PERSPECTIVE

# Urokinase-type plasminogen activator promotes synaptic repair in the ischemic brain

The central nervous system has a very high energy requirement. Accordingly, despite representing only 2% of the body's mass, the brain uses 20% of the total oxygen consumption. Importantly, because most of this energy is used to maintain synaptic activity, even a mild decrease in its supply to the brain has deleterious implications for synaptic function. For example, only one minute of interruption of the cerebral blood flow during an acute ischemic stroke (AIS) is enough to destroy approximately 14 billion synapses (Saver, 2006). Importantly, because synaptic dysfunction leads to functional impairment, cerebral ischemia is one of the leading cause of disability in the world. Unfortunately, to this date there is no effective therapeutic strategy to promote neurological recovery among AIS survivors. Here we will discuss recent data indicating that binding of the serine proteinase urokinase-type plasminogen activator (uPA) to its receptor (uPAR) promotes synaptic repair in the ischemic brain, and will argue that this discovery has significant translational implications for the treatment of the rapidly growing number of patients that survive an AIS.

**The uPA/uPAR system:** uPA is a serine proteinase assembled by an amino-terminal domain homologous to the epidermal growth factor that harbors the binding site to uPAR, a kringle domain that contains a sequence that interacts with plasminogen-activator inhibitor-1 (PAI-1), and a catalytic carboxylic-terminal that has the protease domain assembled by the specific amino acids triad His204, Asp255 and Ser356. uPAR is a glycosylphosphatidylinositol (GPI)-anchored protein assembled by three domains, D1, D2 and D3, with high affinity for uPA, pro-uPA (the inactive single-chain protein), and uPA's amino terminal factor (ATF, formed by the first two domains of uPA).

uPA binding to uPAR on the plasma membrane regulates the activity of the plasminogen activation system on the cell surface by a sequence of carefully orchestrated steps that begin with the binding of pro-uPA and uPA to uPAR, and is followed by uPA-catalyzed conversion of plasminogen into plasmin, and plasmin-induced cleavage and activation of pro-uPA (Smith and Marshall, 2010). However, despite the importance of these events, a growing body of experimental evidence indicates that besides catalyzing the conversion of plasminogen into plasmin on the cell surface, uPA binding to uPAR also activates intracellular signaling pathways including the Ras-mitogen-activated protein kinase (MAPK), the Tyr kinases focal adhesion kinase (FAK) and Src, and the Rho family of small GTPases (Blasi and Carmeliet, 2002).

Significantly, because uPAR is a protein anchored to the external surface of the plasma membrane, it needs to interact with transmembrane co-receptors to activate intracellular signaling pathways. In line with this observation, a growing number of co-receptors for uPAR have been identified over the last two decades, including integrins, the low-density lipoprotein receptor-related protein-1 (LRP1), ENDO180, platelet-derived growth factor receptor-β (PDGF-β), and epidermal growth factor receptor (EGFR) (Smith and Marshall, 2010).

**uPA and uPAR in the brain:** The expression of uPA in the brain varies with the developmental stage. Hence, while all identifiable neurons express uPA during the early phases of development (Dent et al., 1993), in the adult brain only well circumscribed groups of neurons in the hippocampus and other subcortical areas are immunoreactive for this protease (Sappino et al., 1993). Interestingly, with exception of oligodendrocytes, glial cells do not express uPA (Sumi et al., 1992; Dent et al., 1993). The expression of uPAR also varies with development, and whereas this receptor is found in all neuronal extension and in astrocytes during early developmental stages, its presence in the adult brain is detected only in subgroups of astrocytes and few growth cones and dendritic spines (Wu et al., 2014; Diaz et al., 2017).

It has long been recognized that remodeling, stress, injury and inflammation increase the expression of uPAR (Smith and Marshall, 2010). In line with these observations, we found that although during the acute stages of an ischemic injury the expression of uPA and uPAR are almost undetectable, during the recovery phase neurons but not astrocytes release uPA, and astrocytes recruit uPAR to their plasma membrane (Wu et al., 2014; Diaz et al., 2017). These data strongly suggest the existence of an uPA/uPAR-mediated neuron-astrocyte cross talk during the recovery phase from an acute ischemic injury.

The role of uPA binding to uPAR during development has been extensively characterized, and several studies have demonstrated that uPA promotes neuritogenesis, neuronal migration, axon growth and neurite branching (Semina et al., 2016). In contrast, the biological effect of uPA/uPAR binding in the adult brain is less well understood. However, our *in vivo* and *in vitro* studies indicate that binding of neuronal uPA to astrocytic uPAR promote synaptic repair and improvement in neurological function following an AIS (Wu et al., 2014; Merino et al., 2016; Diaz et al., 2017). The translational relevance of these observations is underscored by data indicating that binding to uPAR not only of endogenous uPA but also of intravenously administered recombinant uPA (ruPA) promotes neurorepair in the ischemic brain (Wu et al., 2014; Merino et al., 2016), thus indicating that intravenous treatment with ruPA may be a potential therapeutic strategy to promote neurological recovery in patients that have survived an AIS.

**Astrocytic activation: the first step of uPA-induced synaptic repair:** A large body of experimental evidence indicates that astrocytes not only modulate neurotransmission and provide nutrients and structural support to the brain, but also that they play a central role in neurorepair after an ischemic injury. Indeed, we have found that hypoxia promotes the recruitment of uPAR to the astrocytic plasma membrane and that uPA/uPAR binding induces cellular hypertrophy and a series of well-defined genetic and biochemical changes known as astrocytic activation (Pekny and Pekna, 2014; Diaz et al., 2017). Furthermore, our data indicate that the effect of uPA on astrocytic activation is mediated by extracellular signal-regulated kinase 1/2 (ERK 1/2) activation, and does not require plasmin generation, as it is also observed upon treatment with uPA's ATF, which due to its lack of a proteolytic domain is unable to catalyze the conversion of plasminogen into plasmin.

Astrocyte activation can be promoted by several signaling pathways that converge on the signal transducer and activator of transcription 3 (STAT3), a transcription factor that controls the expression of several astrocytic phenotypic markers. We found that uPA binding to uPAR induces ERK 1/2-regulated STAT3 activation, and that ERK1/2-modulated activation of this transcription factor mediates the effect of uPA on astrocytic activation.

The effect of astrocytic activation in the ischemic brain has been debated. Indeed, although it was originally believed that astrocytic activation has a deleterious effect in the ischemic brain, recent studies with genetically modified mice indicate that this may not be the case, and that astrocytic activation may instead play a protective role in neurons that have suffered an acute ischemic insult (Li et al., 2008). These data agree with our observations that uPA binding to uPAR promotes both, astrocytic activation and synaptic recovery in the ischemic brain. Furthermore, because neurons but not astrocytes release uPA during the recovery phase from an ischemic injury, these results also suggest a model in which owning to their ability to release uPA after an acute injury, wounded neurons are capable to induce astrocytic activation around the injured synapse, and once there activated astrocytes release a soluble factor that promotes synaptic recovery.

**uPA and the tripartite synapse:** The term "tripartite synapse" conceptualizes the fact that the intimate contact between neurons and astrocytes in the brain allows astrocytes to have a direct effect on synaptic function. Thus, based on this observation and our data, we proposed a model in which binding of uPA released by an injured neuron to uPAR recruited to the plasma membrane of activated astrocytes has a direct effect on the synapse that has suffered an acute hypoxic injury. To test this hypothesis, we exposed wild-type (Wt) neurons to 5 minutes of oxygen and glucose deprivation (OGD; our previous studies indicate that this period of OGD is enough to cause a significant decrease in the number of synaptic contacts without inducing cell death). Simultaneously, we exposed Wt astrocytes to 3 hours of OGD to induce the recruitment of uPAR to their plasma membrane. Then we recovered those neurons exposed to 5 minutes of OGD in the presence of astrocytes either previously activated

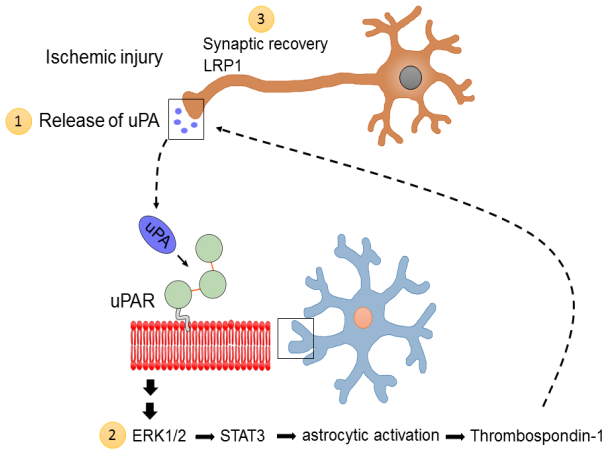


**Figure 1 Schematic representation of urokinase-type plasminogen activator (uPA)-urokinase-type plasminogen activator receptor (uPAR) induced synaptic recovery in the ischemic brain.**
Binding of uPA released by neurons to uPAR recruited to the astrocytic plasma membrane during the recovery phase from an acute ischemic injury induces extracellular signal-regulated kinase 1/2 (ERK 1/2)-mediated signal transducer and activator of transcription 3 (STAT3)-regulated astrocytic activation. Activated astrocytes release thrombospondin-1 (TSP-1) which upon binding to lipoprotein receptor-related protein-1 (LRP1) in the synapse promote synaptic recovery.

by exposure to 3 hours of OGD or maintained under physiological conditions. Twenty-four hours later the number of intact synaptic contacts was quantified in each experimental group. We found that incubation with astrocytes previously activated by OGD, but not with astrocytes kept under normoxic conditions, induces the recovery of synaptic contacts in neurons that have been exposed to an acute hypoxic injury. Importantly, this effect was not observed when neurons were exposed to astrocytes genetically deficient on uPAR, or when Wt astrocytes were added to neurons genetically deficient on uPA. In summary, these observations suggest a model in which binding of uPA released by neurons to uPAR recruited to the plasma membrane of astrocytes promotes the recovery of synapses of neurons that have suffered an acute hypoxic injury. To determine the *in vivo* relevance of these observations, we quantified the number of intact synaptic contacts in the brain of Wt and *Plat*$^{GFDhu/GFDhu}$ mice (in which a 4 aminoacids substitution in uPA's growth factor domain precludes its biding to endogenous uPAR while preserving other functions of the protease and its receptor) 24 hours after 30 minutes of transient occlusion of their middle cerebral artery. Our data show that binding of endogenous uPA to uPAR promotes synaptic recovery in the ischemic brain (Diaz et al., 2017).

**Astrocytic thrombospondin-1 (TSP-1) and synaptic low-density lipoprotein receptor-related protein-1 (LRP1) mediate uPA-induced synaptic repair:** Thrombospondins (TSPs) are large conserved extracellular glycoproteins that mediate interactions between the intracellular space and the extracellular. TSPs have several functions including wound healing, angiogenesis, and synaptogenesis. Interestingly, TSP-1, one of the five TSPs found in mammalians, induces synaptogenesis and is produced by astrocytes *via* STAT3 activation. In line with these observations, we found that uPA increases the abundance of astrocytic TSP-1, and that TSP-1 blocking antibodies abrogates the synaptic reparative effect of activated astrocytes (Diaz et al., 2017). Together, these data suggest a model where astrocytes release TSP-1 in response to uPA binding to uPAR. Because LRP1 binds to the N-terminal domain of TSP-1, then we postulated that LRP1 is the receptor for astrocytic TSP-1 that promotes neurorepair in the injured synapse. Our hypothesis was confirmed by the observation that LRP1 is abundantly found in the synapse, and that LRP1 antagonism with the receptor-associated protein (RAP; a chaperone that antagonizes the binding of LRP1 to its ligands abrogates the synaptic protective effect of uPA.

**Proposed model of uPA-induced synaptic recovery in the ischemic brain:** Based on the data discussed above, we propose a model in which during the recovery phase from an acute ischemic injury neurons release uPA and astrocytes recruit uPAR to their plasma membrane. Our results indicate that binding of neuronal uPA to astrocytic uPAR promotes ERK 1/2-mediated STAT3-regulated astrocytic activation, and that activated astrocytes release TSP-1 which upon it binding to LRP-1 promote synaptic recovery of neurons exposed to an acute hypoxic/ischemic injury. More importantly, our data indicate that treatment with ruPA may be an effective therapeutic strategy to promote synaptic recovery and functional improvement in the ischemic brain (**Figure 1**).

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