growth factor, NMDA: *N*-methyl-d-aspartate, P: postnatal day, PAR: protease activated receptor, PCR: polymerase chain reaction, PKA: protein kinase A, PKC: protein kinase C, PSF: PTB-associated splicing factor, SR: scavenger receptor, SRCR: scavenger receptor cysteine-rich, TFIID: transcription factor IID, TMPRSS: transmembrane protease, serine, tPA: tissue plasminogen activator

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