

Is tissue plasminogen activator a threat to neurons?

The clot-busting drug tissue plasminogen activator (tPA) is currently the only FDA-approved therapy for acute stroke. However, increasing evidence suggests that tPA can also contribute to excitotoxic neuronal damage in animal models of stroke. (pages 59–64)

Stroke, which results from a rupture or obstruction (as by a clot) of an artery of the brain, causes devastating complications and represents a global health problem. According to the National Center for Health Statistics, over 3.3 million people in the United States alone suffer from stroke symptoms. The clot-busting blood protease tissue plasminogen activator (tPA), which has been successfully used to treat myocardial infarction, has been approved for treatment of occlusive stroke. However, tissue-plasminogen activator (tPA) is also believed to have direct effects on neurons, an important factor to consider in evaluating tPA's therapeutic potential for stroke victims. In this issue, Nicole *et al.*¹ report that the proteolytic activity of tPA affects neuronal N-methyl-D-aspartate (NMDA) receptor-mediated signaling.

In the blood, tPA converts plasminogen to plasmin, a protease that dissolves blood clots². As the first treatment targeted at the precipitating event in occlusive stroke (blood vessel blockage), tPA represents a significant therapeutic step forward. However, results from murine stroke models suggest that tPA may also mediate neuronal death^{3–6}. In a mouse model of stroke using an intraluminal suture, rather than a blood clot, to cause temporary occlusion of the middle cerebral artery, intravenous injection of tPA produced larger infarcts, indicating that tPA can increase stroke-induced injury⁶. Moreover, in the same model, tPA-deficient mice exhibited approximately 50% smaller cerebral infarcts than wild-type mice with similar genetic backgrounds. Although there has been some controversy surrounding these results, the work has been replicated and several different groups have

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reported that in addition to its beneficial clot-busting action, tPA can induce neuronal damage^{4,5,7,8}.

A large amount of basic research has recently been focused on the role of tPA in neuronal function and cell survival. The findings of Nicole *et al.*¹ expand the scope of tPA's complex effects by showing that it can potentiate NMDA-receptor-mediated neurotoxicity. The NMDA receptor is a glutamate-gated ion channel that is highly permeable to Ca⁺⁺. However, excessive influx of Ca⁺⁺ via this route can lead to a series of dire cellular consequences, including free radical formation, injury and cell death.

Nicole *et al.*¹ used co-immunoprecipitation experiments to demonstrate an apparently direct association between tPA and the NR1 subunit of the NMDA receptor. They conclude that tPA contributes to overactivation of NMDA receptors and exacerbation of neuronal death. Activation of the thrombin receptor PAR1 has also been shown to potentiate NMDA receptor function⁹, so a link between blood pro-

teases and NMDA receptors has already been established.

Nicole *et al.*¹ also observed that tPA cleaves the NR1 subunit of the NMDA receptor, removing a fragment of approximately 15–20 kD from its amino terminus (Fig. 1). Ca⁺⁺ imaging results suggest that this cleavage leads to the increased activity of the NMDA receptor. The authors, however, have not ruled out the possibility that the co-immunoprecipitation of NR1 and tPA is accompanied by other unidentified proteins that could be involved in the cleavage of the NR1 subunit. An analysis of recombinant NR1 proteolytic cleavage fragments, along with an NR1 mutational analysis, could be used to identify tPA's cleavage site. Interestingly, the serine protease thrombin also cleaves the NR1 subunit in both neurons and recombinant receptors⁹, suggesting NR1 may have structural determinants that enhance its interactions with blood-derived proteases.

It is tempting to extrapolate these results into a simple pathway in which tPA potentiation of NMDA-receptor function alone accounts for the induction of neuronal death; however, there are many other effects of tPA that merit consideration. For example, tPA has been observed to interact with the low-density lipoprotein receptor-related protein to influence neuronal function¹⁰. In addition, tPA and any plasmin that is formed by tPA proteolysis of plasminogen may cleave or activate other cell surface substrates. These include laminin, matrix metalloproteinases⁵ and elements of the immune complement system, which would affect cell adhesion, function and survival after NMDA receptor overactivation.

Furthermore, Nicole *et al.*¹ have not provided evidence that tPA cleavage of NR1 directly alters NMDA receptor func-

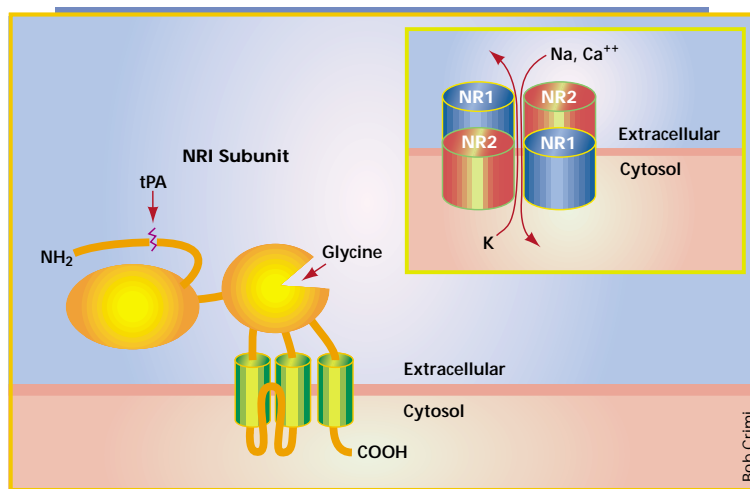


Fig. 1 The NMDA receptor is probably composed of four subunits (inset), one of which, NR1, is illustrated here. NR1 also contains a glycine binding site and an intracellular C-terminus. Nicole *et al.*¹ report that tPA cleaves the NR1 subunit near the N-terminus. This truncated NMDA receptor potentiates the Ca⁺⁺ influx by an unidentified mechanism. The authors suggest that tPA cleavage and increased Ca⁺⁺ influx enhances NMDA receptor-mediated neuronal damage.



tion. NR1 cleavage may inactivate the receptor, have no effect on receptor function, or somehow enhance receptor activity. Future studies must directly assay the effects of tPA through patch-clamp recordings of different cells that express wild-type NMDA receptors, a truncated recombinant form of the NMDA receptor, or a mutated recombinant NMDA receptor that is resistant to tPA proteolysis.

It will also be important to elucidate the mechanism by which alteration of NMDA receptor function affects neuron survival. Nicole *et al.*¹ report that tPA treatment enhances the NMDA-evoked Ca⁺⁺ influx, which could be blocked by co-application of an NMDA receptor antagonist. If accumulation of intracellular Ca⁺⁺ underlies the enhanced neuron death observed in the *in vitro* excitotoxicity assay, these findings suggest that neuronal survival hangs in a very precarious balance, such that even modest increases in the Ca⁺⁺ load induce cell death. Given these questions, the picture of just what tPA is doing seems likely to get murkier before it becomes clear.

Nevertheless, the study suggests interesting new molecular relationships and

generates working hypotheses to frame important questions. Despite the need for additional work to understand the link between tPA, NMDA receptors and cell death, the findings are an important step forward. The observed interactions between NR1 and tPA are intriguing from both a biochemical and functional perspective, since tPA has been shown to be released by neuronal PC12 cells in a Ca⁺⁺ dependent fashion¹¹. If we are able to eventually understand the mechanism by which tPA interacts with neurons to induce neurodegeneration, we may identify new targets for drugs that promote neuroprotection during stroke therapy.

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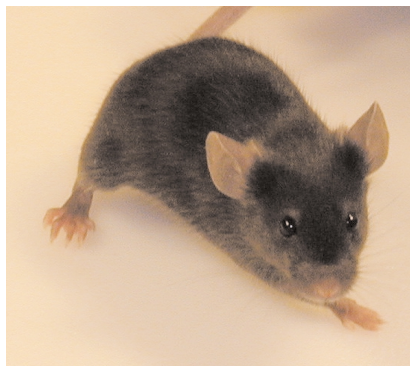
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Amyloid β vaccination: reduced plaques and improved cognition

Studies in three different transgenic mouse models suggest that the amyloid β -protein contributes to memory loss in Alzheimer disease. Immunization with an amyloid β -peptide fragment reduces learning and memory impairments in mice, and this approach may eventually be used to prevent and/or treat this disease in people.

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evidence that learning and memory defects are related to A β accumulation, and two of the reports^{2,3} show that A β immuniza-



Memory deficits in Tg2576 transgenic mice, which overexpress mutant APP, can be reversed by A β_{1-42} vaccination.

tion can reduce these deficits.

The abundant evidence that A β aggregation is an essential early event in AD pathogenesis has prompted an intensive search for therapeutics that target A β . This search has been aided immeasurably by transgenic mouse models of AD. These models overexpress mutant forms of APP and Presenilin 1 (PS1), which cause early onset familial AD. This overexpression causes an age-dependent accumulation of A β plaques that are quantitatively and qualitatively similar to those of the AD brain, but does not reproduce the complete phenotype observed in human AD brain, as neurofibrillary tangles do not develop and there is considerably less neuron and synapse loss. The current AD transgenic models are an excellent tool for evaluating therapies that target A β , and they can be used to determine if age-dependent

Alzheimer disease (AD) is the most common form of dementia in the elderly. Currently there is no effective treatment for this disorder, which is projected to affect 22 million people worldwide by 2025. Two lesions (senile plaques and neurofibrillary tangles) are invariably found in the brains of AD patients. Neurofibrillary tangles are composed primarily of abnormally phosphorylated τ -proteins. The major protein component of senile plaques is the amyloid β -protein (A β), a secreted protein released through stepwise proteolytic cleavage of the amyloid β -protein precursor (APP). Most A β has 40 amino acids (A β_{40}) but a minor, slightly longer form (A β_{42}) is believed to be the major pathogenic species in AD. Three studies, published in the 21/28 December issue of *Nature* by Chen *et al.*¹, Janus *et al.*² and Morgan *et al.*³, provide new evi-