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The plasminogen activation system in neuroinflammation☆

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ABSTRACT

The plasminogen activation (PA) system consists in a group of proteases and protease inhibitors regulating the activation of the zymogen plasminogen into its proteolytically active form, plasmin. Here, we give an update of the current knowledge about the role of the PA system on different aspects of neuroinflammation. These include modification in blood–brain barrier integrity, leukocyte diapedesis, removal of fibrin deposits in nervous tissues, microglial activation and neutrophil functions. Furthermore, we focus on the molecular mechanisms (some of them independent of plasmin generation and even of proteolysis) and target receptors responsible for these effects. The description of these mechanisms of action may help designing new therapeutic strategies targeting the expression, activity and molecular mediators of the PA system in neurological disorders involving neuroinflammatory processes. This article is part of a Special Issue entitled: Neuro Inflammation edited by Helga E. de Vries and Markus Schwaninger.

1. Introduction

The plasminogen activator (PA) system refers to a group proteins involved in the regulation of the activation of the zymogen plasminogen to the active serine-protease plasmin. This includes the two plasminogen activators (tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA)) and a series of inhibitors of their activity, principally plasminogen activator type 1 (PAI-1) and neuroserpin (NS) (Fig. 1).

The PA system has two classical functions: the regulation of extracellular matrix (ECM) degradation in most tissues, and the regulation of fibrinolysis in the bloodstream. In the ECM, plasmin activates the pro-forms of matrix metalloproteases to their active form, which are able to degrade ECM components. This function is important for prevention of fibrosis, but also in migration and cell process growth, in which ECM is a physical obstacle. In the bloodstream, plasmin directly cleaves fibrin into fibrin degradation products. This function is important for the prevention of fibrin clot formation responsible for vascular occlusions and is also the basis of thrombolysis (i.v. injection of recombinant tPA), the only approved pharmacological treatment of ischemic stroke.

Neuroinflammation is a common feature to several CNS diseases such as stroke, Alzheimer's disease and multiple sclerosis (MS). A neuroinflammatory response occurs from the acute phase of ischemic stroke and is characterized by the production of inflammatory mediators (cytokines, chemokines and adhesion molecules), overt blood brain barrier leakage and leukocyte entry to the CNS (early neutrophil infiltration, followed by monocytes). Alzheimer's disease is considered a chronic neuroinflammatory disease, in which misfolded and aggregated proteins trigger microglial activation, leading to the release of inflammatory mediators which contribute to disease progression. Neuroinflammation is also a key feature in the pathology of multiple sclerosis: adhesion molecules and inflammatory cytokines lead to the infiltration of neutrophils, monocytes and lymphocytes through an altered blood–brain barrier, which trigger autoimmune attack against myelin.

In the recent years, the spectrum of actions of the PA system has extended far beyond these classical functions. In particular, the PA system has been reported to influence several cellular and molecular determinants of inflammation, making it a key player in the regulation of neuroinflammation. Because tPA is the active molecule used for thrombolysis in stroke, part of the studies presented here have addressed the effects of the PA system on neuroinflammation in the context of stroke. However, the different actors of the PA system can also display unforeseen effects, some of them independent of the activation of plasminogen, in a more general context of neuroinflammation, with implications in other pathologies, such as multiple sclerosis or Alzheimer's disease. In this review, we will explain how the PA system acts on several aspects of inflammation, such as blood–brain barrier function, leukocyte diapedesis, intraparenchymal fibrinolysis, microglial activation or neutrophil functions. We will focus on the cellular targets and the molecular mechanisms responsible for these effects.

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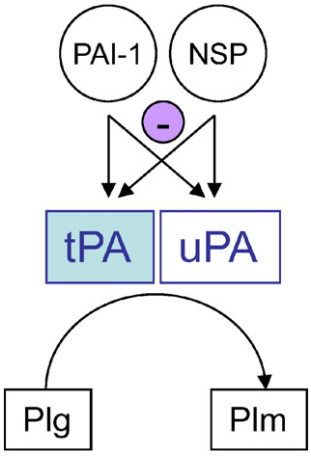

Fig. 1. The plasminogen activator system. Tissue-type- and Urokinase- plasminogen activators (tPA and uPA) activate the inactive zymogen plasminogen (Plg) into active plasmin (Plm). The activity of tPA and uPA is inhibited by serine proteases inhibitors (serpins), mainly Plasminogen activator inhibitor −1 (PAI-1) and neuroserpin (NS).

1.1. Sources of the different actors of the PA system in the brain

1.1.1. Tissue-type plasminogen activator (tPA)

The presence of a fibrinolytic/plasminogen converting activity in the brain was reported as early as in the 70's [1,2]. Ten years later, this activity was reported to in fact result from two actors, identified as being tPA and uPA [3].

Endothelial cell forming microvessels (<100 μm of diameter) are the main source of tPA in the brain [4,5]. However, virtually all cell types within the central nervous system are potential sources of tPA. The expression of tPA by neurons has been largely documented *in vitro* and *in vivo* [6,7,8,9]. The presence of tPA mRNA and protein/activity in cultured astrocytes is also well documented [7,9,10,11,12] but in a lesser extent *in vivo* [13,14]. Oligodendrocytes had formerly been shown to be devoid of tPA activity *in vitro* [15], but they have recently been shown to display a positive immunostaining for tPA *in vivo* [16].

The ability of microglia to produce tPA remains debated. Indeed, some authors reported the absence of tPA mRNA in microglia [16,7,10] while others detected it [8,17,18,19,20]. Similar controversial results exist regarding tPA protein and activity: for instance, cultured microglia display tPA activity [21], while *in vivo*, no tPA immunostaining is reported in microglia of the hippocampus [22]. A possible explanation for these discrepancies is that, as discussed later in this review, tPA expression, although low in basal conditions, could be induced in microglia during inflammatory processes.

Other sources of tPA in the central nervous system are perivascular mast cells [23], pericytes [24,25], infiltrating leukocytes [26]and blood-derived tPA. This highlights the role for tPA at the blood–brain interface. Indeed, tPA can cross the blood–brain barrier towards the injured brain and aggravate the extent of neuronal loss [27,28]. Low density lipoprotein receptor-related protein (LRP), a transmembrane protein involved in endocytosis, has been implicated in the extravasation of tPA across the blood–brain barrier. *In vitro* data suggest that this passage occurs via LRP-dependent transcytosis under normal conditions, or via a LRP-independent pathway under ischemic-like conditions [29]. These data underline the fact that tPA present in the brain can originate from synthesis by brain cells as well as from entry from the circulation.

1.1.2. Urokinase plasminogen activator (uPA)

uPA expression in the healthy brain is low, mainly restricted to astrocytes and to a few populations of neurons [30,31,32]. Its expression in the brain is however enhanced in pathological conditions such as epilepsy [32] or inflammatory lesions in multiple sclerosis [33]. The proteolytic activity of uPA is regulated by its binding to the cell surface receptor uPA receptor (uPAR). uPAR is a glycosyl phosphatidylinositol (GPI)-anchored protein which binds uPA and its pro-form (pro-uPA).

1.1.3. Plasminogen

As discussed throughout this review, while some functions of tPA in the brain occur by a direct effect on target effectors, some others require the activation of plasminogen into plasmin. Therefore, the issue of the origin of plasminogen in the healthy and injured brain appears critical and has been a subject of debate in the last years, particularly regarding data obtained from plasminogen knockout mice. The question whether plasminogen is synthesized by brain cells and/or comes from the circulation by crossing the BBB is still open. Also, the possibility has been raised that plasminogen synthesis could be silenced in basal conditions and up-regulated following neuronal activity or brain injury.

The main source of plasminogen in the body is the liver, from where it is released into the circulation with an estimated concentration on a micromolar range [34]. In the brain, plasminogen is secreted by neuroendocrine tissues [35,8] and expressed in the cortex, hippocampus and cerebellum [36]. In addition, plasminogen mRNA and protein were detected in the mouse in neurons -but not in glial cells- of the hippocampus, [8,37,38]. *In vitro*, cortical neurons subjected to nerve growth factor (NGF) application reveal an increased expression of the mRNA encoding for plasminogen [39]. Overall, while plasmin activity in the normal rat brain is low, it is possibly increased during axonal growth [40], after brain injury [41], or throughout regenerative events such as spine pruning [42].

Consequently, plasminogen/plasmin activity within the brain parenchyma may be involved in processes related to brain development, learning and memory, brain diseases and brain recovery, as revealed by studies using plasminogen knockout animals. However, only a relatively low number of studies describe brain-related phenotypes for plasminogen knockout mice in health and disease:

Most of the brain-related phenotypes for plasminogen knockout mice have been described during experimentally-induced brain lesions. For instance, Plasminogen knockout are resistant to excitotoxic neurodegeneration induced by kainate infusion [43]. In these conditions, plasminogen was postulated to act by the proteolytic degradation of the extracellular matrix [8,44], thus promoting anoikis, a mechanism of cell death due to loss of anchorage.

1.1.4. Serine proteases inhibitors: PAI-1 and Neuroserpin

The two main tPA inhibitors in the brain are plasminogen inhibitor-1 (PAI-1) and neuroserpin (NS). PAI-1 is an irreversible inhibitor of tPA and uPA activity. PAI-1 is synthesized mainly by endothelial cells in the vasculature but also by astrocytes in the brain [9]. NS is a transient inhibitor of tPA and uPA activity. It is expressed principally in the neurons of the central and peripheral nervous systems [45]. NS forms short-lived and unstable complex with plasminogen activators leading to the cleavage of NS and the liberation of the active enzyme *in vitro* [46]. Thus neuroserpin may be considered as a “plasminogen activator buffer”. When cleared, the NS–tPA complexes removed from the synaptic space by astrocyte-derived LRP receptors are not necessarily degraded [47]. Further studies should be investigated to understand the fate of NS–tPA complexes cleared from the synaptic space. A possible role of NS in the recycling of tPA may be further investigated. In fact, it seems that even if NS has a lower inhibitory constant than PAI-1, it plays a central role in the bioavailability of tPA in the brain by buffering its presence and its activity.

NS expression can be regulated in pathological conditions, such as in the brain of Alzheimer's disease, possibly in relation to thyroid hormone response [48].

To summarize the information available concerning the expression of the actors of the PA system in the brain, it can be stated that the PA system is upregulated in the brain as a response to inflammation. Accordingly, the actors of the PA system may be used as biomarkers in