TISSUE PLASMINOGEN ACTIVATOR AND UROKINASE PLASMINOGEN ACTIVATOR IN HUMAN EPILEPTOGENIC PATHOLOGIES

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Abstract—A growing body of evidence demonstrates the involvement of plasminogen activators (PAs) in a number of physiologic and pathologic events in the CNS. Induction of both tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) has been observed in different experimental models of epilepsy and tPA has been implicated in the mechanisms underlying seizure activity. We investigated the expression and the cellular distribution of tPA and uPA in several epileptogenic pathologies, including hippocampal sclerosis (HS; *n*=6), and developmental glioneuronal lesions, such as focal cortical dysplasia (FCD, *n*=6), cortical tubers in patients with the tuberous sclerosis complex (TSC; *n*=6) and in gangliogliomas (GG; *n*=6), using immuno-cytochemical, western blot and real-time quantitative PCR analysis. TPA and uPA immunostaining showed increased expression within the epileptogenic lesions compared to control specimens in both glial and neuronal cells (hippocampal neurons in HS and dysplastic neurons in FCD, TSC and GG specimens). Confocal laser scanning microscopy confirmed expression of both proteins in astrocytes and microglia, as well as in microvascular endothelium. Immu-

noblot demonstrated also up-regulation of the uPA receptor (uPAR; *P*<0.05). Increased expression of tPA, uPA, uPAR and tissue PA inhibitor type mRNA levels was also detected by PCR analysis in different epileptogenic pathologies (*P*<0.05). Our data support the role of PA system components in different human focal epileptogenic pathologies, which may critically influence neuronal activity, inflammatory response, as well as contributing to the complex remodeling of the neuronal networks occurring in epileptogenic lesions. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

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The plasminogen (fibrinolytic) system comprises the inactive proenzyme, plasminogen, that can be converted to the active enzyme plasmin by two serine proteases, the tissue-type plasminogen activator (tPA) and the urokinase-type plasminogen activator (uPA) (Collen, 1999). TPA and uPA elicit various cellular responses, involving the activation of distinct signaling pathways. While several of these pathways have been described (Maupas-Schwalm et al., 2004; Benarroch, 2007), their interactions and the link to specific biological responses are only partly understood. Attention has been recently focused on the role of uPA receptor (uPAR) indicating that it may act as signaling receptor, also independently of uPA-mediated proteolysis (for review see Smith and Marshall, 2010). In the CNS, increasing evidence suggests a crucial role of the plasminogen system in a broad range of physiological and pathological processes ranging from neuronal development, cell migration and invasion, cell growth and apoptosis, immune responses, inflammation, angiogenesis and regulation of synaptic remodeling and neuronal plasticity (Seeds et al., 1999; Tsirka, 2002; Powell et al., 2003; Alfano et al., 2005; Benarroch, 2007).

TPA is widely expressed by many types of neurons in the human CNS, including the neocortical and hippocampal pyramidal neurons (Teesalu et al., 2004). Activation of the plasminogen system, involving neurons, reactive glial cells and vascular endothelium, as source of plasminogen activators, has been reported in different neurological disorders such as stroke and other forms of acute brain injury, as well as in patients with inflammatory disorders (Gveric et al., 2001; Teesalu et al., 2002; Benarroch, 2007). A complex deregulation of the plasminogen system may also be involved in neurodegenerative disorders, such as Alzheimer's disease (Tucker et al., 2002; Fabbro and Seeds, 2009).

Several experimental findings identified a role for tPA in the mechanisms underlying seizure activity (Tsirka et al., 1995; Pawlak and Strickland, 2002; Benarroch, 2007). Interestingly, induction of plasminogen activators (PAs) has been

Table 1. Summary of clinical details of cases studied according to pathology

Pathology type (pm or s)	Number of cases	Mean age at surgery (range/y)	Localization	Mean duration of epilepsy (range/y)
HS (pm)	6	26.3 (12–42)	Temporal	17 (11–32)
Non-HS (s)	5	29.5 (18–41)	Temporal	15.2 (6–22)
FCD IIB (s)	6	27.3 (14–48)	Temporal (4) Frontal (2)	19.2 (5–25)
Cortical tubers (TSC; s)	6	17.8 (5–35)	Frontal (3) Temporal (2) Parietal (1)	13.5 (2.8–34)
Ganglioglioma (GG; s)	6	32 (16–49)	Temporal	16.1 (12–26)
Control neocortex (pm)	6	31.6 (18–35)	Temporal	—

HS, Hippocampal Sclerosis; FCD, Focal Cortical Dysplasia; TSC, Tuberous Sclerosis; pm, post-mortem; s, surgical specimens.

observed in different experimental models of epilepsy (Lukasiuk et al., 2003; Gorter et al., 2006; Lahtinen et al., 2006) and gene expression profile analysis of gangliogliomas (GG) revealed that both tPA and uPA represent one of the most up-regulated genes in these epileptogenic lesions (Aronica et al., 2008). In rat hippocampus, tPA and uPA were both activated at one day after induction of status epilepticus (SE) and were still elevated during epileptogenesis (Gorter et al., 2006, 2007; Lahtinen et al., 2006). Expression of tPA mRNA was still increased in the chronic phase in the CA3 region (Gorter et al., 2007). Recently, the cellular distribution of uPA and uPAR has been characterized in rat hippocampus during epileptogenesis (Lahtinen et al., 2006). However, whether the up-regulation of plasminogen activator proteins persists in the chronic phase in epileptic human brain is unclear. Moreover, a detailed analysis of tPA and uPA cellular expression in human epileptogenic pathologies is still lacking. Because of the functional redundancy among PAs (Carmeliet and Collen, 1995), characterization of the expression in human tissue is important for the correct interpretation of the experimental observations.

In the present study we have examined the tPA and uPA tissue distribution, evaluated their degree of expression and defined their cellular origin in common causes of human focal chronic refractory epilepsy.

EXPERIMENTAL PROCEDURES

Subjects

The human cases included in this study were obtained from the files of the departments of neuropathology of the Academic Medical Center (AMC, University of Amsterdam), the VU University Medical Center (VUMC) in Amsterdam, the University Medical Center in Utrecht (UMC) and the Neuromed Neurosurgery Center for Epilepsy, Pozzilli-Isernia, Italy. We examined a total of 29 surgical epilepsy specimens, six hippocampal sclerosis (HS), five hippocampal surgical specimens of patients without HS (non-HS; with a focal lesion not involving the hippocampus proper; no appreciable neuronal loss and reactive gliosis are observed in the hippocampus), six focal cortical dysplasia (FCD) type IIB, six cortical tubers from patients with Tuberous Sclerosis Complex (TSC), six ganglioglioma (GG). In six patients (two FCD, two GG and two TSC) a significant amount of perilesional tissue (normal-appearing cortex/white matter adjacent to the lesion) was resected as well. This material was carefully inspected by microscopy, using both histological and immunocytochemical stainings (HE, luxol-PAS, GFAP, vimentin, neurofilament, neuronal nuclear protein, NeuN and phosphorylated ribosomal pro-

tein S6 and CD34) to differentiate the lesion (tumor or malformation) from the perilesional control cortex, defined as cortical region adjacent the lesion, but histologically normal (i.e. not containing tumor cells, dysplastic neurons and without appreciable neuronal loss and reactive gliosis). Informed consent was obtained for the use of brain tissue and for access to medical records for research purposes. Tissue was obtained and used in a manner compliant with the Declaration of Helsinki. The clinical characteristics derived from the patient's medical records are summarized in Table 1. Patients underwent therapeutic surgical resection for refractory epilepsy and had predominantly medically intractable complex partial seizures.

The HS specimens include four cases of classical HS (grade 3; Wyler et al., 1992; Mesial Temporal Sclerosis (MTS) type 1a; Blumcke et al., 2007) and two cases of severe HS (grade IV; Wyler et al., 1992; MTS type 1b; Blumcke et al., 2007). For the FCD we followed the classification system proposed by Palmini et al. for grading the degree of FCD (Palmini et al., 2004). All patients with cortical tubers fulfilled the diagnostic criteria for TSC (Gomez et al., 1999). For the GG we used the revised WHO classification of tumors of the CNS (Louis et al., 2007). The patients undergoing epilepsy surgery predominantly had medically intractable complex partial seizures. In addition, normal-appearing control cortex/white matter was obtained at autopsy from six young adult control patients (male/female: 3/3; mean age 31; range 18–35), without a history of seizures or other neurological diseases. All autopsies were performed within 12 h after death.

Human tissue preparation and immunocytochemistry

The tissue was fixed in 10% buffered formalin (autopsy tissue, for 2 weeks; surgical specimens, for 24 hours). To detect differences in labeling related to technical variables such as tissue fixation, we also tested the antibodies in specimens of selected regions (temporal cortex/ hippocampus) collected at autopsy and immediately fixed in formalin for 24 h (same fixation time used for the surgical specimens). Formalin fixed, paraffin-embedded tissue was sectioned at 6 μm and mounted on organosilane-coated slides (SIGMA, St. Louis, MO, USA). Specimens were processed for haematoxylin/eosin and Nissl, as well as for immunocytochemical reactions.

Glial fibrillary acidic protein (GFAP; polyclonal rabbit, DAKO, Glostrup, Denmark; 1:4000), vimentin (mouse clone V9, DAKO; 1:400), neuronal nuclear protein (NeuN; mouse clone MAB377, IgG1; Chemicon, Temecula, CA, USA; 1:1000), neurofilament (NF, SMI311; Sternberger Monoclonals, Lutherville, MD; 1:1000), microtubule-associated protein (MAP2; mouse clone HM2; Sigma 1:100), (HLA)-DP, DQ, DR (mouse clone CR3/43; DAKO, Glostrup, Denmark, 1:400), CD68 (mouse clone PG-M1, DAKO; 1:200) and CD31 (mouse JC/70A; 1:100), were used in the routine immunocytochemical analysis of epilepsy specimens.

For the detection of tPA we used a mouse anti-human tPA (American Diagnostica Inc., Greenwich, CT, USA; 1:50; previously used and characterized; Teesalu et al., 2004), a rabbit