



TISSUE PLASMINOGEN ACTIVATOR, NEUROSERPIN, AND SEIZURES

Ethanol-withdrawal Seizures Are Controlled by Tissue Plasminogen Activator via Modulation of NR2B-containing NMDA Receptors

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Chronic ethanol abuse causes upregulation of *N*-methyl-D-aspartate (NMDA) receptors, which underlies seizures and brain damage on ethanol withdrawal (EW). Here we show that tissue plasminogen activator (tPA), a protease implicated in neuronal plasticity and seizures, is induced in the limbic system by chronic ethanol consumption, temporally coinciding with upregulation of NMDA receptors. tPA interacts with NR2B-containing NMDA receptors and

is required for upregulation of the NR2B subunit in response to ethanol. As a consequence, tPA-deficient mice have reduced NR2B, extracellular signal-regulated kinase 1/2 phosphorylation, and seizures after EW. tPA-mediated facilitation of EW seizures is abolished by NR2B-specific NMDA antagonist ifenprodil. These results indicate that tPA mediates the development of physical dependence on ethanol by regulating NR2B-containing NMDA receptors.

Neuroserpin Portland (Ser52Arg) Is Trapped as an Inactive Intermediate That Rapidly Forms Polymers: Implications for the Epilepsy Seen in the Dementia FENIB

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The dementia, familial encephalopathy with neuroserpin inclusion bodies (FENIB), is caused by point mutations in the neuroserpin gene. We have shown a correlation between the predicted effect of the mutation and the number of intracerebral inclusions, and an inverse relation with the age at onset of disease. Our previous work has shown that the intraneuronal inclusions in FENIB result from the sequential interaction between the reactive center loop of one neuroserpin molecule with β -sheet A of the next. We show here that neuroserpin Portland (Ser52Arg), which causes a severe form of FENIB, also forms loop-sheet polymers but at a faster rate, in keeping with the more severe clinical phenotype. The Portland mutant has a normal unfolding transition in urea and a normal melting tempera-

ture but is inactive as a proteinase inhibitor. This results in part from the reactive loop being in a less accessible conformation to bind to the target enzyme, tissue plasminogen activator. These results, with those of the CD analysis, are in keeping with the reactive center loop of neuroserpin Portland being partially inserted into β -sheet A to adopt a conformation similar to an intermediate on the polymerization pathway. Our data provide an explanation for the number of inclusions and the severity of dementia in FENIB associated with neuroserpin Portland. Moreover, the inactivity of the mutant may result in uncontrolled activity of tissue plasminogen activator, and so explain the epileptic seizures seen in individuals with more severe forms of the disease.

COMMENTARY

Concerted action of extracellular proteases and their modulators are involved in controlling the turnover of proteins of the extracellular matrix. Plasminogen activators are

serine proteases that catalyze the conversion of plasminogen into plasmin. Plasmin, in turn, is a protease involved directly or indirectly in the degradation of most extracellular proteins. Two plasminogen activators have been identified in mammals: urokinase and tissue plasminogen activator (tPA). Plasminogen

activators are synthesized by a wide variety of cell types, including trophoblasts, monocytes/macrophages, and epithelial and endocrine cells.

One of the first tPA actions to be discovered is thrombolytic activity, stemming from its ability to degrade the insoluble fibrin mesh of a thrombus into soluble degradation products (1). tPA has been identified in the endothelium of the blood-brain barrier, where it appears to affect the cerebrovascular permeability and the vascular tone (2). tPA also is produced in neurons and glia in various CNS regions and is particularly abundant in the hippocampus, hypothalamus, cerebellum, and amygdala (3).

Neuroserpin is a member of the serpin (serine proteinase inhibitors) family and is expressed primarily in the brain (2), where it reacts preferentially with tPA by inhibiting its function. tPA, together with neuroserpin, is rapidly released from neurons in response to neuronal depolarization (4). Evidence indicates that tPA or plasmin-mediated extracellular proteolysis in the adult CNS may play a role in the structural changes associated with activity-dependent plasticity, including long-term potentiation, learning, kindling, and epileptogenesis (2). A role for tPA and neuroserpin also has been proposed in some CNS pathologies, including ischemic stroke, dementia, and multiple sclerosis (2).

In 1993, Qian and colleagues (5) showed that tPA is induced by pentylenetetrazol in the rodent brain within 1 hour of seizure onset. In addition, tPA^{-/-} mice show a higher threshold for the onset of seizures after systemic delivery of pentylenetetrazol or kainic acid (6), and these mice also are resistant to neurodegeneration induced by excitotoxins. These findings strongly suggest that tPA activity and its related downstream events regulate neuronal excitability in a way that alters seizure susceptibility and is involved in the associated neuronal cell loss. However, tPA-dependent plasminogen activation appears to mediate the action of tPA only in neurodegeneration but not in seizures.

Work by Yepes et al. demonstrates that endogenous tPA is involved in the generalization of seizures induced in rodents by intraamygdala injection of kainic acid and that this activity is plasminogen independent (7). The authors showed that tPA activity and neuroserpin expression were increased in the injected amygdala within 10 minutes after kainic acid delivery and that both subsequently increase in other forebrain regions involved in the spread of seizure activity. When neuroserpin was injected into the hippocampus immediately before intraamygdala application of kainate, the progression and generalization of seizures was attenuated. In particular, neuroserpin blocked the development of EEG seizures in the hippocampus, and the associated cell death was reduced concomitantly. Because neuroserpin is a natural inhibitor of tPA in the brain, these findings support the

hypothesis that tPA is involved in the generalization, but not in the induction, of seizures.

Plasminogen^{-/-} mice are resistant to neurodegeneration, but they do not show altered sensitivity to seizures. Therefore, tPA-dependent plasminogen activation appears to mediate the action of tPA only in neurodegeneration but not in seizures. One potential synaptic substrate for mediating the effect of tPA on seizures is represented by the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors, which are crucially involved in the propagation of seizures. Thus, tPA has been reported to potentiate NMDA-receptor signaling by interacting with its specific subunits NR1 and NR2B. The functional interaction between tPA and NMDA receptors was supported by the *in vivo* evidence that intrastriatal injections of recombinant tPA results in potentiation of NMDA-induced excitotoxic lesions (8).

The article by Pawlak et al. provides additional *in vivo* evidence linking tPA and NMDA-receptor activities. The study shows that tPA interacts with NR2B-containing NMDA receptors, by using a mouse model of ethanol intoxication. tPA is induced in the hippocampus and amygdala of mice during ethanol consumption, and its presence is required for the upregulation of the NR2 subunit in response to ethanol intoxication. Pawlak and colleagues suggest that tPA is one of the factors that facilitates the development of physical dependence on ethanol and that this effect is due to its functional interaction with the NMDA receptors. The authors' conclusions were supported by the following findings: 1) the severity of ethanol withdrawal symptoms (i.e., hypolocomotion and handling-induced seizures) was drastically decreased in tPA^{-/-} mice but not in plasminogen^{-/-} mice; and 2) the injection of tPA in tPA^{-/-} mice during the withdrawal symptoms increased seizure severity, and this effect was blocked by ifenprodil, a specific inhibitor of NR2B subunit. This study suggests that the effect of tPA on NR2B subunit is nonproteolytic but rather is a result of physical binding to a still unidentified site. This interaction also allows phosphorylation of the NR2B subunit and is required for receptor upregulation, thus leading to a potential state of hyperexcitability, uncovered by the rapid withdrawal of ethanol.

The experimental evidence on the involvement of endogenous tPA in seizures is supported by human findings. Thus, genetic point mutations in the neuroserpin gene are associated with aberrant deposition of neuroserpin in neurons, resulting in the formation of intracytoplasmic bodies containing the mutant protein. A human neuroserpin gene mutation, which causes an arginine-for-serine substitution at residue 52 (S52R), is characterized by generalized seizures, myoclonus, and progressive dementia, that is, progressive myoclonus epilepsy. Studies by Takao and Belorgey (9) provide a detailed analysis of the

distribution of the brain lesions associated with this mutation. In addition, Belorgey and colleagues show that the number of intracellular neuroserpin inclusions in cortical and subcortical neurons is inversely correlated with age at onset of the disease. Importantly, they provide evidence that the mutated neuroserpin is inactive as a proteinase inhibitor, suggesting that the inactivity of this enzyme, which is, at least in part, due to changes in this conformation, may result in uncontrolled activity of tPA and, thus, explain the epileptic seizures observed in the individuals carrying the mutation. In keeping with this observation, epilepsy is far more common with Ser52Arg neuroserpin than with other mutations of this gene, such as Ser49Pro neuroserpin, which remains partly active as a proteinase inhibitor. In summary, the clinical evidence suggests that the inactivity of Ser52Arg neuroserpin in individuals carrying this mutation may exacerbate the intrinsic ability of the intracellular inclusion to cause epilepsy.

tPA and neuroserpin are therefore among the numerous endogenous factors that may contribute to the mechanisms of hyperexcitability in the CNS. Although they subservise physiologic roles inside and outside the CNS, an imbalance in their relative production or function may lead to exaggerated tPA activity in brain, thus resulting in harmful effects, including seizures and neuronal cell loss. These studies suggest new avenues for therapeutic intervention. Neuroserpin, because of its unique role as a modulator of tPA, could represent a potential target for epilepsy therapy. The identification of novel tPA substrates also may suggest new downstream treatment approaches.

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