Tissue plasminogen activator in central nervous system physiology and pathology

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Summary

Although conventionally associated with fibrin clot degradation, recent work has uncovered new functions for the tissue plasminogen activator (tPA)/plasminogen cascade in central nervous system physiology and pathology. This extracellular proteolytic cascade has been shown to have roles in learning and memory, stress, neuronal degeneration, addiction and Alzheimer's disease. The current review considers the different ways tPA functions in the brain.

Keywords

Tissue-type plasminogen activator; plasminogen; central nervous system; LTP; excitotoxicity

Abbreviations

PA: plasminogen activator; tPA: tissue-type plasminogen activator; uPA: urokinase-type plasminogen activator; PAI-1: plasminogen activator inhibitor-1; LRP: low-density lipoprotein receptor-related protein; CNS: central nervous system; CPEB: cytoplasmic polyadenylation element binding; LTP: long-term potentiation; LTD: long-term depression; mRNA: messenger ribonucleic acid; BDNF: brain-derived neurotrophic factor; NMDA: N-methyl-D-aspartate; RAP: receptor-associated protein; ECM: extracellular matrix; BBB: blood brain barrier; MMP: matrix metalloproteinase; EtOH: ethanol; ERK: extracellular signal-regulated kinase; GAP: growth-associated protein; CRF: corticotropin-releasing factor; MCAO: middle cerebral artery occlusion; $A\beta$: beta-amyloid

Introduction

The plasminogen activator (PA)/plasminogen proteolytic cascade is known to be important for thrombolysis (1, 2). There are two mammalian PAs: tissue-type (tPA) and urokinase-type (uPA) (3). PAs are serine proteases that cleave a specific peptide bond within the zymogen plasminogen to generate the active protease, plasmin, which is capable of degrading numerous substrates. In the vasculature, plasmin efficiently breaks down fibrin clots, aiding in hemostasis and vascular patency. This protease cascade is tightly regulated by the actions of serine protease inhibitors (serpins), of which plasminogen activator inhibitor-1 (PAI-1) and neuroserpin are the major cognate serpins for tPA, while plasmin is inhibited by α_2-antiplasmin (2). Furthermore, tPA activity can be attenuated by rapid clearance through low-density lipoprotein receptor-related protein (LRP)-mediated endocytosis and augmented by binding annexin-II or to fibrin within a clot (4, 5).

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Infusion of PAs, such as recombinant tPA and its derivatives, is used for lysis of fibrin clots to help restore blood flow following myocardial infarction or thrombotic stroke (2). Although tPA can be used for the treatment of stroke, there is a narrow time-frame and limited patient population for which thrombolytics are appropriate, since delayed delivery of tPA can lead to neuronal damage or cerebral hemorrhage, worsening the outcome for the patient.

The function of tPA, however, is not limited to the initiation of thrombolysis. Recent research has shown that the tPA/plasminogen system has other roles within the central nervous system (CNS). Although the presence of uPA has been confirmed in the brain, its role has not been fully investigated. Therefore, this review will focus on the various functions of tPA in brain physiology and pathology.

tPA/plasminogen system in CNS physiology

tPA expression and regulation

tPA is highly expressed in the adult mouse brain in regions involved in learning and memory (hippocampus), fear and anxiety (amygdala), motor learning (cerebellum), and autonomic and endocrine functions (hypothalamus) (6–13). Both neurons and microglial cells express tPA. Plasminogen, PAI-1, and neuroserpin are also present in the brain indicating that the components of the tPA/plasminogen cascade are present in the CNS (9, 13–15). In neurons, the expression of tPA can be under translational control; tPA expression is regulated by the binding of the cytoplasmic polyadenylation element binding (CPEB) protein which leads to the extension of tPA mRNA polyadenylation and a subsequent increase in tPA protein synthesis (16). This regulation of tPA expression allows for a rapid increase in activity in response to specific stimuli (as seen in synaptic plasticity), and the presence of cognate serpins of tPA in the CNS allows for swift and specific inactivation. The localization to axon terminals and the highly regulated axonal release of tPA is consistent with a proteolytic-dependent role for tPA in the CNS in which PA cascade activation alters the extracellular matrix (ECM) and modifies synapses, such as during seizure, kindling, and long-term potentiation (LTP) (6, 17). While tPA also has been visualized in dendrites, the release of this protein from these structures has yet to be demonstrated (16).

There is an additional level of complexity to tPA expression in neuronal cells. In neurons, tPA is not constitutively secreted but contained in vesicles. tPA can be released from secretory vesicles after membrane depolarization or stimulation (18–21). This mechanism of regulated tPA secretion allows for rapid localized increase in tPA activity at the synapse.

The role of tPA in synaptic plasticity

Long-term potentiation (LTP), considered to be a molecular correlate of learning, refers to remodeling of neuronal connections leading to increased synaptic strength after repetitive excitatory stimulation (22). tPA was identified as an immediate-early gene whose mRNA transcription is induced shortly after synaptic activity such as LTP (6). The possibility that this protease participates in synaptic plasticity is strengthened by the fact that tPA is elevated after LTP. Indeed, either inhibition of proteolytic activity or deficiency in tPA affects the late-phase of LTP (L-LTP) or long-term depression (LTD), while increased tPA facilitates LTP and learning (21, 23). Another action of the tPA/plasminogen system that contributes to LTP generation is cleavage of the pre-cursor form of brain-derived neurotrophic factor (proBDNF) to its mature form (mBDNF) (24). The application of mBDNF, derived from tPA/plasmin cleavage, to hippocampal slices can rescue impaired L-LTP in both tPA-deficient and plasminogen-deficient mice. This work reveals an interaction between the tPA/plasminogen axis and BDNF and suggests a potential impact of this interaction on LTP. These observations

suggest further studies to define plasmin/proBDNF binding and identify putative mBDNF receptors for LTP propagation.

One of the molecules with which tPA purportedly interacts to enhance LTP is the NR1 subunit of the N-methyl-D-aspartate (NMDA) receptor. Using a neuronal cell culture model, Nicole et al. reported that tPA cleaves NR1, enhancing NMDA-mediated intracellular calcium levels and neuronal degeneration (25). In this model, tPA cleaves at amino acid Arg260 of the NR1 subunit leading to NMDA signaling enhancement; mutation of Arg260 to Ala260 abrogates tPA-induced NMDA activity augmentation (26). However, the direct cleavage of NR1 by tPA has not been observed in other studies (27, 28). An interaction between tPA and NR2B-containing NMDA receptors has been reported during ethanol withdrawal-induced seizures (29). Therefore, although tPA can interact with either NR1 or NR2B, the specific outcome of these interactions as they pertain to LTP have yet to be delineated.

An additional tPA receptor affecting synaptic plasticity is LRP. An antagonist of LRP, receptor-associated protein (RAP), hinders tPA-induced L-LTP and prevents rescue of synaptic potentiation by addition of tPA in tPA-deficient mice (17). Additionally, the binding of tPA to LRP can lead to the up-regulation of matrix metalloproteinase-9 (MMP-9), a protease which can degrade the ECM contributing to either synaptic plasticity or neuronal degeneration (30). Interaction between LRP and tPA also contributes to blood-brain barrier (BBB) breakdown (31). tPA-induced opening of the BBB is evident in both plasminogen-deficient and MMP-9-deficient mice as measured by Evans blue dye extravasation, implying that neither plasminogen nor MMP-9 is necessary for tPA to produce its effect on vascular permeability. However, the tPA-clearing receptor LRP is involved since both RAP and anti-LRP antibodies can block this specific tPA activity. The exact mechanism by which tPA alters the BBB is still not clear and is subject to further investigation.

The function of tPA in plasticity *in vivo* has been scrutinized in mice either deficient for or over-expressing this protease since Qian and co-workers showed that tPA mRNA was up-regulated in the rat hippocampus after seizure, kindling, and LTP (6). Although several studies do not indicate a role for tPA in traditional hippocampal-based behavioral tests, mice overexpressing tPA in the CNS show enhanced LTP in hippocampal slices and exhibit improved hippocampal-dependent spatial memory formation as measured by both Morris water maze and homing hole board tests (32). In these studies, overexpression of tPA led to increased synaptic activity *in vivo*, which is similar to published *in vitro* results. Thus, despite a lack of a learning phenotype in tPA-deficient mice in several learning paradigms, behavioral results from tPA-overexpressing mice indicate a role for this protease in memory (23, 33).

tPA has also been studied in the amygdala, a brain region that regulates reponse to fear and anxiety (10, 12). Acute restraint stress studies in mice indicate that one function of this protease is in amygdala-dependent learning in fear response. tPA is induced in the medial and central amygdala after acute restraint stress, where it promotes stress-induced post-synaptic (phosphorylation of ERK1/2) and axonal (amplified GAP-43 expression) neuronal changes (10). tPA-deficient mice display impaired response to stress and abnormal circulating levels of corticosterone during the recovery period after stress. They also do not exhibit stress-induced anxiety as measured by the elevated-plus maze test. These results indicate that tPA contributes to proper control of hormonal stress response and has a significant role in emotional learning. Further research into the role of tPA in stress has revealed that this protease is elevated after the infusion of corticotrophin-releasing factor (CRF), an important hormone for triggering the stress response, into the lateral ventricle (34). The activation of tPA by CRF in the central and medial amygdala leads to an increase of c-fos immunoreactivity, a measure of neuronal activation. However, the function of tPA seems to be independent of plasmin production during

stress-induced anxiety, since plasminogen-deficient mice, unlike tPA-deficient mice, do not show lower levels of anxiety in the elevated plus-maze after restraint stress or abnormal c-fos expression after CRF infusion into the ventricle. As the function of tPA in stress and the amygdala have been uncovered, the next challenge is to identify new therapies for anxiety-related disorders in the context of the tPA/plasminogen axis.

Another area of the brain in which tPA has been shown to have a role is the cerebellum, which is responsible for motor learning. Seeds and colleagues reported the up-regulation of tPA mRNA in rats after learning a complicated motor task (traversing a runway by grabbing horizontal irregular pegs) (7). Additionally, even though there were no apparent consequences, the rate of cerebellar granule cell migration seems hindered in tPA-deficient mice, since more granule cells are present in the molecular layer of the cerebellum in these mice as compared to age-matched controls (8). Consistent with the importance of proteolytic action, wild-type mice infused with tPA inhibitors PAI-1 or tPA-STOP have deficits in cerebellar motor learning (11).

In addition to its roles in the hippocampus, amygdala, and cerebellum, the tPA/plasminogen system is also involved in the recovery of function in the visual cortex after reverse occlusion. The development of the visual pathway is dependent on activity and leads to ocular dominance, a condition in which certain cortical neurons become selectively connected to one specific eye. Ocular dominance progression can be enhanced by addition of norepinephrine, which leads to increased tPA mRNA (35). Preventing visual stimulation of an eye leads to monocular deprivation, a state of diminished cortical response in the closed eye. Reverse occlusion is the rescue of visual function in the previously deprived eye by opening this eye and closing the formerly open eye, and this process occurs by the formation of connections from the lateral geniculate nucleus of the thalamus to the visual cortex. Muller and Griesinger have shown the tPA/plasminogen proteolytic cascade is necessary for reverse occlusion, since inhibition of either protease prevents proper formation of thalamocortical connections for visual rescue (36). Other research has extended this finding, indicating that tPA/plasminogen activity allows for reorganization of connections in the visual cortex, again highlighting this proteolytic cascade's contribution in synaptic plasticity (37). More specifically, two recent reports show that during monocular deprivation, tPA/plasminogen activity helps in structural remodeling by pruning dendritic spines and reorganizing the ECM (38, 39).

Addiction can be considered a form of adaptive synaptic plasticity. In models of morphine and ethanol addiction, tPA expression is elevated in the nucleus accumbens and limbic system, respectively. The rewarding effects of morphine can be measured by the conditioned place-preference test, which associates the rewarding or aversive effect of a drug to placement into a specific compartment. Both the rewarding effect of morphine and morphine- related dopamine release are diminished in tPA-deficient and plasminogen-deficient mice when compared to wild-type mice (40). Although the effector substrate of plasmin in morphine addiction is not yet identified, the role of plasmin in this pathway is evident.

In addition to morphine addiction, ethanol consumption and withdrawal also elevate tPA activity. Ethanol inhibits NMDA receptor activity, and NMDA receptor numbers increase as an adaptive response. The rapid removal of ethanol relieves this inhibition on the expanded population of NMDA receptors, and when tPA acts on this large number of NR2B-containing NMDA receptors, it can result in hyperexcitation and seizures. However, in this instance, the primary effect of tPA does not appear to be plasminogen activation but rather interaction with the NR2B subunit of the NMDA receptor in a non-proteolytic manner (29). Additionally, tPA-deficient mice also have reduced ethanol withdrawal seizures (see below). This is an example

of the progression of a physiological role for tPA (synaptic plasticity in addiction) resulting in a pathological consequence (seizure induction upon ethanol withdrawal).

tPA function in CNS pathology

The role of tPA in neurotoxicity

While the understanding of physiological functions of tPA in the CNS has expanded, so has understanding of its roles in pathological situations. The participation of the tPA/plasminogen axis has been defined in neuronal degeneration due to excitotoxicity. Injection of kainate, an excitotoxic glutamate analog, into the CA1 region of the hippocampus of wild-type mice leads to neuronal damage, and this damage is reduced in either tPA-deficient or plasminogen-deficient mice (41–43). Neurodegeneration is prevented in wild-type mice co-injected with both kainate and α_2 -antiplasmin, supporting the role of plasmin in this neuronal injury paradigm. Further investigation identified laminin, a component of the ECM, as a substrate of plasmin proteolysis, and laminin cleavage combined with glutamate excitotoxicity results in neuronal degeneration due to anoikis (43–46). Plasmin-cleaved laminin fragments or anti-laminin antibodies infused into the hippocampus of either wild-type or plasminogen-deficient mice disrupts the ECM, sensitizing these mice to kainate excitotoxicity (46). The disruption of the ECM by laminin fragments indicates that the ECM is an active structure, whose components can be replaced or competed against by excess exogenous laminin. The dynamic nature of the ECM would also permit the remodeling necessary during synaptic plasticity, and it has been reported that cleavage of laminin by plasmin can hinder LTP in rat hippocampal neurons (47).

While the tPA/plasminogen axis contributes to excitotoxic neuronal degeneration, tPA is also involved in other CNS pathologies. The role of tPA has been studied in Alzheimer's disease (AD), stroke, infarct formation, and seizure spreading. tPA expression is elevated during seizures, a state of synchronous, pathological hyperactivity in the CNS. Comparable to observations in the hippocampus, delivery of kainate into the rat amygdala results in damage to hippocampal neurons (48). The neurodegeneration observed in the hippocampus after kainate injection into the amygdala is tPA-dependent, since neuronal damage can be attenuated by neuroserpin delivery into the brain (48). Additionally, seizure spreading is plasminogen-independent, since seizure inception in plasminogen-deficient mice was similar to wild-type mice, and seizure onset is also delayed by neuroserpin. The tPA/plasminogen system also contributes to handling-induced seizures after ethanol withdrawal in mice, and these seizures are attenuated in tPA-deficient mice (29).

The role of tPA in infarct formation has also been described. tPA-deficient mice have reduced infarct volume and neuronal damage as compared to control mice subjected to middle cerebral artery occlusion (MCAO) (49, 50). The infusion of recombinant tPA or delivery of tPA-expressing adenovirus into tPA-deficient mice also led to increased infarct size after MCAO, indicating a role for tPA in this process. The observation that thrombolytics can increase the infarct size after MCAO is independent of plasminogen activation since plasminogen-deficient mice displayed larger infarcts post-MCAO. In accordance with the contribution of tPA to infarct formation, PAI-1-deficient mice have increased infarct size while neuroserpin-injected mice have diminished infarcts after MCAO. Therefore, regulating tPA activity might be beneficial in controlling infarct injury after stroke (50–52).

tPA in Alzheimer's disease

AD is the most common cause of dementia and cognitive decline in the elderly (53). One of the characteristic pathologies of AD is the deposition of a 39–43 amino acid peptide called β-

amyloid (Aβ) in the CNS parenchyma, leading to the activation of the inflammatory response. The PAI-1 gene is induced during inflammation, which results in depressed tPA activity in transgenic AD mouse models (54–56). Furthermore, the tPA/plasminogen system has been implicated in the degradation of A β in the parenchyma of both mice and humans (56–60). Diminished tPA/plasmin activity in the AD brain can contribute to a deleterious cycle in which increased A β concentration leads to elevation of PAI-1, further depressing tPA/plasmin activity and resulting in inefficient A β degradation. Concomitant with inflammation-induced increase in PAI-1 levels, decreased plasmin activity in AD brains may also be due to diminished plasminogen availability in lipid rafts, areas in neurons where plasminogen and plasminogen-binding molecules reside (61). These rafts are disturbed in AD brains, consequently resulting in decreased plasmin activity. These observations indicate that molecules within the tPA/plasminogen proteolytic axis are viable therapeutic targets to delay the progression of AD.

Serpins in CNS pathology

There is a significant elevation in PAI-1 and neuroserpin expression during pathological insult to the brain. The increase of PAI-1 in the brain is seen in neurological disorders such as AD and dementia, after kainate injection into the CA1 region of the hippocampus, and after restraint stress. Escalation of PAI-1 expression normally corresponds to a depression of tPA activity, and it is thought to be a regulatory response to increased tPA, since excessive tPA activity can contribute to CNS pathology, such as by worsening neuronal damage. However, PAI-1 could interact with tPA, not as a serpin but as a binding partner to mediate its effect on receptors including the NMDA receptors. Clearly, tPA can function either as a protease or modulator of cell signaling, and PAI-1 can influence both of these activities. The elevation of neuroserpin, the other major tPA inhibitor in the CNS, has also been implicated in regulation of seizure spreading, control of BBB integrity, and emotional learning (15, 62, 63).

Conclusions

The function of the tPA/plasminogen proteolytic cascade has been conventionally assigned to fibrinolysis. However, existing research, especially using mice deficient in specific components of the fibrinolytic system, has extended the role of tPA and plasminogen from thrombolytic enzymes to encompass functions in both CNS physiology and pathology (Table I). Identification of novel substrates for either tPA or plasmin in the CNS would extend understanding of the influence of this proteolytic cascade. New lessons learned about the function of the tPA/plasminogen system can be utilized to identify novel therapies for slowing the pathogenesis of disorders such as AD or attenuating the severity of ethanol withdrawal-induced seizures. Further clarification of the contributions of this proteolytic cascade to LTP (such as the identification of the interaction domains between tPA and NR2B or proBDNF and plasmin) might help ease cognitive decline. The potential applications of new functions of the tPA/plasminogen proteolytic cascade highlight the importance and complexity of this system.

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PROCESS	MECHANISM	REFERENCE
LTP	proBDNF>mBNDF	[24]
LTP	LRP	[17]
Visual function recovery	-	[35-39]
Enhance LTP	NR1 Arg260	[21, 25, 26]
Open BBB	LRP	[31]
Increase MMP-9 activity	LRP	[30]
Stress	-	[10, 34]
Excitotoxicity	Laminin cleavage	[41-46]
Alzheimer's disease	Aβ degradation	[56-61]
Increased stroke damage		[49-52]
EtOH withdrawal seizure	NR2B	[29]
Seizure spreading	-	[48]

PHYSIOLOGY → Plg-dependent / Plg-independent

tPA

PATHOLOGY → Plg-dependent / Plg-independent

Table I. Summary of known functions of tissue-type plasminogen activator in the CNS
For motor learning [7, 8, 11], it is not clear whether the process is plasminogen-dependent or -independent. Abbreviations listed on page 659.