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Plasmin System, Alzheimer's Disease and Stroke

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The role of the plasmin system in the nervous system is generating increasing interest. The conventional role of plasmin being a fibrinolytic has been overshadowed by the realisation that cleavage of nonfibrin substrates plays a part in many pathophysiological settings. The focus has been on understanding the significance of localization of the plasmin system in dictating substrate specificity and thereby explaining the diverse processes influenced by plasmin, actions that cannot be attributed to fibrinolysis alone. The clinical relevance of the plasmin system is brought to the fore by focussing on its role in two neurological disorders. Alzheimer's disease appears to be caused by the accumulation of amyloid-beta. This laboratory and others have shown that plasmin degrades amyloid-beta. Therefore, we hypothesize that dysfunction of the plasmin axis facilitates amyloid-beta accumulation and hence enhances Alzheimer's disease risk. In stroke and excitotoxic disease models, plasmin, directly or indirectly through matrix metalloproteinases, degrades proteins essential for maintaining the blood brain barrier and cell-extracellular matrix interactions and may thereby contribute to neuronal loss. Thus, a better understanding of the role of plasmin system in neuronal pathology may yield a rich dividend in terms of novel therapeutic targets for neurodegenerative disorders.

Key Words: Plasmin, Alzheimer's disease, Stroke, Matrix metalloproteinase, Blood brain barrier, Urokinase-type plasminogen activator, Tissue-type plasminogen activator, Amyloid-beta

Introduction

In this review we will briefly introduce the plasmin system and two neurological disorders in which this proteolytic cascade may play a key role. Although Alzheimer's disease (AD) and stroke have varied aetiologies, altered proteolysis is likely a common thread. We will elaborate on the interplay between plasmin and these disorders at the cellular and molecular level and identify promising research areas.

The Plasmin System

The plasmin system is a closely interacting set of proteases and their cognate inhibitors. The protease components of this system consist of urokinase-type plasminogen activator (uPA) and the closely related tissue-type plasminogen activator (tPA), each of which acts to convert plasminogen to the active protease, plasmin. This protease system has three primary inhibitors. Both uPA and tPA are inhibited by plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2), while the principal plasmin inhibitor is alpha-2-antiplasmin (a2-AP) (Andreasen et al. 2000). In the brain, tPA, uPA and plasminogen are expressed by neurons; tPA, uPA, PAI-1 and PAI-2 by activated microglia and reactive astrocytes (Akiyama et al. 1993, Tsirka et al. 1996, Dietzmann et al. 2000).

Abbreviations:
a2-AP, Alpha-2-antiplasmin; *AD*, Alzheimer's disease; *Aß*, Amyloid-beta; *APP*, Amyloid--beta protein precursor; *BBB*, Blood brain barrier; *ECM*, Extracellular matrix; *GPI*, Glycosyl phosphatidyl inositol; *IDE*, Insulin degrading enzyme; *MMP*, Matrix metalloproteinase; *PAI-1*, Plasminogen activator inhibitor-1; *PAI-2*, Plasminogen activator inhibitor-2; *tPA*, Tissue-type plasminogen activator; *uPA*, Urokinase-type plasminogen activator; *uPAR*, Urokinase-type plasminogen activator receptor

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Historically, this system of serine protease zymogens and their serpin inhibitors has been described in terms of its ability to mediate the breakdown of fibrin clots within the vasculature. Indeed, "knockout" studies have demonstrated that members of plasmin system are not necessary for grossly normal development but are needed for normal life span and vascular homeostasis. Mice deficient in uPA or tPA manifest only a modest excess in fibrin deposits. In contrast, mice deficient in both uPA and tPA are similar to plasminogen-deficient mice, suffering from very extensive fibrin deposits and concomitant thrombotic emboli, with death typically occurring by six months of age (Bugge et al. 1995, Carmeliet et al. 1994). That death is primarily due to fibrin deposition was demonstrated convincingly by showing that mice deficient in both plasminogen and fibrinogen are largely rescued from their vascular pathology (Bugge et al. 1996). We interpret these data as suggesting that uPA and tPA complement one another in contributing to fibrin clearance because uPA or tPA deficiency is much less deleterious than plasminogen deficiency.

Dramatic advances have been made in our understanding of the biochemistry of the plasmin system over the past decade. Plasmin, uPA and tPA are each serine-dependent proteases with similar but not identical structural motifs. The amino-terminal portion of each contains one or more kringle domains, which are ~100 amino acid structural domains mediating protein-protein interactions; the carboxyl-terminal portion contains the serine protease domain. These proteins are initially secreted from cells as single-chain zymogens and converted to two-chain, maximally active proteases by internal proteolytic cleavage. These serine proteases cleave the inactive zymogen plasminogen at the Arg^{560}-Val^{561} peptide bond to yield the active serine protease plasmin. Networks of intra- and inter-chain disulfide bonds maintain the activated proteases (Andreasen et al. 2000). Active plasmin proteolytically degrades a variety of target molecules.

Plasmin is activated in a spatially limited manner by three mechanisms. First, the actions of uPA and tPA, as well as plasmin, are sharply restricted due to the relative abundance of their cognate inhibitors, PAI-1, PAI-2 and a2-AP. Second, transcription of the activators is regulated at the cellular level. Third, the plasmin activators are often localized because of their binding to specific extracellular proteins. More specifically, tPA is localized to fibrin clots by virtue of it's kringle domains binding directly to fibrin aggregates. In addition to localizing tPA activity, this binding to fibrin aggregates enhances tPA self-activation and results in a dramatic increase in the affinity of tPA for plasminogen (Andreasen et al. 2000). Both of these actions promote tPA's ability to convert plasminogen to active plasmin. In summation, this mechanism effectively localizes active tPA and hence plasmin production to the site of fibrin aggregates, consistent with tPA's critical role in fibrinolysis. Interestingly, amyloid ß (Aß) aggregates can substitute for fibrin-aggregates in activating tPA (Kingston et al. 1995).

The other plasminogen activator, uPA, is localized by a different mechanism. While uPA has been reported to bind to fibrin aggregates as well (Yoshimoto et al. 1996), this interaction appears much less robust than that between tPA and fibrin (Andreasen et al. 2000). Rather, binding of uPA to the uPAR receptor (uPAR) confers focused pericellular localization to this plasminogen activator. The uPA receptor is a cell surface, glycosyl phosphatidyl inositol (GPI) anchored receptor that is expressed by many cell types, including neurons. As with other GPI-anchored proteins, uPAR is enriched in lipid raft microdomains on the cell surface. This receptor is located at specific cell-substratum contact sites as well as the leading edges of migrating cells (Reuning et al. 1998, Andreasen et al. 2000). This interaction is also regulatory in that uPA conversion from an inactive zymogen to an active protease is enhanced by uPA binding to uPAR. The result of this binding is that constitutively expressed plasminogen is converted to plasmin at discrete microdomains on the cell surface. Plasmin in turn degrades not only fibrin, but also activates matrix metalloproteinase (MMP) zymogens that then further degrade the extracellular matrix (ECM). This ECM degradation at the leading edge of migratory cells facilitates cell migration

The ability of the plasmin system to degrade ECM may contribute to both the normal and pathophysiologic actions of plasmin. Mice genetically deficient in plasmin system components have been used to provide evidence implicating the plasmin system in normal events occurring within the central nervous system such as neurite outgrowth, neuronal migration, and long term potentiation (Baranes et al. 1998, Madani et al. 1999, Pittman et al. 1989, Pittman et al. 1995, Seeds et al. 1999, Zhuo et al. 2000) as well as pathologic events ranging from tumour metastases to wound healing, cell migration, and vascular remodelling (Akassoglou et al. 2000, Bezerra et al. 2001, Degen 1999, Drew et al. 2001, Romer et al. 1996). Quite recently, Powell et al. (2001) found that mice deficient in uPAR have decreased levels of active hepatocyte growth factor in the basal forebrain due to decreased activation of the latent form. Moreover, these mice manifested decreased numbers of parvalbumin positive interneurons, which the author attributes to altered cell migration (Powell et al. 2003).

As with many other genes, the effects of a plasmin system genetic deficiency become especially apparent when mice are pathologically challenged. For example, the use of mice deficient for plasminogen or tPA allowed Tsirka and her colleagues to clearly implicate this system in neuronal death after stroke and seizure (Chen & Strickland 1997, Tsirka et al. 1996, 1997b, Wang et al. 1998, Wu et al. 2000). The basic model derived from this work is that stress-induced increases in plasminogen activator expression lead to localized conversion of constitutively expressed plasminogen to plasmin, and, in turn, inappropriate degradation of the extracellular matrix, and neuronal death (discussed further below).

Alzheimer's Disease

AD is a progressive neurodegenerative disorder that afflicts four million people in the United States and many millions more worldwide. There is no definitive cure or intervention presently available. Symptomatic treatment and healthcare costs are in excess of $100 billion per year (DeKosky & Orgogozo 2001). This will increase in the future as the number of cases in the United States alone is projected to reach 15 million over several decades.

The complex aetiology of AD encompasses both environmental and genetic risk factors. The existence of environmental risk factors is suggested by twin studies, e.g., while many identical twins have some degree of concordance for AD, there is clear differential susceptibility to AD (Breitner et al. 1992, Raiha et al. 1996). Anti-inflammatory drug-use constitutes an 'environmental' factor that appears to lower AD risk, although clinical trials with a cyclo-oxygenase-2 inhibitor suggest that such drugs do not ameliorate existing disease (reviewed in McGeer et al. 1996, Pasinetti 2002). Also consistent with a role for inflammation in AD is that certain polymorphisms in the inflammatory mediator interleukin-2 have been associated with AD (Mrak & Griffin 2000). A role for oestrogen as a neuroprotectant has been controversial (Wise et al. 2001).

One hypothesis for a common final pathway of AD aetiology has been termed the 'amyloid cascade'. Aß is a 39-42 amino acid peptide that spontaneously aggregates and that accumulates in AD brain. According to the amyloid cascade hypothesis, a chronic imbalance between Aß production and clearance leads to a gradual accumulation of Aß aggregates which initiate a complex, multistep cascade that includes gliosis, inflammatory changes, neuritic/synaptic change, neurofibrillary tangles and neuronal death (Hardy and Selkoe 2002).

Aß itself is contained entirely within the amyloid precursor protein (APP), a type 1 transmembrane protein. Three enzymes – the alpha, beta and gamma 'secretases' – proteolyze APP to liberate Aß (Selkoe & Podlisny 2002). The alpha secretase cleaves in the middle of the Aβ sequence (between amino acids 16 and 17) while the beta and gamma secretases define the amino and carboxyl termini of Aβ. The product of beta and gamma secretase is the highly pathological Aβ which is rapidly turned over under normal conditions with a half-life of 1-2.5 hours in the mouse brain (Savage et al. 1998). Different signalling pathways and GPI anchored proteins (Sambamurti et al. 1999) can influence APP processing. Interestingly, one of the alternatively spliced forms of the APP contains a Kunitz proteinase inhibitor domain that has been reported to be capable of inhibiting activated plasmin (van Nostrand et al. 1994). Although this

might suggest that an overexpression of the APP containing the Kunitz proteinase inhibitor domain could modulate plasmin activity in AD brain, the preponderance of evidence is that differential alternative splicing of the APP mRNA between AD and control brain tissue does not occur (reviewed in Selkoe 2001).

Plasmin System in Alzheimer's Disease

Strikingly, the plasmin system is induced by and degrades protein aggregates, while AD is marked by the inappropriate accumulation of protein aggregates. This has lead us and others to consider whether the plasmin system may have a role in AD. We can consider this evaluation as a two-step process. First, if the plasmin system contributes to AD, the plasmin system should be altered in a comparison of AD versus control brain tissue. Second, a mechanistic hypothesis that links this alteration in plasmin activity to phenotypic changes seen in AD should be identified and evaluated. These issues provide a useful framework for discussion.

Plasmin System may be Altered in AD

Several lines of evidence suggest that plasmin system is altered in AD. At the global level, Ledesma et al. (2000) in a limited study sample found that total plasmin activity appears decreased in AD. A decreased plasmin activity could reflect an imbalance in activators and inhibitors. Although global inductions in uPA and tPA mRNA occur in aged APP transgenic mice (Tucker et al. 2000a), counterbalancing increases in PAI-1 and a2-AP at the mRNA level also occur in these mice (Bondada & Estus, unpublished observations). Similarly, PAI-1 protein is reported to be increased in cerebrospinal fluid of AD patients (Sutton 1994). Hence, the overall apparent decreased plasmin system activity in AD reported by Ledesma et al. may reflect an increased proportion of inhibitors relative to activators. This speculation assumes that the usual inhibitor excess is influenced significantly by increased inhibitor production. This assumption may be reasonable in that the brain is relatively poorly characterized with respect to these activator/inhibitor ratios and prior assumptions derived from the vasculature literature may not be directly applicable to the central nervous system.

Global information may not accurately reflect local proteolytic milieu since sites of plasmin activation are localized by tPA binding to fibrin or amyloid, or uPA binding to uPAR. Both activators and inhibitors are altered locally in AD. Rebeck and colleagues reported that uPA, tPA and PAI-1 accumulate in the AD brain around senile plaques (Rebeck et al. 1995). Activated microglia and reactive astrocytes are hallmarks of AD, and both are known to increase production of tPA, uPA, PAI-1, and PAI-2 (Akiyama et al. 1993, Dietzmann et al. 2000). Hence, although plasmin activity may be globally decreased in AD, local pockets of increased activity may exist that are of biological significance.

Possible Mechanistic Links between the Plasmin System and AD Neuropathology

The second aspect of this discussion is whether a mechanistic hypothesis linking altered plasmin activity to phenotypic AD could be identified and evaluated. Recently, an unexpected possible action of the plasmin system with potential relevance to AD was identified, namely Aß degradation. Others had reported that plasmin could act upon Aß (Van Nostrand & Porter 1999). We augmented these data, reporting that *uPA* and *tPA* are induced in *in vitro* and *in vivo* models of Aß accumulation. Subsequently, we found that plasmin degrades both non-aggregated and aggregated Aß with physiologically relevant rates (Tucker et al. 2000a). This is remarkable in that Aß aggregates are considered strongly resistant to proteolytic degradation. More recently, others reported that plasmin in lipid rafts, presumably activated by uPA bound to GPI-anchored uPAR, cleaves APP at the alpha-secretase position (Ledesma et al. 2000). This precludes Aß formation and again suggests a role for localized plasmin in Aß clearance. Overall, we can conclude from these observations that decreased plasmin activity may contribute to AD via decreased Aß clearance. We hasten to bring to the attention of the reader that we recognize that this model is Aß-centric and, given the suggested roles of plasmin system in neuronal migration, long-term potentiation, neuronal toxicity, and cell-cell interactions, there are multiple other possibilities whereby plasmin could impact AD (Seeds et al. 1999, Tsirka et al. 1995, Baranes et al. 1998, Madani et al. 1999). However, the apparent decrease in

plasmin activity in AD (Ledesma et al. 2000) would be inconsistent with a direct role of plasmin in neurotoxicity. Moreover, given that the plasmin system in the vasculature is induced by protein aggregates and mediates their clearance, the scientific law of parsimony would suggest that the most probable mechanism to explain a decreased plasmin activity as contributing to AD is via decreased Aß clearance.

In addition to the plasmin system, two additional Aß degrading enzymes have been proposed to regulate Aß clearance *in vivo*, including insulin degrading enzyme (IDE) and neprilysin (Kurochkin et al. 1994, Howell et al. 1995, McDermott & Gibson 1997, Qiu et al. 1998, Chesneau et al. 2000, Iwata et al. 2000, 2001, Vekrellis et al. 2000, Shirotani et al. 2001). Whether any or all of these enzymes modulate Aß *in vivo* is unclear. The strongest evidence is for neprilysin, where studies showed neprilysin-deficient mice had increased endogenous murine Aß levels (Iwata et al. 2001). Whether IDE alters Aß *in vivo* has not been reported. Among these enzymes, only plasmin has been shown to degrade aggregated Aß fibrils (Tucker et al. 2000a). In summary, we propose that multiple enzymes contribute to Aß clearance. Neprilysin may be the predominant enzyme that clears soluble Aß. Plasmin may be the predominant enzyme that is activated by insoluble Aß and that clears insoluble Aß.

Considering the findings discussed here, a model for plasmin in AD can be proposed. We propose that during normal aging, Aß aggregates produced occasionally during APP catabolism lead to the enhanced expression of plasminogen activators relative to plasmin system inhibitors, which overall leads to increased localized plasmin activity and degradation of Aß aggregates (figure 1). More specifically, we propose that Aß accumulation leads to localized uPA and tPA induction. Both uPA and tPA are secreted, and bind to uPAR or Aß aggregates, respectively. Both uPA and tPA convert constitutively expressed plasminogen to plasmin. This localized plasmin activity then degrades the Aß aggregates that initiated the process. This process occurs in normal aging in a fashion analogous to that of fibrin aggregates being cleared by uPA and tPA-dependent processes in the vasculature.

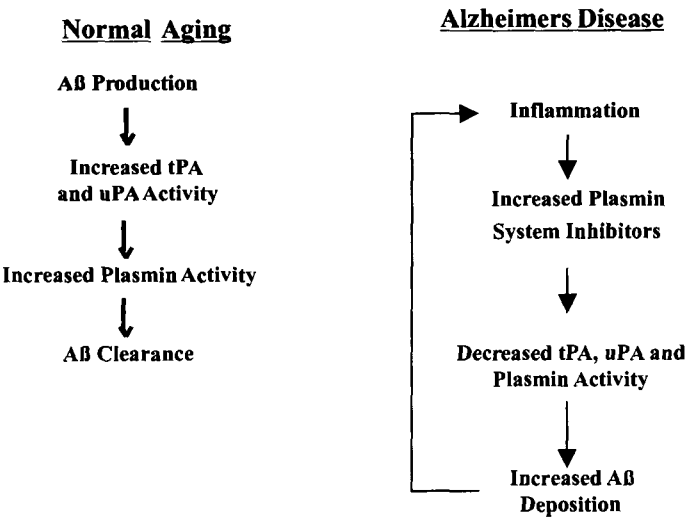

Figure 1 Model of the plasmin system and Aß clearance in normal aging and in AD.

The relevant research findings supporting this model include (i) *uPA* and *tPA* are induced in *in vitro* and *in vivo* models of Aß accumulation (Tucker et al. 2000a; 2000b), (ii) tPA binds to fibrin or Aß via its kringle domains, which leads to tPA activation (Andreasen et al. 2000; Kingston et al. 1995), (iii) uPA and tPA locally activate plasmin, which is very robust at Aß catabolism (Tucker et al. 2000a; 2000b), (iv) uPA and tPA have been localized to the vicinity of senile plaques in AD tissue (Rebeck et al. 1995), (v) PAI-1 and PAI-2 are induced by inflammation in general and in AD in particular, especially in microglia, which are a component of the senile plaques that surround Aß deposits (Akiyama et al. 1993, McGeer & McGeer 1995, Sutton et al. 1994), and (vi) a variety of evidence implicates inflammation in the aetiology of AD, including that patients with a history of robust anti-inflammatory drug use have a much lower incidence of AD (McGeer et al. 1996, Pasinetti 2002). We again note that although this proposed model is Aß-centric, this model is not exclusive of other possible roles of plasmin in AD.

In AD, we propose that an initial inflammatory event upregulates expression of plasmin system inhibitors, i.e., PAI-1, PAI-2 and/or a2-AP. This alters the balance of plasmin system such that Aß aggregates produced normally are not cleared efficiently by the concomitant uPA and tPA induction. Rather, Aß begins to accumulate, augmenting the inflammatory cascade, leading to increased upregulation of PAI-1, PAI-2, and/or a2-AP. These inhibitors in turn increasingly block Aß degradation, fuelling the cycle further. This model suggests that the proteases in the plasmin system are in a balance with their cognate inhibitors. If this balance is shifted away from proteolysis, then Aß accumulation is more likely. Experiments to evaluate this model are outlined in the future directions.

Stroke

A stroke or "brain attack" occurs when blood flow to an area of the brain is interrupted by either a blood clot (ischaemic stroke), or by a vessel rupture (haemorrhagic stroke). When a brain attack occurs, cells in the immediate area (infarct) usually die within minutes. In the hours during and after the stroke, the area surrounding the infarct (penumbra) starts releasing chemicals that set off a chain reaction called the "ischaemic cascade". This chain reaction endangers brain cells in a larger, surrounding area of brain tissue for which the blood supply is compromised but not completely cut off. Without prompt medical treatment the larger number of brain cells in the penumbra will also die. The therapeutic window for intervention is three hours. Beyond this window, reestablishment of blood flow may fail to help and can potentially cause further damage.

According to the United States National Stroke Association (www.stroke.org), every year approximately 750,000 Americans have a new or recurrent stroke. Stroke is the third leading cause of death, killing nearly 160,000 Americans every year. Approximately one-third of all stroke survivors have another stroke within five years. Stroke is a major factor in the late-life dementia that affects more than 40 percent of Americans over age 80. The care giving costs $30 billion annually.

Plasmin System in Stroke

The role of the plasmin system in stroke is multifaceted and depends upon the location of the activated plasmin and, thereby, its substrate. In the vasculature, the plasmin axis can be beneficial by degrading fibrin clots, improving perfusion and thereby ending strokes. Alternatively, plasmin generated in the vicinity of neurons can lead to ECM degradation, promoting neuronal death. Here, we compare these two roles of the plasmin system.

Plasmin in the Ischaemic Vasculature

Seventy-five percent of all stroke cases are due to ischaemia resulting from complete occlusion of an artery (Zerwic et al. 2002). A recombinant form of tPA is the only drug that has received FDA approval for acute ischaemic stroke treatment. The approval of tPA in June 1996 sparked increased public and medical interest in stroke. Recombinant tPA is routinely administered up to three hours from the onset of occlusive stroke to dissolve clots and improve perfusion. The mechanism of action reflects that tPA is relatively inactive until bound to fibrin aggregates. Hence, tPA migrates through the bloodstream until it reaches the fibrin clot responsible for the stroke. tPA binds the aggregate, and the resultant increase in the affinity of tPA towards plasminogen leads to enhanced plasmin formation. Plasmin then acts to degrade the fibrin clot, allowing reperfusion. The relative excess of their specific cognate inhibitors limits the actions of active tPA and plasmin.

Although the benefits of tPA treatment have been dramatic, one of the deleterious side effects of the treatment is haemorrhagic transformation in which blood escapes from the blood vessel into the brain. Blood brain barrier (BBB) disruption appears to be due to plasmin-mediated MMP activation (Gasche et al. 2001). Sumii and Lo (2002) showed that BB-94 (a broad spectrum MMP inhibitor) decreased tPA induced haemorrhage volumes Moreover, the BBB protein ZO-1 is degraded by a member of the MMP family, MMP9, and MMP9 knock out mice are less susceptible to BBB degradation and its sequel of oedema and infarction (Asahi et al. 2001). Hence, these results provide insights into two aspects of tPA function. First, elucidating the molecular mechanisms underlying this action of tPA can allow us to decrease the incidence of haemorrhage while maintaining beneficial tPA actions. Second, this side effect of tPA treatment illustrates that plasmin has many substrates, and therefore must be tightly localized to maximize benefit and minimize harm.

Plasmin in the Ischaemic Parenchyma

An alternative role of the plasmin system in stroke has also generated interest over the past several years. As noted above, tPA, uPA, and plasmin are synthesized in neurons (Tsirka et al. 1996). This low basal level of plasmin activity may aid in matrix remodelling during activity-dependent modulation of synaptic function, an area of current and future exploration. However, excessive plasmin system overactivation in these cell types can be deleterious. More specifically, the plasmin system appears to modulate neuronal loss in both ischaemia and

excitotoxicity models (Chen & Strickland 1997, Tsirka et al. 1997b, Wang et al. 1998, reviewed in Strickland 2001).

The mechanism underlying this neurotoxic action appears to reflect inappropriate overactivation of the plasmin system. Stroke or excitotoxins like kainate markedly induce tPA expression in the brain *in vivo*, and mice lacking tPA are markedly protected from stroke and kainate toxicity (Tsirka et al. 1995, 1997b, Wang et al. 1998). Studies with mice deficient in both plasminogen and fibrinogen revealed that fibrin is not necessary for this plasmin-mediated effect in the hippocampus (Tsirka et al. 1997a). Rather, work by Strickland's group has suggested that plasmin directly or indirectly leads to degradation of laminin, an ECM component key for neuronal survival. Indeed, this group proceeded to show that this plasmin-dependent laminin loss is a critical aspect of the neuronal death (Chen & Strickland 1997). Interestingly, disruption of cell-ECM interaction through the proteolytic degradation of matrix proteins may be a general mechanism of cell death (Meredith et al. 1993, Boudreau et al. 1995). Additionally, plasmin or downstream MMPs could also act upon a number of other proteins, leading to their inappropriate degradation, or alternatively activation from latent zymogen status, e.g., hepatocyte growth factor (Powell et al. 2001) or transforming growth factor beta (Dallas et al. 2002). An additional mechanism to account for deleterious actions of tPA in stroke is suggested by the recent report that tPA directly or indirectly leads to proteolysis of the NMDA receptor that accentuates receptor response to stimulation (Nicole et al. 2001).

Although the data suggesting a toxic role for the plasmin system appears compelling, several contradictory reports have also been published. For example, Nagai et al. (1999) examined stroke volumes in mice individually deficient for tPA, uPA, PAI-1, plasminogen, and a2-AP. Consistent with the possibility that tPA contributed to stroke damage, deficiency of either tPA or PAI-1 was associated with decreased or increased damage, respectively. However, plasminogen appeared protective because deficiency of either plasminogen or a2-AP was associated with increased or decreased stroke volume. Deficiency of the uPAR had no effect. Also, Tabrizi et al. (1999) reported that tPA deficiency was not neuroprotectve in stroke. The weight to assign these various reports is unclear. Confounding experimental variables may account for some of these contradictions. For example, some studies use non-siliconized threads for the occlusion that promote clot formation and hence may find a protective role for tPA and plasminogen that is not noted in studies involving permanent occlusion. Murine strain differences that are known to modulate responses to excitotoxicity may also contribute to these inconsistencies.

In summary, the preponderance of evidence suggests a model wherein the plasmin system contributes to neuroprotection in the vasculature and neurotoxicity in the parenchyma (see figure 2). The contribution of possibly minor pathways such as NMDA receptor degradation relative to the actions of the plasmin system on the ECM remains to be elucidated.

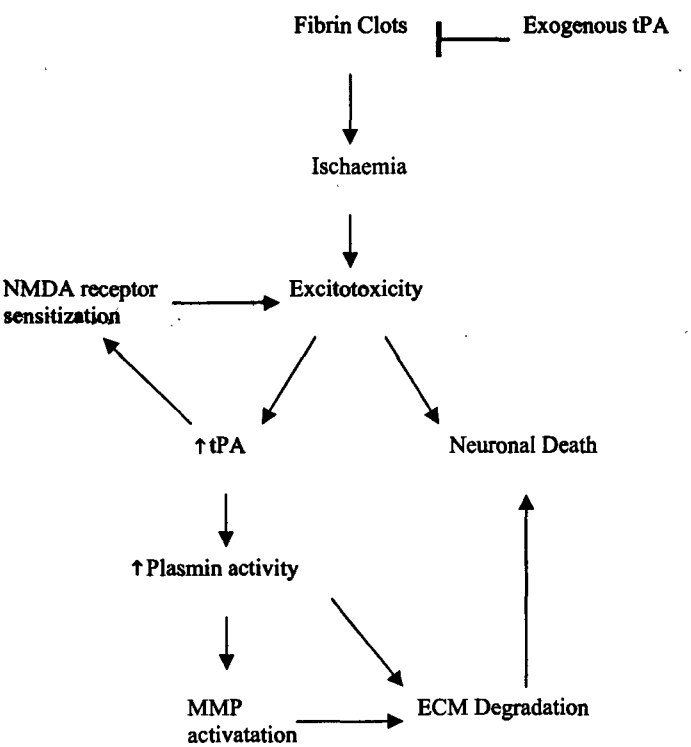

Figure 2 Model of the plasmin system in stroke. This model highlights the dual role of tPA in stroke. When confined to the intravascular compartment, plasmin is beneficial in 'busting' clots and hence ameliorating the ischaemia. But when the localization of this activity escapes from the confines of the vasculature, deleterious effects predominate. The ECM components which support and protect the neurons are compromised either directly through plasmin or indirectly through MMP activation.

Future Directions

Research involving the interface of the plasmin system and neurological disorders involves exciting possibilities. Several lines of investigation are likely to be fruitful with regards to the role of the plasmin system in AD. First, a locus for late onset AD risk was recently identified on chromosome 10 (Bertram et al. 2000, Ertekin-Taner et al. 2000, Myers et al. 2000). Interestingly, the uPA gene is among the approximately 150 genes contained within this locus (Tucker et al. 2002). Hence, the results of single nucleotide polymorphism analyses in AD and control populations may provide insights into whether uPA variants are associated with late onset AD risk. Second, a critical aspect of testing whether the plasmin system contributes to Aß clearance *in vivo* is to quantify Aß levels in mice deficient for members of the plasmin family. Since regulation at the transcriptional and post-translational levels are involved in plasmin system actions, these experiments may require that the mice be APP transgenics, which has been reported to induce at least plasminogen activators (Tucker et al. 2000a, 2000b).

Benefits will accrue in stroke as both diagnostic and therapeutic advances are made. Advances in perfusion-weighted imaging will facilitate the identification of stroke patients suitable for tPA treatment and hence extend the therapeutic window beyond the present 3 hours, thereby benefiting more people (Lindsberg & Kaste 2003). The current tPA-based treatment will likely synergize with advances in other aspects of stroke research. For example, DNA microarray technology is being used unravel the changes in gene expression profiles that accompany ischaemia. This may provide new insights into the pathologic and protective mechanisms in stroke and thereby lead to novel therapies (Ginsberg 2003). In this regard, advances in gene and protein transfer to cerebral blood vessels and ischaemic brain tissue may synergize markedly to maximize the protective effects of tPA on clots while minimizing the deleterious actions of tPA on the vasculature and on neuronal survival directly. In this regard, a recent paper demonstrating that intracisternally injected neuroserpin, a member of the serpin family capable of inhibiting tPA at pharmacologic concentrations, was effective at minimizing haemorrhage in a rat model of embolic stroke (Zhang et al. 2002).

Lastly, emerging evidence suggests that the plasmin system may play a role in additional neurological disorders. First, plasminogen was recently shown to bind disease-associated prion protein, but not the cellular form of prion (Fischer et al. 2000). This finding may lead to advances in diagnosis and perhaps therapy in transmissible spongiform encephalopathies. Second, Tsirka and colleagues have recently used tPA deficient mice in a model of multiple sclerosis to identify a complex role for tPA in this process; tPA deficient mice have a delayed onset of inflammation but a prolonged recovery (Lu et al. 2002). Third, mutations in neuroserpin have been associated with intracellular neuroserpin aggregates that lead to dementia, suggesting that polymorphisms in plasmin system members that are serpins, e.g., PAI-1 and PAI-2, may be found that also lead to dementia (reviewed in Lomas & Carrell 2002). Fourth, although uPAR deficient mice appear to develop normally (Dewerchin et al. 1996), a close evaluation by Powell et al. (2000) found clear decreases in parvalbumin positive neurons in uPAR defient mice and that these ice were prone to anxiety and seizures. Hence, future short-term research in this area will likely reveal a role for uPAR and uPA in migration or survival of these neurons. Moreover, these results suggest that in the longer term, further careful scrutiny of mice deficient in plasmin system members will identify unforeseen roles for these family members in normal development into adulthood.

Conclusion

The plasmin proteolytic cascade culminates in the conversion of plasminogen to plasmin. Although this protease is relatively promiscuous with respect to substrates, physiologic plasmin activity is limited by the discrete localization of its activators and by excess levels of inhibitors. In normal physiologic conditions, the plasmin system maintains vascular homeostasis and may contribute to cell migration and remodelling. In pathological circumstances, the importance of plasmin system localization becomes clear. In stroke, tPA bound to the fibrin clot can effectively localize plasmin activity to the clot, and

hence the plasmin system is beneficial in terms of clot clearance. However, plasmin that escapes this localization may lead to haemorrhage. Moreover, acute overactivation of plasmin system in the interface between neurons and the ECM appears to contribute to the delayed neuronal death that accompanies stroke and excitotoxicity. In normal aging, localized discrete activation of the plasmin system may contribute to Aß clearance. In contrast, in AD, an imbalance in the plasmin system activators and inhibitors may impair Aß clearance and thereby contribute to AD risk. In conclusion, the metamorphosis of the plasmin system from within the fibrinolytic cascade per se to its emergence as a recognized modulator of multiple aspects of physiology and pathology suggests that further work in this area will yield basic science advances and, hopefully, novel therapeutic strategies.

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