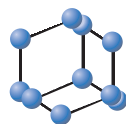


## REVIEW ARTICLE

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SCIENCE

## The Plasminogen Activation System Promotes Neurorepair in the Ischemic Brain

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**Abstract:** The Plasminogen Activation (PA) system was originally thought to exclusively promote the degradation of fibrin by catalyzing the conversion of plasminogen into plasmin *via* two serine proteinases: Tissue-type Plasminogen Activator (tPA) and Urokinase-type Plasminogen Activator (uPA). However, experimental evidence accumulated over the last 30 years indicates that tPA and uPA are also found in the Central Nervous System (CNS), where they have a plethora of functions that not always require plasmin generation or fibrin degradation. For example, plasminogen-dependent and -independent effects of tPA and uPA play a central role in the pathophysiological events that underlie one of the leading causes of mortality and disability in the world: cerebral ischemia. Indeed, recent work indicates that while the rapid release of tPA from the presynaptic compartment following the onset of cerebral ischemia protects the synapse from the deleterious effects of the ischemic injury, the secretion of uPA and its binding to its receptor (uPAR) during the recovery phase promotes the repair of synapses that have been lost to the acute ischemic insult. This restorative role of uPA has high translational significance because to this date there is no effective approach to induce neurorepair in the ischemic brain. Here we will discuss recent evidence that bridges the gap between basic research in the field of the PA system and the bedside of ischemic stroke patients, indicating that uPA and uPAR are potential targets for the development of therapeutic strategies to promote neurological recovery among ischemic stroke survivors.

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## 1. INTRODUCTION

The Plasminogen Activation (PA) system is an enzymatic cascade that promotes the degradation of fibrin by catalyzing the conversion of plasminogen into the broad-spectrum protease plasmin *via* two serine proteinases: Tissue-type Plasminogen Activator (tPA) and urokinase-type Plasminogen Activator (uPA) [1]. The activation of this system is tightly controlled by plasminogen activator inhibitors-1 (PAI-1) and -2 (PAI-2), neuroserpin and  $\alpha_2$ -antiplasmin [2, 3]. Consecutively, plasmin generated by the activity of tPA and uPA cleaves several extracellular matrix (ECM) components, degrades fibrin and activates matrix metalloproteinases (MMPs).

According to the most accepted model of PA system function, the release of tPA maintains the patency of the blood vessels by controlling the degradation of fibrin in the intravascular space, whereas the secretion of uPA regulates

cell migration and ECM turnover by triggering the generation of plasmin on the cell surface [4, 5]. However, a growing body of experimental evidence indicates that this model is significantly more complex than originally thought.

This is the case for the Central Nervous System (CNS), where the PA system plays a pivotal role in a plethora of plasminogen-dependent and -independent events [6] such as neuronal migration [7], neurite outgrowth and remodeling during development [8], synaptic plasticity [9], learning [10, 11], stress-induced anxiety [12], neuroprotection [13], regulation of the permeability of the blood-brain barrier [14], and neuroinflammation [15]. Here we will review recent experimental evidence indicating a novel role for the PA system in the CNS as inductor of neurorepair in the brain that has suffered an acute ischemic injury. We will also discuss the translational relevance that these findings have for patients recovering from an ischemic stroke.

## 2. CEREBRAL ISCHEMIA

Ischemic stroke is caused by the interruption of the blood supply to the brain. Remarkably, the rapid development of pharmacological and neurointerventional strategies to treat

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ischemic stroke patients during the acute stages of this disease has resulted in a significant decrease in mortality [16]. Paradoxically, this has also caused a sharp increase in the number of people that survive an ischemic stroke with different degrees of disability. Unfortunately, in contrast with the treatment of acute ischemic stroke patients, to this date there is no effective therapeutic strategy to promote neurological recovery among ischemic stroke survivors.

Experimental evidence accumulated over the last 20 years indicates that tPA activity transiently increases in the ischemic brain within minutes of the onset of the ischemic injury [3]. This observation has led to a large number of papers reporting either a harmful [17] or a beneficial [13] effect for this rise in tPA. In contrast, little is known to this date on the effect of the delayed increase in uPA activity in the ischemic brain. Below we will discuss experimental data indicating that while the early release of tPA is a neuroprotective response aimed at protecting the synapse from the deleterious effects of the acute ischemic injury, the delayed secretion of uPA induces neuropair and improves neurological outcomes following an acute ischemic stroke. Collectively, the data discussed below indicate that the PA system is a potential target for the development of pharmacological strategies to either protect or promote neurorepair in the ischemic brain.

### 3. TISSUE-TYPE PLASMINOGEN ACTIVATOR (tPA)

TPA is a serine proteinase of 527 residues assembled in an A chain with a finger, an EGF and two kringle domains, and a B chain with a protease domain. TPA is secreted as a single chain molecule (sctPA), but cleavage by plasmin at its Arg561-Val562 peptide bond generates two-chain tPA (tctPA). Importantly, in absence of fibrin tPA is an inefficient activator of plasminogen, and both, sctPA and tctPA, bind to fibrin with comparable affinity [18].

#### 3.1. TPA Expression in the Central Nervous System (CNS)

*In situ* zymography studies indicate that the activity of tPA in the brain is circumscribed to the hippocampus, hypothalamus, thalamus, amygdala, cerebellum and meningeal blood vessels [19, 20]. Interestingly, tPA mRNA has been detected in areas of the brain, such as the cerebral cortex, that do not display tPA-catalyzed enzymatic activity. It has been postulated that this discrepancy between tPA catalyzed-proteolysis and tPA mRNA expression is due either to the localized expression of tPA inhibitors in those areas where tPA-catalyzed proteolysis is not observed, or the accumulation of tPA protein at distant places of its synthesis such as the synapse, or to posttranslational regulation of tPA expression, or to the possibility that in some areas of the brain tPA has plasmin-independent functions. Regardless of the reason for this divergence, the release of tPA plays a central role in several physiological processes such as neuronal migration [7], neurite growth [8], synaptic plasticity [9], learning [10, 11], stress-induced anxiety [12], and neurovascular coupling [21].

#### 3.2. TPA in the Ischemic Brain

A substantial number of studies have detected a rapid increase in the expression of tPA in the brain following dif-

ferent forms of injury, including seizures [9, 14], ischemia [3, 22] and physical trauma [23]. Almost simultaneously it was shown that tPA interacts with glutamate receptors [24] and modulates the postsynaptic response to the excitotoxic release of glutamate [25]. Despite the fact that it was originally argued that tPA had a harmful effect on the injured brain [22], recent evidence indicates that the release of tPA during the acute phase of an ischemic injury may instead be a beneficial response aimed at protecting the structure and functional integrity of the synapse [13, 26, 27]. Taken together, these data indicate that tPA plays a pivotal role in the response of the brain to an acute ischemic injury. In contrast, very few studies have shown that tPA has a neurorestorative role in the brain already damaged by an ischemic insult. Indeed, although it has been described that tPA promotes neurite outgrowth and axonal remodeling after stroke [28], these data are difficult to reconcile with a transient increase in tPA activity only during the early stages of the ischemic injury when the neurorestorative process has not taken place yet.

### 4. UROKINASE-TYPE PLASMINOGEN ACTIVATOR (uPA)

UPA is a serine proteinase of 411 residues assembled in three domains: Epidermal Growth Factor (EGF), kringle and protease domains. The first two domains form the amino-terminal fragment (ATF)[4, 5]. The EGF domain binds to uPA's receptor (uPAR), while the protease domain harbors the catalytic residues His204, Asp255, and Ser356. UPA undergoes N-glycosylation at Asn302 in the proteolytic domain, and O-glycosylation at Thr18 in the EGF domain; and although the effect of these post-translational modifications is unknown, it has been suggested that Asn302 glycosylation makes uPA more susceptible to activation by plasmin, while deglycosylation of the Thr18 residue has a negative effect on uPA's mitogenic properties [29].

UPA is synthesized by vascular endothelial and smooth muscle cells, fibroblasts, monocytes and macrophages [30], and secreted as a single-chain polypeptide, known as pro-urokinase because it was originally thought not to have proteolytic activity. However, subsequent studies found that pro-urokinase can cleave plasminogen into plasmin, which in turn activates pro-urokinase by a cleavage at its Lys158-Ile159 residues, converting the single-chain urokinase into a two-chain form. Importantly, the rate of plasminogen cleavage by two-chain urokinase is over 200-fold higher than the single-chain form [31].

Despite the fact that tPA and uPA activate plasminogen by cleaving its Arg561-Val562 bond, only uPA can do it in the absence of fibrin. This has generated the view that whereas tPA has a fibrinolytic effect in the intravascular space, uPA generates plasmin on the cell surface, and by doing so has a primary role in ECM degradation, cell migration, wound healing, inflammation, embryogenesis and spread of tumor cells and metastasis [32, 33]. As it will be discussed below, in contrast with this model, several of the effects of uPA in the CNS do not require plasmin generation.

#### 4.1. Urokinase-type Plasminogen Activator Receptor (uPAR)

UPAR is a receptor anchored to the plasma membrane by a glycosyl phosphatidylinositol (GPI) tail. It has 313 residues

assembled in three homologous domains, designated D1-D3, connected by a linker region and that together form a concave structure [34]. uPA's growth factor domain (residues 1 - 44) binds to uPAR. Importantly, besides localizing plasmin generation to the cell surface, uPAR is also a signaling receptor that promotes cell survival, proliferation, invasion and motility [4]. However, because uPAR is not a transmembrane protein, it needs transmembrane coreceptors to activate intracellular signaling pathways.

#### 4.2. uPA and uPAR Expression in the Central Nervous System (CNS)

Early work using northern blot and *in situ* zymography assays failed to detect uPA mRNA or uPA activity in the adult brain [19, 20]. In contrast, a different group of investigators detected high levels of uPA mRNA in spinal cord and sensory ganglia during the early stages of development [35]. Subsequent *in situ* hybridization studies detected high levels of uPA mRNA in the developing cerebral cortex, olfactory bulb, hippocampus and cerebellum [36]. Together, these studies suggested a role for uPA on neurite growth during development. Interestingly, *in situ* hybridization studies found uPA mRNA not only during development but also in large neurons of well-defined areas of the adult murine brain, namely the parietal and entorhinal cortex, and subculicular complex [37]. Despite the fact that these data suggested a role for uPA not only during development but also in the physiology of mature neurons, the role of uPA in the normal CNS remained unclear for a long period of time. Interestingly, high levels of uPA mRNA are elevated in CNS tumors, specifically gliomas, and uPA mRNA expression has been associated with malignant progression of these neoplasms [38, 39]. The expression of uPAR in neurons follows a pattern similar to uPA's. Indeed, while uPAR is abundantly found in almost all cell bodies and neurites in days *in vitro* (DIV) 3 neurons, at DIV 7 is detected along axon shafts and growth cones, and at DIV 16 its expression is restricted to few growth cones and dendrites [40].

#### 4.3. uPA and uPAR in the Ischemic Brain

Early *in situ* zymography studies indicated that cerebral ischemia induces a rapid increase in tPA activity in the ischemic brain [3, 22]. In contrast, uPA activity was detected only 72 hours after the end of the ischemic injury [3]. Interestingly, this finding remained unexplored for almost 20 years, when ELISA-based studies showed that although the expression of uPA in the ischemic brain remains unchanged during the acute phase of the ischemic injury, it exhibits a large increase 3 - 24 hours after the end of the ischemic insult [41]. Remarkably, it was found that this increase in uPA expression in the ischemic brain does not have an effect on neuronal survival, as genetic deficiency of uPA (uPA<sup>-/-</sup>) did not have an effect on the volume of the ischemic lesion following transient occlusion of the middle cerebral artery (tMCAO). These findings were further supported by the observation that exposure of wild-type (wt) cerebral cortical neurons to oxygen and glucose deprivation (OGD) conditions induces the release of uPA only 3 - 24 hours after the end of the hypoxic injury, and that genetic deficiency of uPA did not have an effect on OGD-induced neuronal death [41].

In line with these observations, it was observed that during the recovery phase from a hypoxic injury the expression of uPA's receptor (uPAR) increases in growth cones and dendritic spines of cerebral cortical neurons to levels comparable to those observed during development [40].

#### 4.4. uPA Promotes Neurological Recovery Following an Ischemic Stroke

The *in vitro* and *in vivo* studies discussed above indicate that the expression of uPA and uPAR in the brain increase during the recovery phase from an acute ischemic injury, and that this rise does not have an effect on neuronal death. This late release and lack of effect on neuronal survival suggested that uPA may play a role on the neurorestorative process that ensues after the end of the acute ischemic injury. To test this hypothesis, a battery of neurobehavioral tests was performed in wt and uPA<sup>-/-</sup> mice, and in animals genetically deficient on uPAR (uPAR<sup>-/-</sup>) 0 - 7 days after 60 minutes of tMCAO. A sub-group of animals was intravenously treated with recombinant uPA (ruPA) immediately after the end of the ischemic injury at doses comparable to those used in acute ischemic stroke patients [42]. Surprisingly, these experiments showed that in contrast with wt mice, uPA<sup>-/-</sup> and uPAR<sup>-/-</sup> animals fail to recover their neurological function after stroke. However, intravenous treatment with ruPA promoted neurological recovery in wt and uPA<sup>-/-</sup> but not uPAR<sup>-/-</sup> animals. Together, these results indicate that uPA / uPAR binding promotes neurological recovery following an ischemic stroke [41].

To study the mechanism of this effect, diffusion magnetic resonance imaging (dMRI) was used to study changes in the mean diffusivity (MD) and fractional anisotropy (Fa) of water, both determined by the microscopic features of the neuronal tissue [43], in the brain of wt and uPA<sup>-/-</sup> mice 24 hours after tMCAO. These experiments indicated that compared to wt animals, uPA<sup>-/-</sup> mice have a significant increase in the MD and decrease in Fa of water in a thin band of approximately 1 mm of neuronal tissue surrounding the necrotic core [41]. These observations are of remarkable translational relevance because biochemical and anatomical changes in this area have been linked to neurological recovery after an ischemic stroke [44]. Together, these data indicated that the release of uPA during the recovery phase from an ischemic injury has an effect on the microanatomy of the neural tissue that surrounds the ischemic core which, as stated above, is known to drive the process of neurological recovery after an ischemic stroke [45].

#### 4.5. Synaptic Effect of uPA in the Ischemic Brain

Cerebral ischemia has a deleterious effect on the synapse linked to impairment of neurological function [46]. Because the data discussed above indicate that uPA promotes neurological recovery and induces microstructural changes in the area surrounding the necrotic core, then it is conceivable to postulate that uPA/uPAR binding during the recovery phase from an ischemic stroke has a direct effect on the synapse that has been harmed by the ischemic injury. To test this hypothesis, a series of experiments were performed to study the effect of uPA/uPAR binding on the structure of the pre-synaptic (axon) and postsynaptic (dendritic spines) terminals.

#### 4.5.1. UPA/uPAR Binding Promotes Axonal Recovery

Axons are highly vulnerable to cerebral ischemia and their restoration is pivotal for the recovery of neurological function following an acute ischemic stroke [47]. To study whether uPA has an effect on axonal recovery, axons from cerebral cortical neurons were cultured in an *in vitro* system that allows the growth of a homogenous axonal mantle devoid of dendrites and other neuronal structures [48]. A first hint of an effect of uPA on axonal recovery came from the observation that the expression of uPA's receptor (uPAR) was significantly increased in growth cones reemerging from the axonal mantle following a mechanical injury [40]. These data are in agreement with work from several groups describing an increase in uPAR expression following different forms of injury to non-CNS organs [4].

Axonal growth cones are highly dynamic structures that guide the formation of new axons during development, and the regeneration of mature axons after an injury [49]. Thus, the finding of an increase in uPAR expression in axonal growth cones reemerging from a mechanical injury led to study whether uPA/uPAR binding has an effect on axonal repair. To study this possibility, the speed and length of axonal growth were quantified with live microscopy after the axonal mantle of wt and uPAR<sup>-/-</sup> neurons underwent a mechanical injury followed by treatment with uPA or vehicle (control). These experiments showed that treatment with uPA promotes the growth of wt but not uPAR<sup>-/-</sup> axons, and that this effect is independent of uPA's ability to generate plasmin [40].

As stated above, uPAR is a GPI-anchored receptor that needs transmembrane co-receptors to activate cell signaling pathways [34]. In line with these observations, it was found that low-density lipoprotein receptor-related protein-1 (LRP-1)-mediated recruitment of  $\beta$ 1 integrin to the plasma membrane of axonal growth cones was required for uPA/uPAR to induce axonal growth following a mechanical injury. Furthermore, subsequent studies identified that the neurorestorative effect of uPA/uPAR binding was mediated by  $\beta$ 1 integrin-promoted activation of the Rho GTPase Rac-1 [40].

The ability of an axon to regrow after an ischemic lesion *in vivo* is restricted by the formation of a glial cell-derived inhibitory microenvironment in the injured site [50]. Thus, to test the translational relevance of the *in vitro* findings described above, neurological recovery was examined following the induction of an ischemic lesion to the internal capsule [a subcortical structure assembled by a well-defined bundle of descending axons from cerebral cortical neurons [51]] of wt, uPA<sup>-/-</sup>, uPAR<sup>-/-</sup>, and Plau<sup>GFDhu/GFDhu</sup> mice [in which a 4-amino acid substitution into uPA's growth factor domain abrogates its binding to uPAR while maintaining other functions of the protease and its receptor [52]]. A sub-group of each strain of mice was intravenously treated with ruPA immediately after the induction of the ischemic injury. These studies indicated that binding of either endogenous or ruPA to uPAR promotes neurological recovery following the induction of an ischemic lesion to subcortical axons *in vivo* [40].

#### 4.5.2. Effect of uPA on the Postsynaptic Terminal

Dendritic spines are postsynaptic protrusions that receive the input of most of the excitatory synapses in the CNS [53].

Cerebral ischemia has a direct effect on the integrity and receptor composition of dendritic spines. However, this is not a homogenous process, and while spines located inside the necrotic core are irreversibly damaged, those located in a thin band around the necrotic core are replaced by areas of local swelling known as varicosities. Remarkably, if the ischemic area is reperfused in less than 60 minutes from the onset of the ischemic injury, a significant proportion of spines either re-emerge again from these varicosities or are formed *de novo* [54]. The translational importance of these observations derives from the fact that the re-emergence and formation of new dendritic spines in the area surrounding the necrotic core is considered a pivotal event for the recovery process from an acute ischemic stroke [45].

To investigate whether the release of uPA during the recovery phase from an ischemic stroke has an effect on dendritic spines, confocal microscopy was used to perform a tridimensional reconstruction of dendrites from the II-III cortical layers in the area surrounding the necrotic core of wt, uPA<sup>-/-</sup> and uPAR<sup>-/-</sup> mice 24 hours after 60 minutes of tMCAO. These studies detected long filopodia and clusters of dendritic spines in wt but not uPA<sup>-/-</sup> or uPAR<sup>-/-</sup> mice, in which these extensions were smooth and exhibited lack of protrusions [41]. Importantly, intravenous administration of ruPA after the end of the ischemic injury increased the number of dendritic spines and filopodia in wt and uPA<sup>-/-</sup> but not in uPAR<sup>-/-</sup> animals. These observations were further supported by subsequent *in vitro* work indicating that the excitotoxic injury induces the clustering of uPAR on the membrane of those spines that re-emerge from dendritic varicosities.

The observations described above provided a clue on a potential effect of uPA on the reorganization of the actin cytoskeleton in the postsynaptic terminal. Indeed, dendritic spines harbor globular (G) and filamentous (F) actin, and the re-emergence and formation of new dendritic spines requires the formation and stabilization of F-actin [55, 56]. Ezrin, Radixin and Moesin (ERM) are a group of proteins that regulate the reorganization of the actin cytoskeleton. Hence, following their activation by phosphorylation at conserved regulatory threonine residues, ERM proteins link the plasma membrane to the actin cytoskeleton, thus inducing the formation of filopodia, lamellipodia and microvilli in different cell systems [57, 58]. A further step on our understanding of the effect of uPA on the actin cytoskeleton was provided by the finding that uPA induces the local synthesis and phosphorylation of ezrin, but not radixin or moesin, in the postsynaptic terminal of cerebral cortical neurons [59]. Importantly, these studies showed that  $\beta$ 3-integrin is the coreceptor that mediates the effect of uPA/uPAR binding on ezrin, and that this integrin recruits the intercellular adhesion molecule-5 (ICAM-5), which in turn recruits ezrin to the postsynaptic density (PSD) where it undergoes  $\beta$ 3-integrin-mediated phosphorylation (activation) at Thr567. Curiously, these findings show that while  $\beta$ 1 integrin is the co-receptor for uPAR in axons (see above),  $\beta$ 3 integrin mediates the effect of uPAR in the postsynaptic compartment. The importance of this sequence of events was supported by the finding that treatment with uPA induces the formation of F-actin bundles in the postsynaptic terminal, and that siRNA-induced down-regulation of ezrin in the postsynaptic terminal abrogates the

effect of uPA on the formation of F-actin bundles and the re-emergence of dendritic spines [59]. Importantly, *in vitro* work with neurons exposed to OGD conditions and *in vivo* experiments with an animal model of cerebral ischemia indicated that treatment with ruPA induces ezrin-mediated reorganization of the actin cytoskeleton in neurons recovering from a hypoxic/ischemic injury, and that this effect promotes the recovery of synaptic contacts lost to the acute hypoxic/ischemic insult [59].

#### 4.6. The uPA/uPAR System in Astrocytes

A substantial body of experimental evidence indicates that uPAR is abundantly found in astrocytic tumors, and that uPA/uPAR expression and signaling are linked to the malignancy and growth of these neoplasms [38, 39]. In contrast with these observations, it is unknown whether uPA and uPAR play a role in non-malignant astrocytic function. Importantly, it has been reported that during the recovery phase from an ischemic injury neurons but not astrocytes release uPA, while astrocytes recruit uPAR to their plasma membrane [60], suggesting that uPA/uPAR binding in the ischemic brain mediates a cross-talk between astrocytes and the synapse.

##### 4.6.1. UPA/uPAR Binding Induces Astrocytic Activation

Recent work indicates that the intracerebral injection of either uPA or its Amino Terminal Fragment (ATF; devoid of proteolytic activity) increases the expression of Glial Fibrillary Acidic Protein (GFAP) in wt but not uPAR<sup>-/-</sup> animals [60]. These results are of significant importance because increased GFAP expression is a marker of astrocytic activation, which is an evolutionary preserved process whereby these cells develop morphologic, biochemical and genetic changes in response to a variety of stimuli [61]. Despite the fact that it has long been recognized that cerebral ischemia is a potent inductor of astrocytic activation, the role of activated astrocytes in the ischemic brain is still unclear. Indeed, while some studies indicate that astrocytic activation interferes with neuronal recovery from an ischemic injury [62], others have proposed that it has a protective effect [61].

A number of *in vitro* and *in vivo* studies found that hypoxia induces the recruitment of uPAR to the astrocytic plasma membrane, that cerebral ischemia-induced astrocytic activation is significantly attenuated by genetic deficiency of uPA<sup>-/-</sup> or uPAR<sup>-/-</sup>, and that intravenous treatment with ruPA increases the population of GFAP-immunoreactive astrocytes in Wt and uPA<sup>-/-</sup> but not uPAR<sup>-/-</sup> mice during the recovery phase from an acute ischemic stroke [60].

To investigate the effect of uPA/uPAR binding-induced astrocytic activation under hypoxic/ischemic conditions, the number of intact synaptic contacts was quantified with confocal microscopy in wt and uPA<sup>-/-</sup> neurons after 24 hours of recovery from 5 minutes of exposure to OGD conditions in the presence of wt or uPAR<sup>-/-</sup> astrocytes either maintained under hypoxic conditions or previously activated by exposure to 3 hours of OGD. These series of experiments showed that 5 minutes of OGD induces a significant decrease in the number of intact synaptic contacts without causing cell death, and that this effect is attenuated when wt but not uPA<sup>-/-</sup> neurons are recovered in the presence of wt but not uPAR<sup>-/-</sup>

astrocytes previously exposed to OGD conditions. Importantly, wt neurons failed to recuperate their synapses when they were recovered in the presence of either wt astrocytes maintained under normoxic conditions, or uPAR<sup>-/-</sup> astrocytes previously exposed to 3 hours of OGD. In summary, these experiments indicate that binding of uPA released from neurons to uPAR recruited to the astrocytic plasma membrane during the recovery phase from a hypoxic injury promotes the recovery of synapses in neurons that have suffered a hypoxic injury. Subsequent studies showed that this effect was also observed when wt mice were intravenously treated with ruPA following 60 minutes of tMCAO [60].

##### 4.6.2. UPA Promotes the Formation of the Tripartite Synapse

The term “tripartite synapse” conceptualizes the fact that astrocytes enter in direct contact with the synapse, and thus are capable of regulating synaptic function [63]. A further analysis of this concept reveals that astrocytes enter in contact with the synapse *via* activity-dependent formation of highly motile elongations known as Peripheral Astrocytic Processes (PAPs) [64]. Remarkably, it was found that uPAR is abundantly found in PAPs, and that the interaction between uPA and uPAR not only induces the rapid formation of these elongations, but also their direct contact with the synapse. It was also revealed that this effect is mediated by uPA/uPAR-induced local synthesis of ezrin in astrocytes. Furthermore, *in vitro* studies with neurons and astrocytes exposed to OGD conditions, and *in vivo* work with an animal model of cerebral ischemia indicate that binding to uPAR of either endogenous uPA or intravenously administered ruPA induces the formation of PAPs that upon entering in contact with the ischemic synapse promote their recovery [65].

## CONCLUSION

The studies discussed above indicate that the PA system plays a central role in the pathophysiological events that promote neuronal survival and neurorepair in the ischemic brain. More specifically, the evidence available to this date indicate that neurons release tPA during the acute stages of the ischemic injury, and uPA during the recovery phase. Significantly, while the release of tPA is a beneficial response aimed to protect the synapse from the deleterious effects of the acute ischemic injury, uPA promotes the recovery of those synapses that have been functionally and structurally damaged by the ischemic insult. In summary, the data examined here indicates that the PA system is a potential target for the development of a therapeutic strategy to protect the synapse (tPA) and promote its recovery (uPA/uPAR) in neurons that have suffered an acute ischemic injury.

## LIST OF ABBREVIATIONS

CNS	=	Central nervous system
DIV	=	Days <i>in vitro</i>
dMRI	=	Diffusion magnetic resonance imaging
Fa	=	Fractional anisotropy
GFAP	=	Glial fibrillary acidic protein
MD	=	Mean diffusivity of water
OGD	=	Oxygen and glucose deprivation

PA	= Plasminogen activation
PAPs	= Peripheral astrocytic processes
tMCAO	= Transient middle cerebral artery occlusion
tPA	= Tissue-type plasminogen activator
uPA	= Urokinase type plasminogen activator
uPA <sup>-/-</sup>	= Genetically deficient in uPA
uPAR	= Urokinase-type plasminogen activator receptor
uPAR <sup>-/-</sup>	= Genetically deficient in uPAR
wt	= Wild-type

## CONSENT FOR PUBLICATION

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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