SYMPOSIUM

New Functions for an Old Enzyme: Nonhemostatic Roles for Tissue-Type Plasminogen Activator in the Central Nervous System

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Tissue-type plasminogen activator (tPA) is a highly specific serine proteinase that activates the zymogen plasminogen to the broad-specificity proteinase plasmin. Tissue-type plasminogen activator is found not only in the blood, where its primary function is as a thrombolytic enzyme, but also in the central nervous system (CNS), where it promotes events associated with synaptic plasticity and acts as a regulator of the permeability of the neurovascular unit. Tissue-type plasminogen activator has also been associated with pathological events in the CNS such as cerebral ischemia and seizures. Neuroserpin is an inhibitory serpin that reacts preferentially with tPA and is located in regions of the brain where either tPA message or tPA protein are also found, indicating that neuroserpin is the selective inhibitor of tPA in the CNS. There is a growing body of evidence demonstrating the participation of tPA in a number of physiological and pathological events in the CNS, as well as the role of neuroserpin as the natural regulator of tPA's activity in these processes. This review will focus on nonhemostatic roles of tPA in the CNS with emphasis on its newly described function as a regulator of permeability of the neurovascular unit and on the regulatory role of neuroserpin in these events. Exp Biol Med 229:1097–1104, 2004

Key words: tissue-type plasminogen activator; neuroserpin; plasminogen; cerebral ischemia; seizures; cerebral edema

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Tissue-type plasminogen activator (tPA) is a highly specific serine proteinase and one of the two principal plasminogen activators. The primary substrate for tPA *in vivo* is the zymogen plasminogen, which tPA activates to the broad-specificity proteinase plasmin. Outside the central nervous system (CNS), tPA is primarily a thrombolytic enzyme, as plasmin's principal substrate is fibrin. However, within the CNS, the roles of tPA and plasmin are not well characterized, and their primary substrates are not known.

Tissue-type Plasminogen Activator and Neuroserpin in the Central Nervous System

Under normal circumstances, tPA is expressed at low levels in specific areas of the CNS (1). However, its synthesis is increased by events that require synaptic plasticity, such as long-term potentiation, kindling, seizures, and motor learning (2–5). During normal mouse development, tPA mRNA is expressed in tissues derived from neural ectoderm (2). In the adult nervous system, tPA is detected mainly in the hippocampus, hypothalamus, cerebellum, amygdala (1, 6, 7), and sympathetic nerves (8). It has been suggested that in the CNS, tPA mRNA is translationally controlled, so the protein is made only on demand in individual synapses (1). However, it has also been demonstrated that tPA is rapidly released from the cell in response to neuronal depolarization (9). This suggests the presence of a "storage" pool of tPA ready to be released to the synaptic cleft in response to stimuli that are associated with rapid changes at the synaptic level (5).

Neuroserpin is a Selective Regulator of tPA Activity in the CNS. Neuroserpin is an axonally secreted member of the *serpin* (*ser*ine *p*roteinase *in*hibitors) family

(6, 10, 11) that is primarily expressed in the brain (Fig. 1; Refs. 6, 12). Sequence analysis of neuroserpin's cDNA as well as biochemical studies have demonstrated that neuroserpin is a fully inhibitory serpin that reacts preferentially with tPA (6, 11). During development of the normal mouse, neuroserpin mRNA is expressed in neurons throughout the CNS (13) as well as in the olfactory epithelium and in the ganglia of the cranial and the sympathetic and parasympathetic nerves (13). In the adult nervous system, neuroserpin is detected in neurons in the same places where tPA is detected (i.e., hippocampus, hypothalamus, cerebellum, amygdala, and sympathetic nerves; Figs. 2 and 3; Refs. 1, 6), supporting the biochemical evidence that neuroserpin is the selective inhibitor of tPA in the CNS. As demonstrated for tPA (9), neuroserpin is rapidly released from the cell in response to neuronal depolarization (14), indicating the presence of a "storage" pool that, as with tPA, is ready to be released to the synaptic cleft in response to stimuli (5).

Nonhemostatic Roles for tPA and Neuroserpin in the Central Nervous System

Tissue-type plasminogen activator and its inhibitor, neuroserpin, have been implicated in a number of nonhemostatic events in CNS, both during development and in response to physiological and pathological processes. The best-characterized physiological events associated with tPA activity in the CNS are synaptic plasticity (15–17), learning (3, 18, 19), and behavior (4, 20), whereas a role for tPA and neuroserpin has also been described in the pathogenesis of diseases such as cerebral ischemia (21, 22), dementia (23, 24), seizures (5), and multiple sclerosis (25). The first part of this review will focus on a discussion of the role of tPA and neuroserpin in these known physiological and pathological events, whereas the second part will focus on a recently identified role for tPA and neuroserpin in the CNS: the regulation of permeability of the neurovascular unit.

Tissue-type Plasminogen Activator and Neuroserpin in Physiological Events in the CNS

Synaptic Plasticity. Neuronal plasticity is associated with critical physiological and pathological processes in the developing and mature CNS. One of the most important characteristics of this phenomenon is an activity-dependent remodeling of neuronal connectivity and synaptic transmission. There is a growing body of evidence supporting the hypothesis that both tPA (15) and neuroserpin (26, 27) play a significant role in the changes associated with the development of synaptic plasticity in the CNS.

Learning and Memory. Long-term potentiation (LTP) is considered to be a cellular correlate of the changes that accompany learning and memory, which are examples of naturally occurring neuronal plasticity. The importance of

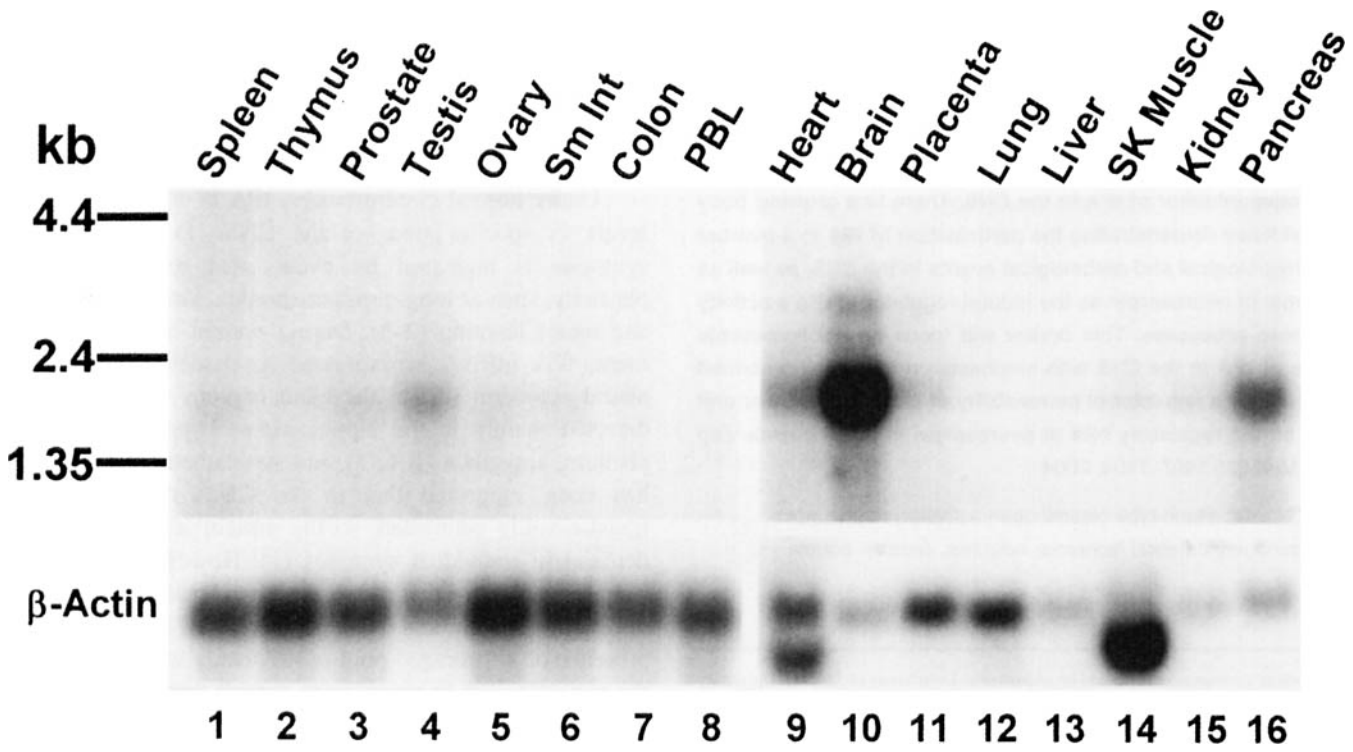

Figure 1. Northern blot analysis of RNA from human tissues. The top panel in each section shows the neuroserpin message, and the lower panel shows actin as a control for RNA loading. Each tissue is indicated above the lane. Sm Int, small intestine; PBL, peripheral blood lymphocytes. (Reprinted with permission from Ref. 6).

tPA in LTP has been shown by observations that tPA is induced during LTP (3), lack of tPA or its inhibition affects the late phase of LTP (19), and treatment with tPA facilitates LTP (18). Moreover, tPA has been shown to be upregulated in the cerebellum of rats during learning of a complex motor skill (28), and the absence of tPA in $tPA^{-/-}$ mice results in a significant reduction in the rate and extent of learning (29).

Behavior and Anxiety. Although the role of tPA and neuroserpin in behavior is less well defined, it has been demonstrated that $tPA^{-/-}$ mice are impaired in their ability to respond to a negative stimulus (19) as well as to stress-inducing situations (30). Likewise, animals either lacking or overexpressing neuroserpin exhibit a reduction in locomotor activity in novel environments and in the response to anxiety-like situations, as well as a neophobic response to novel objects (20). It should be kept in mind, however, that although animals overexpressing neuroserpin have a significant decrease in tPA activity (31), in neuroserpin-deficient mice ($Ns^{-/-}$), tPA's proteolytic activity remains unchanged (20). These findings indicate that the role of neuroserpin in behavior may be independent of its effect on tPA activity.

Tissue-type Plasminogen Activator and Neuroserpin in Pathological Events in the CNS

There is a growing body of evidence demonstrating that tPA and neuroserpin are also involved in pathological events in the CNS. The following section will discuss briefly the role of tPA and neuroserpin in neurodegeneration, seizures, and cerebral ischemia.

Excitotoxic Cell Death. Excitotoxicity involves a cascade of molecular and cellular events that result in cell death in pathological processes such as seizures and cerebral ischemia. The importance of tPA in this process was demonstrated by the observation that both genetic deficiency of tPA (32, 33) and the infusion of neuroserpin directly into the brain (hippocampus; Ref. 5) results in a significant resistance to cell death induced by either the direct injection of an excitotoxin into the brain or the generation of a seizure. This former effect seems to be mediated by plasminogen, as either genetic deficiency of plasminogen ($Plg^{-/-}$) or infusion of α_2-antiplasmin also result in a significant neuroprotective effect when the excitotoxin is directly infused into the hippocampus (33). However, it is not known whether seizure-induced cell death is Plg dependent.

There is also ample evidence of excitotoxic cell death during cerebral ischemia (34, 35). Genetic deficiency of tPA (21) or treatment with neuroserpin results in a significant decrease in ischemia-induced cell death (22, 31). Conversely, the reduction of plasminogen activity either by plasminogen gene inactivation or treatment with α_2-antiplasmin results in a significant increase in the volume of the ischemic lesion after middle cerebral artery occlusion (MCAO; Ref. 36). These results demonstrate that tPA mediates cell death induced by both the direct injection of excitotoxin into the hippocampus and cerebral ischemia. However, in excitotoxin-induced cell death, tPA acts through a plasminogen-dependent mechanism (37), whereas in ischemia-induced cell death, this mechanism is plasminogen independent (36).

Seizures. Expression of the gene for tPA is induced in events in which neuronal activity is increased, such as seizures (3). The spreading of a seizure throughout the CNS occurs in parallel with an increase in tPA activity, followed by a surge in neuroserpin antigen in the regions of the brain affected by the abnormal electrical activity (5). Moreover, the progression of a seizure throughout the brain is slowed either by the absence of tPA or by treatment with neuroserpin and remains unchanged in the absence of either plasminogen or plasminogen activator inhibitor 1 (PAI-1; Ref. 5). These results strongly support the hypothesis that the interaction between tPA and neuroserpin has a direct affect on the development of structural changes at the synaptic level necessary for the progression of electrical activity throughout neuronal circuits. This effect of tPA is independent of plasminogen, indicating the presence of an as-yet-unidentified substrate for tPA in the CNS, and the effect is not modified by the absence of PAI-1 in $PAI\text{-}1^{-/-}$ mice, indicating a unique role for neuroserpin as a modulator of tPA's activity in the CNS.

Cerebral Ischemia. In cerebral ischemia, tPA seems to have both beneficial (38) and deleterious (21) effects. Early experiments with an animal model of stroke demonstrated that the use of tPA as a thrombolytic reduces the extent of the neurological damage when given a few hours after the onset of cerebral ischemia (39). More recent studies showed that treatment with tPA in patients with acute ischemic stroke resulted in a 35% increase in the proportion of patients free of disability at 3 months (40). However, although the thrombolytic effect of intravascular tPA is desirable for the treatment of patients with acute ischemic stroke, several studies have demonstrated that excessive tPA within the CNS promotes neuronal death (21, 22, 36). Although one study has indicated that tPA deficiency increases cerebrovascular fibrin deposition, with subsequent worsening in brain injury following transient MCAO (41), several animal models have demonstrated that both genetic deficiency of tPA (21, 36) and its inhibition with the natural tPA inhibitor neuroserpin (22, 31) are associated with a significant increase in neuronal survival and a decrease in infarct volume following either transient or permanent MCAO.

Early after the onset of cerebral ischemia, there is a transient increase in tPA activity, followed by a surge in neuroserpin antigen in the ischemic area surrounding the necrotic core (22). Likewise, neuronal depolarization results in secretion of tPA (9) and an increase in the neuronal transcription of neuroserpin (14). Thus, it is possible to postulate that the neuronal depolarization associated with cerebral ischemia *in vivo* leads to an

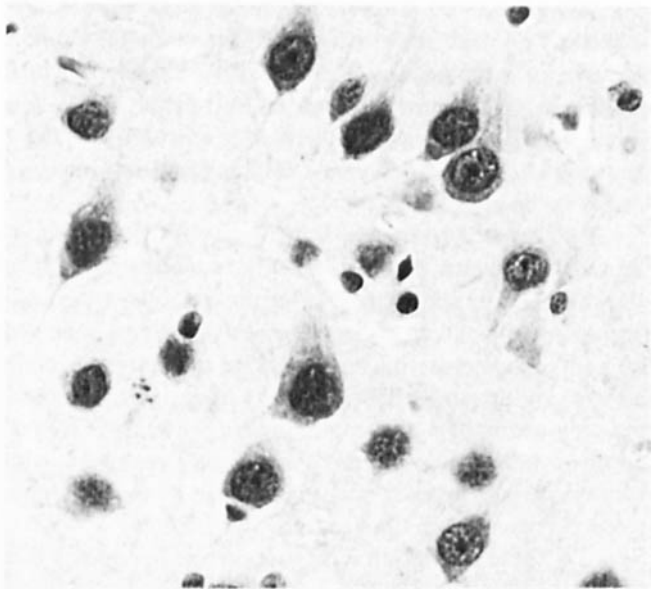

Figure 2. Immunohistochemical staining for neuroserpin in a histological section of the frontal cortex from a normal rat brain. Original magnification is ×40.

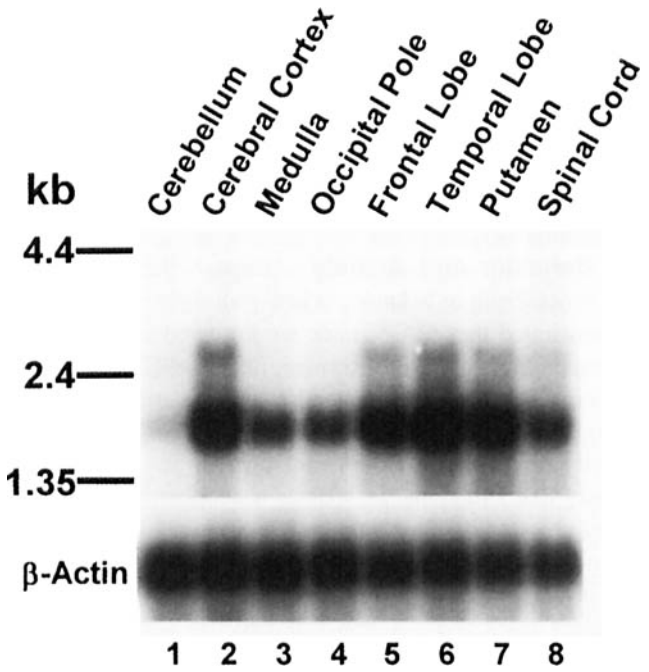

Figure 3. Northern blot analysis of RNA from adult central nervous system. The top panel shows the neuroserpin message, and the lower panel shows actin as a control of RNA loading. Each anatomical location is indicated above the lane. (Reprinted with permission from Ref. 6).

increase in the release of tPA from neurons and microglial cells, followed by a compensatory surge in neuroserpin. If the ischemic insult is too prolonged, then the effect of tPA overcomes the balancing action of its inhibitor, neuroserpin, and the unopposed neurotoxic effect of tPA results in cell death.

Tissue-type Plasminogen Activator and Neuroserpin Regulate the Permeability of the Neurovascular Unit

The Neurovascular Unit. The neurovascular unit is composed of endothelial cells, astrocytes, neurons, and a contractile apparatus of either smooth muscle cells or pericytes (42). Regulation of the permeability of this structure is a necessary part of normal physiology; however, in pathological situations such as acute cerebral ischemia, excessive increases in the permeability of the neurovascular unit lead to the passage of fluids from the intravascular compartment into the extravascular space with the development of vasogenic edema (43, 44). In the neurovascular unit, tPA antigen has been identified in the cerebrovascular endothelium (45), in some neurons (46), and in glial cells (47). On the basis of the currently available evidence, tPA seems to have a dual role in the neurovascular unit: a hemostatic one as a local fibrinolytic enzyme in the microcirculatory bed (48, 49) and a nonhemostatic role responsible for the regulation of the permeability of this structure (50, 51). In the remainder of this review, we focus on the nonhemostatic role of tPA and neuroserpin as regulators of the permeability of the neurovascular unit.

Tissue-type Plasminogen Activator Regulates the Permeability of the Neurovascular Unit Under Physiological Conditions. The role of tPA as a regulator of cerebrovascular permeability is supported by two recent observations: (i) intraventricular infusion of tPA, in the absence of cerebral ischemia, induces, in a dose-dependent manner, an increase in cerebrovascular permeability, as measured by quantification of Evans Blue dye extravasation (51); and (ii) administration of tPA in rats is associated with a significant decrease in cerebrovascular resistance (52). Tissue-type plasminogen activator has been reported to associate with two nonplasminogen ligands in the CNS. These receptors are the NR-1 subunit of the NMDA receptor (53), although this association remains controversial (54), and an LDL receptor family member presumed to be LDL receptor-related protein (LRP; Ref. 55). The reported effect of the intraventricular administration of tPA on cerebrovascular permeability is not modified by blockade of the NMDA receptor but is significantly inhibited by either antibodies to the LRP or by the LRP antagonist, the receptor-associated protein (RAP), indicating the presence of a receptor-mediated process (51). Likewise, in contrast with tPA, intraventricular infusion with uPA does not induce a comparable increase in vascular permeability, indicating that in the CNS, tPA and LRP play an unique role in regulating vascular tone and cerebrovascular permeability.

Tissue-type Plasminogen Activator Induces Opening of the Blood-Brain Barrier Under Pathological Conditions. Analysis of tPA activity and blood-brain barrier (BBB) permeability, using a highly sensitive assay of *in situ* zymography and fluorescence microscopy (51), demonstrated that as early as 1 hr after MCAO, there is a

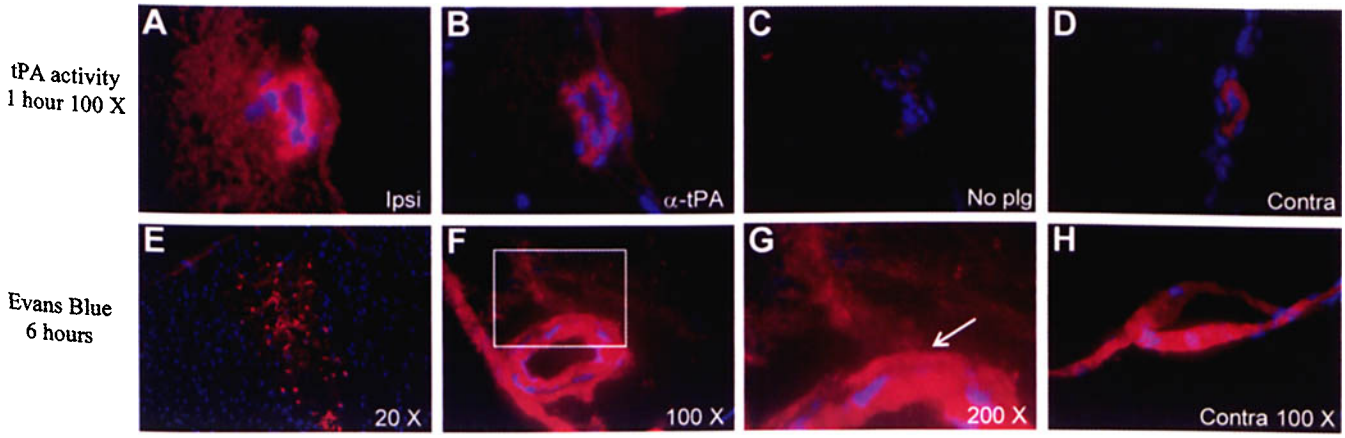

Figure 4. Temporal and spatial relationship between tissue-type plasminogen activator (tPA) activity and vascular permeability following middle cerebral artery occlusion (MCAO) in wild-type C57BL/6J mice. MCAO was performed as described elsewhere (51). Panels A–D show tPA activity in red by *in situ* zymography and cell nuclei in blue (DAPI) 1 hr after MCAO. Panel A demonstrates that by 1 hr after MCAO, there is significant tPA activity surrounding a vessel bordering the necrotic area. Panels B and C show the same vessel in adjacent sections (5 μm), but with either anti-tPA antibodies included (B) or without the addition of Plg (C) in the overlay; and Panel D shows the background tPA activity surrounding a vessel in a corresponding area in the contralateral hemisphere from the same section shown in Panel A. In each case, the original magnification was ×100. Panels E–H show Evans Blue extravasation in red and cell nuclei in blue (DAPI) 6 hrs after MCAO. Panel E shows a low magnification of the entire ischemic area. Panel F shows Evans Blue extravasation from a vessel located in a vessel bordering the necrotic area similar to the one seen in Panel A, and Panel G shows the electronic magnification of the box in Panel F. The arrow indicates an area of Evans Blue leakage outside of the internal elastic lamina of the vessel. Panel H shows Evans Blue adhering to the vessel wall, but no extravasation in a vessel from the same section seen in F and G but located in the corresponding region of the contralateral hemisphere. (Reprinted with permission from Ref. 51).

robust surge in tPA activity associated with blood vessels and the perivascular tissue in the area surrounding the necrotic core, which is followed by an increase in vascular permeability in the same area 5 hrs later (Fig. 4). These results demonstrate that in cerebral ischemia, there is a link between perivascular tPA and increases in vascular permeability (51).

Matrix metalloproteinases (MMPs) are zinc-dependent enzymes that are upregulated following MCAO (56). Some studies have suggested that during ischemic stroke MMPs can promote proteolytic degradation of the vascular basement membrane with subsequent "breakdown" of the BBB and vasogenic edema, and that MMP-9-deficient mice ($MMP\text{-}9^{-/-}$) are partially protected from cerebral ischemia, purportedly because of preservation of the integrity of the BBB (57, 58). A link between tPA, MMP-9, and BBB permeability following MCAO was also indicated by the observation that thrombolytic treatment with tPA in an animal model of embolic stroke is associated with significant increases in MMP-9 expression, cerebrovascular permeability, and vasogenic edema (59, 60). Likewise, treatment with neuroserpin before the administration of tPA results in a significant decrease in tPA-induced increase in BBB permeability (60). Subsequent studies demonstrated that administration of tPA to endothelial cell cultures results in upregulation of MMP-9 (61) and that ischemia-induced MMP-9 activity is significantly decreased by both genetic deficiency of tPA and treatment with neuroserpin (51). The interaction between tPA and MMP-9 in cerebral ischemia was also shown to be independent of the presence of plasminogen, as plasminogen-deficient mice ($Plg^{-/-}$) exhibited an increase in MMP-9 activity similar to that observed in wild-type animals following MCAO (51). Subsequent studies using analysis of the extravasation of Evans Blue Dye as a marker of increases in BBB permeability 6 hrs after permanent MCAO in wild-type, $tPA^{-/-}$, and $Plg^{-/-}$ mice (51) demonstrated that early after MCAO there was a significant increase in BBB permeability in wild-type animals that was significantly decreased in both

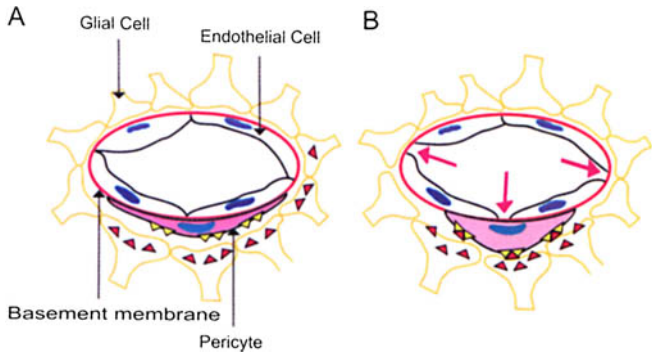

Figure 5. Schematic description of the proposed role of a tissue-type plasminogen activator (tPA) as a regulator of the permeability of the neurovascular unit. In this model, a preformed pool of tPA (red triangles) is located in the glial compartment surrounding the neurovascular unit, and LDL receptor-related protein (LRP) (yellow triangles) is found on the contractile apparatus (pericyte or smooth muscle cells). When there is an increase in metabolic demands or during pathological conditions such as cerebral ischemia (Panel B), tPA is released from the glial compartment and interacts with LRP located in the contractile apparatus. This interaction is likely to result in the activation of an intracellular signaling cascade, with subsequent changes in the cytoskeletal structure and increases in the permeability of the interendothelial junctions (red arrows). The final result is abnormal passage of fluids and harmful substances from the intravascular space into the brain with development of vasogenic edema and cell death.

$tPA^{-/-}$ and wild-type mice treated with neuroserpin. In contrast, ischemia-induced "breakdown" of the BBB was not reduced in animals deficient in plasminogen ($Plg^{-/-}$), MMP-9 ($MMP\text{-}9^{-/-}$), or urokinase-type plasminogen activator ($uPA^{-/-}$) (51). These results indicate that in the early stages of cerebral ischemia, tPA has a unique role in "opening" the BBB by a mechanism that is independent of both plasminogen and MMP-9. Thus, the reported increase in MMP-9 activity following MCAO (56, 59) may be a result of the passage of MMP-9 from the blood into the ischemic area through an already open BBB.

Conclusion

Tissue-type plasminogen activator has many functions in the CNS other than its classical fibrinolytic role. Most of these functions are independent of plasminogen (5, 51), indicating the existence of an as-yet-unidentified substrate for tPA in the CNS. On the basis of recent studies (51, 52), tPA also has been shown to act as a regulator of permeability of the neurovascular unit under both physiological and pathological conditions. We have proposed a model (Fig. 5) in which, in response to increased metabolic demands under both physiological (i.e., learning) and pathological (i.e., cerebral ischemia) conditions, there is release of tPA, most likely from the glial cells surrounding the neurovascular unit. This tPA interacts with LRP, most likely in the contractile apparatus (pericyte or smooth muscle cells), where it may activate an intracellular signaling pathway that leads to changes in either cytoskeletal structure or the interendothelial junctions. Under physiological conditions, this increase in permeability assures the passage of oxygen and nutrients to the brain. Neuroserpin is released into the synaptic space in response to the increase in tPA activity. In this way, tPA's effect is rapidly neutralized. However, when the stimulus is persistent, such as during cerebral ischemia, the increasing concentrations of tPA overcome neuroserpin, leaving the effect of tPA on the neurovascular unit unopposed. This results in the pathological passage of fluids and harmful substances such as MMP-9 into the brain, with the resulting development of vasogenic edema and cell death. This model opens the possibility for two potential therapeutic strategies aimed at maintaining the integrity of the neurovascular unit during pathological conditions: inhibition of tPA activity with neuroserpin, or blocking the interaction with LRP by the therapeutic administration of RAP.

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1. Sappino AP, Madani R, Huarte J, Belin D, Kiss JZ, Wohlwend A, Vassalli JD. Extracellular proteolysis in the adult murine brain. J Clin Invest 92:679–685, 1993.
2. Carroll PM, Tsirka SE, Richards WG, Frohman MA, Strickland S. The mouse tissue plasminogen activator gene 5′ flanking region directs appropriate expression in development and a seizure-enhanced response in the CNS. Development 120:3173–3183, 1994.
3. Qian Z, Gilbert ME, Colicos MA, Kandel ER, Kuhl D. Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. Nature 361:453–457, 1993.
4. Seeds NW, Williams BL, Bickford PC. Tissue plasminogen activator induction in Purkinje neurons after cerebellar motor learning. Science 270:1992–1994, 1995.
5. Yepes M, Sandkvist M, Coleman TA, Moore E, Wu JY, Mitola D, Bugge TH, Lawrence DA. Regulation of seizure spreading by neuroserpin and tissue-type plasminogen activator is plasminogen-independent. J Clin Invest 109:1571–1578, 2002.
6. Hastings GA, Coleman TA, Haudenschild CC, Stefansson S, Smith EP, Barthlow R, Cherry S, Sandkvist M, Lawrence DA. Neuroserpin, a brain-associated inhibitor of tissue plasminogen activator is localized primarily in neurons. Implications for the regulation of motor learning and neuronal survival. J Biol Chem 272:33062–33067, 1997.
7. Ware JH, DiBenedetto AJ, Pittman RN. Localization of tissue plasminogen activator mRNA in the developing rat cerebellum and effects of inhibiting tissue plasminogen activator on granule cell migration. J Neurobiol 28:9–22, 1995.
8. Jiang X, Wang Y, Hand AR, Gillies C, Cone RE, Kirk J, O'Rourke J. Storage and release of tissue plasminogen activator by sympathetic axons in resistance vessel walls. Microvasc Res 64:438–447, 2002.
9. Gualandris A, Jones TE, Strickland S, Tsirka SE. Membrane depolarization induces calcium-dependent secretion of tissue plasminogen activator. J Neurosci 16:2220–2225, 1996.
10. Osterwalder T, Cinelli P, Baici A, Pennella A, Krueger SR, Schrimpf SP, Meins M, Sonderegger P. The axonally secreted serine proteinase inhibitor, neuroserpin, inhibits plasminogen activators and plasmin but not thrombin. J Biol Chem 273:2312–2321, 1998.
11. Osterwalder T, Contartese J, Stoeckli ET, Kuhn TB, Sonderegger P. Neuroserpin, an axonally secreted serine protease inhibitor. EMBO J 15:2944–2953, 1996.
12. Schrimpf SP, Bleiker AJ, Brecevic L, Kozlov SV, Berger P, Osterwalder T, Krueger SR, Schinzel A, Sonderegger P. Human neuroserpin (PI12): cDNA cloning and chromosomal localization to 3q26. Genomics 40:55–62, 1997.
13. Krueger SR, Ghisu GP, Cinelli P, Gschwend TP, Osterwalder T, Wolfer DP, Sonderegger P. Expression of neuroserpin, an inhibitor of tissue plasminogen activator, in the developing and adult nervous system of the mouse. J Neurosci 17:8984–8996, 1997.
14. Berger P, Kozlov SV, Cinelli P, Krueger SR, Vogt L, Sonderegger P. Neuronal depolarization enhances the transcription of the neuronal serine protease inhibitor neuroserpin. Mol Cell Neurosci 14:455–467, 1999.
15. Krystosek A, Seeds NW. Plasminogen activator release at the neuronal growth cone. Science 213:1532–1534, 1981.
16. Krystosek A, Seeds NW. Plasminogen activator secretion by granule neurons in cultures of developing cerebellum. Proc Natl Acad Sci U S A 78:7810–7814, 1981.
17. Krystosek A, Seeds NW. Normal and malignant cells, including neurons, deposit plasminogen activator on the growth substrata. Exp Cell Res 166:31–46, 1986.
18. Baranes D, Lederfein D, Huang YY, Chen M, Bailey CH, Kandel ER. Tissue plasminogen activator contributes to the late phase of LTP and to synaptic growth in the hippocampal mossy fiber pathway. Neuron 21:813–825, 1998.
19. Huang YY, Bach ME, Lipp HP, Zhuo M, Wolfer DP, Hawkins RD, Schoonjans L, Kandel ER, Godfraind JM, Mulligan R, Collen D, Carmeliet P. 1996. Mice lacking the gene encoding tissue-type plasminogen activator show a selective interference with late-phase long-term potentiation in both Schaffer collateral and mossy fiber pathways. Proc Natl Acad Sci U S A 93:8699–8704, 1996.
20. Madani R, Kozlov S, Akhmedov A, Cinelli P, Kinter J, Lipp HP,

Sonderegger P, Wolfer DP. Impaired explorative behavior and neophobia in genetically modified mice lacking or overexpressing the extracellular serine protease inhibitor neuroserpin. Mol Cell Neurosci 23:473–494, 2003.
21. Wang YF, Tsirka SE, Strickland S, Stieg PE, Soriano SG, Lipton SA. Tissue plasminogen activator (tPA) increases neuronal damage after focal cerebral ischemia in wild-type and tPA-deficient mice. Nat Med 4:228–231, 1998.
22. Yepes M, Sandkvist M, Wong MK, Coleman TA, Smith E, Cohan SL, Lawrence DA. Neuroserpin reduces cerebral infarct volume and protects neurons from ischemia-induced apoptosis. Blood 96:569–576, 2000.
23. Davis RL, Holohan PD, Shrimpton AE, Tatum AH, Daucher J, Collins GH, Todd R, Bradshaw C, Kent P, Feiglin D, Rosenbaum A, Yerby MS, Shaw CM, Lacbawan F, Lawrence DA. Familial encephalopathy with neuroserpin inclusion bodies. Am J Pathol 155:1901–1913, 1999.
24. Davis RL, Shrimpton AE, Holohan PD, Bradshaw C, Feiglin D, Collins GH, Sonderegger P, Kinter J, Becker LM, Lacbawan F, Krasnewich D, Muenke M, Lawrence DA, Yerby MS, Shaw CM, Gooptu B, Elliott PR, Finch JT, Carrell RW, Lomas DA. Familial dementia caused by polymerization of mutant neuroserpin. Nature 401:376–379, 1999.
25. Gveric D, Herrera B, Petzold A, Lawrence DA, Cuzner ML. Impaired fibrinolysis in multiple sclerosis: a role for tissue plasminogen activator inhibitors. Brain 126:1590–1598, 2003.
26. Muller CM, Griesinger CB. Tissue plasminogen activator mediates reverse occlusion plasticity in visual cortex. Nat Neurosci 1:47–53, 1998.
27. Parmar PK, Coates LC, Pearson JF, Hill RM, Birch NP. Neuroserpin regulates neurite outgrowth in nerve growth factor-treated PC12 cells. J Neurochem 82:1406–1415, 2002.
28. Seeds NW, Basham ME, Ferguson JE. Absence of tissue plasminogen activator gene or activity impairs mouse cerebellar motor learning. J Neurosci 23:7368–7375, 2003.
29. Siconolfi LB, Seeds NW. Mice lacking tissue plasminogen activator and urokinase plasminogen activator genes show attenuated matrix metalloproteases activity after sciatic nerve crush. J Neurosci Res 74:430–434, 2003.
30. Pawlak R, Magarinos AM, Melchor J, McEwen B, Strickland S. Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. Nat Neurosci 6:168–174, 2003.
31. Cinelli P, Madani R, Tsuzuki N, Vallet P, Arras M, Zhao CN, Osterwalder T, Rulicke T, Sonderegger P. Neuroserpin, a neuroprotective factor in focal ischemic stroke. Mol Cell Neurosci 18:443–457, 2001.
32. Tsirka SE, Gualandris A, Amaral DG, Strickland S. Excitotoxin-induced neuronal degeneration and seizure are mediated by tissue plasminogen activator. Nature 377:340–344, 1995.
33. Tsirka SE, Rogove AD, Bugge TH, Degen JL, Strickland S. An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus. J Neurosci 17:543–552, 1997.
34. Mattson MP. Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders. Neuromolecular Med 3:65–94, 2003.
35. Sattler R, Tymianski M. Molecular mechanisms of glutamate receptor-mediated excitotoxic neuronal cell death. Mol Neurobiol 24:107–129, 2001.
36. Nagai N, De Mol M, Lijnèn HR, Carmeliet P, Collen D. Role of plasminogen system components in focal cerebral ischemic infarction: a gene targeting and gene transfer study in mice. Circulation 99:2440–2444, 1999.
37. Tsirka SE, Bugge TH, Degen JL, Strickland S. Neuronal death in the central nervous system demonstrates a non-fibrin substrate for plasmin. Proc Natl Acad Sci U S A 94:9779–9781, 1997.
38. Marler JR, Goldstein LB. Medicine. Stroke—tPA and the clinic. Science 301:1677, 2003.
39. Zivin JA, Fisher M, DeGirolami U, Hemenway CC, Stashak JA. Tissue plasminogen activator reduces neurological damage after cerebral embolism. Science 230:1289–1292, 1985.
40. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 333:1581–1587, 1995.
41. Tabrizi P, Wang L, Seeds N, McComb JG, Yamada S, Griffin JH, Carmeliet P, Weiss MH, Zlokovic BV. Tissue plasminogen activator (tPA) deficiency exacerbates cerebrovascular fibrin deposition and brain injury in a murine stroke model: studies in tPA-deficient mice and wild-type mice on a matched genetic background. Arterioscler Thromb Vasc Biol 19:2801–2806, 1999.
42. Lo EH, Broderick JP, Moskowitz MA. tPA and proteolysis in the neurovascular unit. Stroke 35:354–356, 2004.
43. Baker RN, Cancilla PA, Pollock PS, Frommes SP. The movement of exogenous protein in experimental cerebral edema. An electron microscopic study after freeze-injury. J Neuropathol Exp Neurol 30:668–679, 1971.
44. Garcia JH, Lossinsky AS, Kauffman FC, Conger KA. Neuronal ischemic injury: light microscopy, ultrastructure and biochemistry. Acta Neuropathol (Berl) 43:85–95, 1978.
45. Levin EG, del Zoppo GJ. Localization of tissue plasminogen activator in the endothelium of a limited number of vessels. Am J Pathol 144:855–861, 1994.
46. Salles FJ, Strickland, S. Localization and regulation of the tissue plasminogen activator-plasmin system in the hippocampus. J Neurosci 22:2125–2134, 2002.
47. Siao C-J, Fernandez SR, Tsirka SE. Cell type-specific roles for tissue plasminogen activator released by neurons or microglia after excitotoxic injury. J. Neurosci 23:3224–3242, 2003.
48. Tran ND, Schreiber SS, Fisher M. Astrocyte regulation of endothelial tissue plasminogen activator in a blood-brain barrier model. J Cereb Blood Flow Metab 18:1316–1324, 1998.
49. Zlokovic BV, Wang L, Sun N, Haffke S, Verrall S, Seeds NW, Fisher MJ, Schreiber SS. Expression of tissue plasminogen activator in cerebral capillaries: possible fibrinolytic function of the blood-brain barrier. Neurosurgery 37:955–961, 1995.
50. Thiex R, Mayfrank L, Rohde V, Gilsbach JM, Tsirka SE. The role of endogenous versus exogenous tPA on edema formation in murine ICH. Exp Neurol 189:25–32, 2004.
51. Yepes M, Sandkvist M, Moore EG, Bugge TH, Strickland DK, Lawrence DA. Tissue-type plasminogen activator induces opening of the blood-brain barrier via the LDL receptor-related protein. J Clin Invest 112:1533–1540, 2003.
52. Nassar T, Akkawi S, Shina A, Haj-Yehia A, Bdeir D, Tarshis M, Heyman SN, Higazi AA. The in vitro and in vivo effect of tPA and PAI-1 on blood vessel tone. Blood 103:897–902, 2004.
53. Nicole O, Docagne F, Ali C, Margaill I, Carmeliet P, MacKenzie ET, Vivien D, Buisson A. The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. Nat Med 7:59–64, 2001.
54. Matys T, Strickland S. Tissue plasminogen activator and NMDA receptor cleavage. Nat Med 9:371–372, 2003.
55. Bu G, Williams S, Strickland DK, Schwartz AL. Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator. Proc Natl Acad Sci U S A 89:7427–7431, 1992.
56. Rosenberg GA, Navratil M, Barone F, Feuerstein G. Proteolytic cascade enzymes increase in focal cerebral ischemia in rat. J Cereb Blood Flow Metab 16:360–366, 1996.
57. Asahi M, Asahi K, Jung JC, del Zoppo GJ, Fini ME, Lo EH. Role for matrix metalloproteinase 9 after focal cerebral ischemia: effects of gene knockout and enzyme inhibition with BB-94. J Cereb Blood Flow Metab 20:1681–1689, 2000.
58. Asahi M, Wang X, Mori T, Sumii T, Jung JC, Moskowitz MA, Fini ME, Lo EH. Effects of matrix metalloproteinase-9 gene knock-out on

the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. J Neurosci 21:7724–7732, 2001.

59. Aoki T, Sumii T, Mori T, Wang X, Lo EH. Blood-brain barrier disruption and matrix metalloproteinase-9 expression during reperfusion injury: mechanical versus embolic focal ischemia in spontaneously hypertensive rats. Stroke 33:2711–2717, 2002.
60. Zhang Z, Zhang L, Yepes M, Jiang Q, Li Q, Arniego P, Coleman TA, Lawrence DA, Chopp M. Adjuvant treatment with neuroserpin increases the therapeutic window for tissue-type plasminogen activator administration in a rat model of embolic stroke. Circulation 106:740–745, 2002.
61. Wang X, Lee SR, Arai K, Lee SR, Tsuji K, Rebeck GW, Lo EH. Lipoprotein receptor-mediated induction of matrix metalloproteinase by tissue plasminogen activator. Nat Med 9:1313–1317, 2003.