Neurovascular disease

The story of an exceptional serine protease, tissue-type plasminogen activator (tPA)

L’histoire d’une sérine protéase exceptionnelle, l’activateur tissulaire du plasminogène

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ABSTRACT

The only acute treatment of ischemic stroke approved by the health authorities is tissue recombinant plasminogen activator (tPA)-induced thrombolysis. Under physiological conditions, tPA, belonging to the serine protease family, is secreted by endothelial and brain cells (neurons, astrocytes, microglia, oligodendrocytes). Although revascularisation induced by tPA is beneficial during a stroke, research over the past 20 years shows that tPA can also be deleterious for the brain parenchyma. Thus, in this review of the literature, after a brief history of the discovery of tPA, we reviewed current knowledge of mechanisms by which tPA can influence brain function in physiological and pathological conditions.

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RÉSUMÉ

Le seul traitement aigu autorisé de l’accident vasculaire cérébral ischémique est la thrombolysy par l’activateur tissulaire du plasminogène recombinant (tPA). Le tPA est une sérine protéase sécrétée de manière physiologique par les cellules endothéliales et cérébrales (neurones, astrocytes, microglie, oligodendrocytes). Bien qu’il existe un effet bénéfique du tPA dans le cadre de la revascularisation cérébrale après AVC ischémique, les recherches de ces 20 dernières années montrent que le tPA peut-être également délétère pour le parenchyme cérébral. Ainsi, dans cette revue de la littérature, après un bref rappel historique sur la découverte du tPA, nous passerons en revue les connaissances actuelles des mécanismes par lesquels le tPA peut influencer le fonctionnement cérébral en conditions physiologique et pathologique.

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1. The history of tPA

In ancient Greece, Hippocrates had already observed that blood from dead people was not clotted. It is in 1844 that Andral put forward the idea that clotted blood can become again liquid, giving to the scientific community the first data on the fibrinolytic system [1]. In 1893, Denys & Marbaix and Dastre determined that this process was the consequence of a proteolytic mechanism that they name fibrinolysis (for fibrin and lysis) [2,3]. In 1902, Conradi brought the first observations of degradation of blood clots by adding extracts of different organs [4]. In 1904, Hedin identified a proteolytic activity in the serum globulin fraction, that was later identified as the fraction containing the plasminogen, the precursor of the enzyme responsible of the fibrin clot lysis, plasmin [5]. This inactive precursor plasminogen presents in the circulation can be activated by bacterial extracts (the proteins involved in this process were identified later as streptokinase and staphylokinase). This observation was later confirmed and refined by Fleisher & Loeb in 1915 and by Astrup in 1947, providing the concept of thrombolysis (degradation of pre-formed clots) [6,7]. It was only in 1979 that the effector of this cascade of events, tissue-type plasminogen activator (tPA) was purified and characterized first in circulation and then in the uterus [8,9]. The first use in humans was conducted in 1981 in Rotterdam by W. Weimar on patients with thrombosis of the renal vein. This led, in 1983, to the cloning of tPA and its recombinant production [10]. 32 years later, tPA is now used in clinic to promote fibrinolysis, especially at the acute phase of ischemic stroke either alone [11] or combined with thrombectomy [12]. In parallel of its well-known roles in the circulation, tPA also displays critical functions in the brain parenchyma. Here, we reviewed our present knowledge of the mechanisms and functions of tPA in the central nervous system (CNS), both in physiological and pathological conditions.

2. Structure and functions of tPA

tPA is a glycoprotein belonging to the superfamily of serine proteases and is a member of the chymotrypsin family. In 1983, Pennica has cloned and produced a functional recombinant tPA, composed of 527 amino acids, with a molecular mass of 70 kDa. The access to the tPA nucleotide sequence has allowed the identification of potential glycosylation sites and disulfide bridges, and therefore the determination of its three dimensional structure [10]. tPA is a protein composed of five domains, maintained in its conformation thanks to 17 disulfide bridges (Fig. 1A). The N-terminal end of tPA starts by a finger domain (also called fibronectin domain, Fig. 1A–C). This domain is involved in the binding of tPA to fibrin, resulting in the formation of a ternary complex with plasminogen [13]. Through this finger domain tPA can also interacts with several membrane receptors including the Low Density Lipoprotein Receptor-related Protein (LRP) [14] and Annexin II [15]. The second domain, called epidermal growth factor-like domain, due to its homology with the epidermal growth factor (EGF, Fig. 1A–C), allows tPA to activate EGF receptor [16]. The sequence continues by the Kringle 1 and the Kringle 2 domains (respectively: K1 and K2, Fig. 1A–C). These domains are characterized by an active site having a high affinity for lysine (lysine binding site; LBS), which is composed of two hydrophobic amino acids (Trp242 and Trp253) forming a pocket in the tertiary structure of the protein. The precise role of the K1 domain is not well known. Although its LBS domain is not functional, the glycosylation at the Asp117 is important for the uptake-clearance of tPA by liver endothelial cells via the mannose receptor [17]. The K2 domain contains a functional LBS. The K2 domain is reported to be involved in the capacity of tPA to bind and activate substrates and/or receptors such as plasminogen, the PDGF-CC (Platelet Derived Growth Factor-CC) [18] and the NMDAR (N-methyl-D-Aspartate Receptor) [19,20]. All these domains (Finger, EGF, K1 and K2) form the heavy chain of tPA (A-chain). The second chain, the light chain (B-chain), consists of a single large domain containing the catalytic activity of the protease (Fig. 1A–C). The catalytic triad is composed of the amino acids His322, Asp71 and Ser478 [10,21], that enables the activation of plasminogen to plasmin. Like all serine proteases, tPA exists in two forms: single chain tPA (sc-tPA) and two chains (tc-tPA). In contrast to the other known serine proteases, which are inactive under their single chain form, the two forms of tPA are proteolytically active [22] (Fig. 1D). The processing of sc-tPA into tc-tPA is mediated by other proteases such as plasmin [23] or kallikreins [24,25]. In the absence of an allosteric regulator such as fibrin, tc-tPA is catalytically more active than sc-tPA [23,26]. However, in the presence of fibrin, both sc-tPA and tc-tPA display the same catalytic or fibrinolytic activity [27].

3. Cellular localization of tPA in the brain

tPA was primarily identified in the blood circulation [8,28]. In this compartment, it is mainly produced and released by endothelial cells [29]. In the brain parenchyma, tPA has been reported to be expressed and synthesized by most of the cell types: astrocytes [30], neurons [31] (Figs. 2A and 3), oligodendrocytes [16] and microglia [32]. In neurons, the presence of tPA can be observed in dendrites and synapses [33], where tPA is stored in pre-synaptic vesicles [34] and released in the synaptic cleft following a depolarization [35] (Fig. 2A). Astrocytes are capable to regulate the quantity of tPA at the synaptic cleft. Indeed, tPA can be endocytosed by astrocytes involving its Finger domain and mediated by LRP receptors [36] (Fig. 2A). Interestingly, astrocytes are also capable to release the tPA previously recaptured by a mechanism involving PKC through a mechanism dependent of the activation of kainate receptors by the glutamate present at the synaptic cleft [36] (Fig. 2A).

4. tPA inhibition in the brain

Only few studies have correlated the levels of tPA mRNA with its enzymatic activity in the brain parenchyma. For example, although tPA mRNA can be detected in the entire hippocampus, no proteolytic activity can be found in the CA1 region [37]. This strongly suggests the presence and differential expression of
inhibitors of tPA proteolytic activity in these regions: the serine proteases inhibitors (serpins). Currently, four serpins have been described to inhibit tPA proteolytic activity: type 1 and 2 plasminogen activator inhibitor (PAI-1 and -2), neuroserpin (NS) and the protease nexin-1 (PN-1) [38,39]. In the brain parenchyma, PAI-1 and NS are the two main inhibitors of tPA (Fig. 2B). PAI-1 is a suicide inhibitor that forms irreversible complexes with tPA [40]. In the brain parenchyma, PAI-1 is expressed at very low levels by neurons and astrocytes under physiological conditions [30,41], but dramatically over-expressed by reactive astrocytes [42,43]. In contrast of PAI-1, NS is highly expressed under physiological conditions by both neurons and astrocytes [38,42]. NS also acts as a substrate of tPA but without formation of an irreversible protease-serpin complex, as previously reported for tPA and PAI-1[44]. In the brain parenchyma, NS mRNA are detected from the early embryonic to adult stages, and are widely distributed in numerous brain structures [45]. Interestingly, accumulation of NS in neuronal bodies is associated with a familial encephalopathy, named FENIB (Familial Encephalopathy with Neuroserpin Inclusion Bodies), due to the presence of a mutated form of NS [46]. NS have been also described to play a role in the etiology of epilepsy and schizophrenia [47,48].

5. tPA-dependent functions in the brain parenchyma

5.1. Development and cellular migration
tPA is highly expressed in embryonic regions undergoing cell migration and tissue remodeling (Fig. 4). For example, Krystosek and Seed evidenced that tPA was secreted by granule neurons in the cerebellum during development [49]. In this structure, the expression of tPA mRNA and tPA proteolytic activity closely correlate with the neuronal migration phase [50,51] (Fig. 4). Accordingly, mice lacking tPA display a delayed neuronal migration when compared with their wild type littermates [52]. A recent study also showed that Pituitary adenylate cyclase-activating polypeptide (PACAP) induces tPA release implicated in the extracellular matrix (ECM) degradation, during the neuronal migration [53].

5.2. Long-term potentiation
Chemical or high frequency stimulation of hippocampal neurons led to an increase of synaptic transmission efficiency.
This phenomenon, initially discovered in the hippocampus and called long-term potentiation [54] (LTP), is considered as the molecular and cellular support of learning and memory processes [55]. The induction of LTP on hippocampal slices leads to a rapid increase of tPA mRNA levels in granular cells of the dentate gyrus [56]. On hippocampal slices, administration of tPA inhibitors (PAI-1 or tPA stop) was reported to inhibit the late phase of LTP either induced by forskolin or a tetanic stimulation [57]. In addition, tPA deficient mice present an altered late LTP in CA1 [58], based on modification of the GABAergic neurotransmission [59], tPA-dependent ECM degradation [57], its ability to activate the neurotrophic factor proBDNF into mBDNF [60] and to cleave reelin [61], are three putative mechanisms through which tPA could contribute to synaptic plasticity and tissue remodeling. Interestingly, only the sc-tPA is capable to increase the late phase of LTP, a mechanism dependent of NMDAR [62] (Fig. 3).

5.3 Behavioral processes

According to its localization in the hippocampus, and to its involvement in synaptic plasticity, tPA has been described as an important actor of spatial learning (results summarized in Fig. 4 and Table 1). When tested in an object recognition task, tPA deficient mice display an impairment to react to a spatial configuration change, without impairment of the detection of non spatial changes [63]. Similarly tPA deficient mice show impaired spatial learning abilities in a two-trial place recognition task in a Y-Maze [64], as well as in a Morris water maze task [65]. In the hippocampus, the interaction between tPA and the NMDA receptor seems to be the key mechanism by which tPA play its role in spatial learning [64,66].

tPA is also expressed in the amygdala and in the Bed Nucleus of the Stria Terminalis (BNST) [67–69]. According to these localizations, tPA plays roles in stress response, anxiety and learned fear (results summarized in Table 1). For instance, tPA is involved in the acquisition and retention of a contextual and/or cued learned fear, even if some results found in the literature are sometimes ambiguous [58,63,70]. tPA is also involved in the mediation of stress effects. Although restraint stress or Corticotrophin-Releasing Factor-induced response lead to an exacerbation of anxiety-like behaviors in wild type animals, all these effects are abrogated in tPA deficient mice [67–69]. Accordingly, NS deficient mice display an increased anxiety-like behavior, assessed using a zero-maze task, compared to their wild-type littermates [71].
5.4. Neuronal death and survival

Several studies have reported that neuroserpin and PAI-1 protected neurons against NMDARs over-activation-induced toxicity [30,72,73]. Accordingly, exogenous tPA has been reported to induce excitotoxic neuronal death mediated by over-activation of NMDARs [74,75] (Figs. 3 and 4), by plasmin-dependent or -independent mechanisms [62,74,76]. For instance, tPA is capable to interact with the GluN1 subunit of NMDAR via its LBS localized on the K2 domain [19,20]. This interaction allows the cleavage of the amino-terminal domain of the GluN1 subunit, leading to the enhancement of NMDAR signaling by sc-tPA [74,77]. However, other authors did not detect such tPA-mediated cleavage of the GluN1 subunit, despite enhancement of NMDAR function by exogenous tPA in cortical cultures [78]. In addition, LRP receptors could act as tPA co-receptors, which in turn enhance Ca^{2+} influx through NMDARs [78]. Interestingly, in response to tPA, LRP1 has been described as able to assemble a co-receptor system to initiate cell-signaling; this system is composed of LPR1, NMDAR, and TrkB receptors [79].

However, it is interesting to note that, in addition to its role in excitotoxic neuronal death, both in vivo, in vitro and ex vivo studies also suggest that tPA may have pro-survival and anti-apoptotic effects on both neurons and oligodendrocytes [16,80–84] (Fig. 3). Two candidates have been proposed as receptors mediating the pro-survival effects of tPA: Annexin II and EGF receptor (EGFR) [16,81,85] (Fig. 3). Despite the heterogeneity of the paradigms used, most of these studies propose that these trophic effects of tPA occurs independently of its proteolytic activity, with the activation of either PI3 K/ Akt-, AMPK- or mTor-HIF-1alpha-dependent signaling pathways [16,86].

Although the deleterious effects of tPA have been extensively reported from animal models (especially its neurotoxicity), it is still a debate in human. Only few papers have reported indirect proofs of this neurotoxicity. For example, in 2015 Alvarez showed that thrombolysis with rt-PA may increase the likelihood of epileptic seizures at the acute phase of ischemic stroke patients, independently of the recanalization rate or of symptomatic intracerebral hemorrhages [87]. This paper supports previous work from Tsirka et al. in 1995 demonstrating in preclinical models that tPA promoted seizures [88]. Tisserand also reported, in human, that a tPA treatment was associated with increased lesion volumes in the grey matter and reduced damages in the white matter following cerebral ischemia [89]. These observations are also supported by data obtained from experimental models of stroke [16].
Fig. 4 - tPA, a multifaceted protease with beneficial and deleterious effects. In the green circle are summarized the principal beneficial effects of endogenous/exogenous tPA in physiological situation (on cerebral development and on the regulation of behavioral processes) but also in pathophysiological situation like in stroke, Alzheimer disease or multiple sclerosis. Conversely, in the red circle are summarized the main adverse effects of tPA in pathological conditions such as in stroke and multiple sclerosis. A.β: β-amyloid peptide. BBB: Blood-Brain Barrier; rtPA: recombinant tPA. Numbers in brackets refer to references.

Up to now, there is no clear clinical data to determine, in human, whether tPA is neurotrophic or neurotoxic. Additional studies are needed to further understand the possible differential functions of endogenous versus exogenous tPA on neuronal survival.

5.5. tPA and the homeostasis of the Blood Brain Barrier

In experimental models, the intravenous administration of rtPA 4 hours post-ischemia results in permeabilization of the BBB [73]. The alterations of the integrity of the BBB induced by tPA are due to different molecular mechanisms involving LRP receptors, NMDAR and metalloproteinases (MMPs) (Fig. 3). MMPs, including MMP-9, are overexpressed after a cerebral ischemia [90], leading to an increased degradation of type IV collagen, laminin and fibronectin [91]. In 2008, Cuadrado showed that neutrophils are the main source of MMP-9 following stroke and tPA treatment, a mechanism involved in the permeabilization of the BBB and in the occurrence of hemorrhagic transformations [92]. By interacting with the LRP receptors and subsequent cleavage of their ectodomain, tPA promotes the removal of astrocytic feet from the basal lamina and permeabilization of the BBB [83]. tPA is also capable to interact with and activate PDGF-CC, a mechanism dependent of both its Kringle2 domain and its catalytic activity. Then, cleaved PDGF-CC activates PDGFR-α (Platelet Derived Growth Factor Receptor-alpha) of perivascular astrocytes promoting BBB leakage and bleeding [18,23] (Fig. 3). Accordingly, coinjection of tPA with an inhibitor of PDGFR-α receptors (Imatinib, Glivec) significantly reduced rtPA-induced hemorrhagic transformations [94].

Hemorrhagic transformation induced by rtPA is the most feared complication (Fig. 4). As mentioned above, the state of the BBB and the delay before treatment seems to play a critical role on the intra-cerebral haemorrhage (Fig. 4). Majority of works that have studied the haemorrhagic transformation with intravenous tPA performed within 3 h confirmed the rate of bleeding observed in the NINDS study: 6.4% of patient showed a haemorrhagic transformation [11]. Others important studies showed and confirmed that as compared to placebo, rtPA was more frequently associated with symptomatic intracranial hemorrhage when thrombolysis is performed between 3 and 4.5 hours after the onset of symptoms. [95,96] (Figs. 3 and 4).

5.6. tPA and inflammation

In vivo, a late i.v. administration of rtPA (4 hours post-occlusion) is associated, 24 hours post-ischemia, with an overexpression of cellular adhesion molecule E- and P-selectin, ICAM-1 which are involved in leukocyte infiltration (Fig. 4).

Compared to rtPA alone, rtPA administration combined with the ICAM-1 inhibition reduces lesion volume (28.9 ± 2.7% versus 39.1 ± 3.9%; P < 0.05) [97]. Similarly, the combined injection of statins and rtPA 4 hours post-ischemia inhibits the expression of MMP-9, PAR-1 and ICAM-1 and reduced the lesion volume [98]. The interaction of the finger domain of tPA with annexin II [85] or LRP-1 microglial receptor [99] activates microglia, inducing the production of NO and secretion of tPA by activated microglia [32] (Fig. 3). These processes contribute to the worsening of brain damage. Thus, in ischemic conditions tPA promotes the inflammatory response but is
also beneficial for stimulating both macrophage migration on the sites of axonal degeneration and MMP-9 expression, that promotes axonal regrowth [100] (Figs. 3 and 4).

Targeting brain inflammation could be an interesting therapeutic strategy. However, inflammation processes are necessary to eliminate cell debris and to fight against infections, their uncontrolled inhibition could thus be deleterious [101].

### 5.7. Role in axonal damage, myelinisation and regeneration

tPA was reported protective against axonal damage and to favour axonal regeneration through the proteolytic removal of fibrin deposits in inflammatory conditions [102] (Fig. 4). In neuroinflammatory conditions such as multiple sclerosis (MS), efficient fibrin removal is impaired, and this is associated with a decreased expression of tPA and an increased expression of PAI-1 [103,104]. In experimental autoimmune encephalitis (EAE, a mouse model of MS), tPA deficient mice show more severe symptoms and impaired recovery [105,106], whereas PAI-1 deficient mice show a delayed onset and less severe symptoms [107]. More specifically, tPA action on axonal regeneration is thought to involve the degradation of chondroitin sulphate proteoglycans (CSPGs), a set of ECM proteins with inhibitory action on axon regrowth both in vitro and in vivo [108–110]. Thus, the combination of tPA with chondroitinase ABC promotes axonal regeneration in experimental models of spinal cord injury (SCI) [108]. In addition, tPA has been shown to activate the CSPG-degrading protease ADAMTS-4 (a desintegrin and metalloprotease with thrombospondin domains-4) following SCI, thus promoting axonal growth and functional recovery [109]. It is also interesting to note that tPA, through its EGF domain, was reported to display oligotrophic functions, promoting survival of oligodendrocytes, thus reducing white matter damages following cerebral ischemia [16] (Figs. 3 and 4).

### Table 1 – Roles of tPA in behavioral processes. Numbers in brackets refer to references.

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5.8. tPA and aging

During physiological aging, the cerebral proteolytic activity of tPA decreases [111–113]. Such modifications of the levels of tPA in the brain during physiological aging have functional consequences. For example, in the hippocampus this decrease of tPA levels leads to spatial memory impairments [113]. Interestingly, there is numerous links existing in the literature between the tPA/plasminogen system, tPA inhibitors and Alzheimer Disease (AD; Fig. 4) including activity on A-β peptide burden and degradation (Fig. 4). For instance, plasmin has been shown to promote Aβ degradation [114–116]. In AD, a dramatic increase of PAI-1 was reported, produced by glial cells, thus worsened the decrease of tPA proteolytic activity occurring during physiological aging [111,117,118] (Fig. 2). During cerebral ischemia, the decrease of tPA with physiological aging is associated with reduced ischemic lesion volumes in the grey matter and worsened alterations of the white matter [16,112].

6. Conclusions and perspectives

Tissue-type plasminogen activator (tPA) is an extracellular proteolytic enzyme that was first described for its effects on blood coagulation and extracellular matrix homeostasis. However, during the last twenty years, tPA has been shown to have numerous functions in brain physiology and pathology (Fig. 4). tPA can act on virtually all cell types of the brain, including neurons, endothelial and glial cells. tPA conducts its actions by enzymatic or growth-factor-like effects on various molecular substrates or receptors. Its first described action was the conversion of the zymogen plasminogen into the active enzyme plasmin, but tPA has since been discovered to drive multiple and sometimes even opposite effects by interacting with or activating BDNF, NGF, PDGF-CC Annexin II, LRP, or NMDAR (Fig. 3). It is therefore often referred to as a ‘double-edged sword’. A lot of interest has been therefore given in the last years to the potential clinical relevance of targeting tPA for neuroprotection or modulation of neuronal plasticity in different diseases of the brain such as stroke, multiple sclerosis or Alzheimer’s disease. Thus, a challenging question is whether these effects of tPA, including neurotoxicity, can be exerted by exogenous recombinant tPA when injected to stroke patients in an attempt to achieve reperfusion. New therapeutic strategies for stroke are in development. For example, safer thrombolytics have been produced [20,62]. Antibodies have been also developed to prevent the interaction of tPA with NMDAR capable to extend the therapeutic window of thrombolysis when injected 4 hours after stroke onset in mice [119]. Other approaches such as a direct intra-arterial endovascular reperfusion of rtPA are also promising [120]. Future therapeutic strategies for AD could emerge via the manipulation of tPA and plasmin activity in order to promote the degradation of Aβ. In multiple sclerosis whether tPA can help the axonal regeneration or remyelination is also an opened avenue. Overall, further studies should provide a better understanding of the complex effects of tPA and its endogenous regulators. This should enable a better discrimination between the different actions of tPA. With regard to the involvement of tPA in brain diseases, investigation on new treatments for brain pathologies should gain from these future progresses.

Disclosure of interest

The authors declare that they have no competing interest.

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