On the Regulation of the Serine Protease t-PA and its Inhibitor PAI-1 in the Brain

Akademisk avhandling
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av Karin Hultman

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I. Hultman K, Tjärnlund-Wolf A, Fish RJ, Wilhelmsson U, Rydenhag B, Pekny M, Kruithof EKO, Jern C. **Retinoids and activation of PKC induce tissue-type plasminogen activator expression and storage in human astrocytes.** *Journal of Thrombosis and Haemostasis 2008;6:1796-1803*

II. Hultman K, Blomstrand F, Nilsson M, Wilhelmsson U, Malmgren K, Pekny M, Kousted T, Jern C, Tjärnlund-Wolf A. **Expression of plasminogen activator inhibitor-1 and protease nexin-1 in human astrocytes; response to injury-related factors.** *Journal of Neuroscience Research 2010;88:2441-2449*

III. Hultman K, Björklund U, Hansson E, Jern C. **Potentiating effect of endothelial cells on astrocytic plasminogen activator inhibitor type-1 gene expression in an *in vitro* model of the blood-brain barrier.** *Neuroscience 2010;166:408-415*

IV. Hultman K, Tjärnlund-Wolf A, Odeberg J, Eriksson P, Jern C. **Allele-specific transcription of the PAI-1 gene in human astrocytes.** *Thrombosis and Haemostasis 2010; August 30* [Epub ahead of print]

V. Tjärnlund-Wolf A, Hultman K, Curtis M, Faull RLM, Medcalf RL, Jern C. **Allelic imbalance of tissue-type plasminogen activator (t-PA) gene expression in human brain tissue.** *In manuscript*

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ABSTRACT

On the Regulation of the Serine Protease t-PA and its Inhibitor PAI-1 in the Brain

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The serine protease tissue-type plasminogen activator (t-PA) is the main fibrinolytic enzyme in the vascular system, and thus plays a critical role in the dissolution of thrombi. In recent years, researchers have focused on its role within the brain, where t-PA has been shown to participate in a number of physiological and pathophysiological processes, including various aspects of synaptic plasticity and neurodegeneration. To date, knowledge on how t-PA and its inhibitors are regulated in the brain has mainly been gained from murine *in vitro* and *in vivo* models. However, in view of the species-specific differences in the expression of these genes, the question remains as to how well the results obtained in animal models can be extrapolated to humans. Therefore, the work described in this thesis was directed at improving our knowledge on the regulation of t-PA and its principal inhibitor plasminogen activator inhibitor type-1 (PAI-1) in the human brain.

In this thesis, it is described for the first time that astrocytes have an intracellular storage compartment for t-PA, the levels of which can be increased in response to retinoic acid and protein kinase C activation. Regulated release of t-PA is induced in response to forskolin and histamine, which implies that astrocytes contribute to the extracellular levels of t-PA within the human brain. Expression studies of PAI-1 and of another t-PA inhibitor, protease nexin 1 (PN-1), revealed that the astrocytic expression levels of these inhibitors are regulated in a dynamic manner by injury-related factors, such as cytokines and hypoxia. This response may represent an important protective mechanism to reduce neurotoxicity under conditions of excessive t-PA activity, such as in the acute phase of cerebral ischaemia and in epilepsy. Given the compelling evidence that excessive t-PA activity results in the breakdown of the blood-brain barrier (BBB), the expression profiles of t-PA, PAI-1 and PN-1 were also investigated in a rodent *in vitro* model of the BBB. We report that the cocultivation of astrocytes and cerebrovascular endothelial cells potentiates astrocytic PAI-1 gene expression, and that this response is more pronounced in the presence of pro-inflammatory stimuli, e.g., lipopolysaccharide. These findings imply an important role for intercellular signalling between astrocytes and endothelial cells in the modulation of t-PA activity within the BBB.

As it has been shown that genetic variants, i.e. polymorphisms, at the t-PA and the PAI-1 loci affect the expression of these genes in endothelial cells, we investigated whether this is also the case in the brain. Allele-specific gene expression analyses revealed that polymorphisms located in the regulatory regions of the t-PA and PAI-1 genes affect their expression in human brain tissue and in human astrocytes, respectively. Furthermore, protein-DNA interaction studies demonstrated that an altered binding of transcription factors to the polymorphic sites, which likely serves as the molecular genetic explanation behind these findings. Consequently, these polymorphisms could be used to explore the significance of differences in the expression levels of t-PA and PAI-1 in adequately powered clinical association studies.

Taken together, our findings elucidate the mechanisms through which t-PA and PAI-1 are regulated in the brain. This knowledge is expected to facilitate our understanding of how t-PA is involved in the processes of memory and learning, as well as in various neurological conditions associated with altered t-PA levels.

Key words: gene expression, astrocytes, blood-brain barrier, plasminogen activator inhibitor type-1, polymorphisms, protease nexin-1, tissue-type plasminogen activator, transcriptional regulation

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