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Tissue-type plasminogen activator protects neurons from excitotoxin-induced cell death via activation of the ERK 1/2–CREB–ATF3 signaling pathway

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ABSTRACT

The release of the serine proteinase tissue-type plasminogen activator (tPA) from cerebral cortical neurons has a neuroprotective effect in the ischemic brain. Because excitotoxicity is a basic mechanism of ischemia-induced cell death, here we investigated the effect of tPA on excitotoxin-induced neuronal death. We report that genetic overexpression of neuronal tPA or treatment with recombinant tPA renders neurons resistant to the harmful effects of an excitotoxic injury in vitro and in vivo. We found that at concentrations found in the ischemic brain, tPA interacts with synaptic but not extrasynaptic NMDARs. This effect is independent of tPA's proteolytic properties and leads to a rapid and transient phosphorylation of the extracellular signal regulated kinases 1/2 (ERK 1/2), with ERK 1/2-mediated activation of the cAMP response element binding protein (CREB) and induction of the neuroprotective CREB-regulated activating transcription factor 3 (Atf3). In line with these observations, Atf3 down-regulation abrogates the protective effect of tPA against excitotoxin-induced neuronal death. Our data indicate that tPA preferentially activates synaptic NMDARs via a plasminogen-independent mechanism turning on a cell signaling pathway that protects neurons from the deleterious effects of excitotoxicity.

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Introduction

Excitotoxicity has been linked to cell death in several pathological conditions of the central nervous system (CNS) including cerebral ischemia, trauma and seizures (Choi, 1988). N-methyl-D-aspartate receptors (NMDARs) are calcium-permeable ion channels that activate several intracellular signaling pathways that mediate not only physiological processes such as neuronal plasticity, learning and memory (Mori and Mishina, 1995), but also pathological events such as excitotoxin-induced neuronal death (Choi, 1987). NMDARs are assembled by obligatory NR1 sub-units that interact with NR2A–D subunits. Most of the NR2A-containing NMDARs are located in the synapses and their activation has been coupled to neuronal survival (Liu et al., 2007). In contrast, the majority of NR2B-containing NMDARs are extrasynaptic and linked to the activation of cell-death pathways (Hardingham and Bading, 2010).

Tissue-type plasminogen activator (tPA) is a serine proteinase that is found in the intravascular space and in the CNS where it plays a pivotal role in the development of synaptic plasticity, learning and memory via plasminogen-dependent and -independent mechanisms (Samson and Medcalf, 2006). A link between tPA and NMDARs has been derived from experimental evidence indicating that tPA enhances the effect of NMDA on intracellular calcium concentrations (Nicole et al., 2001). Several studies have reported that treatment with concentrations of tPA greater than 200 nM (Guo et al., 2011; Nicole et al., 2001) potentiates the harmful effect of an excitotoxic injury, which together with other observations (Tsirka et al., 1997), have led to postulate the hypothesis that tPA mediates excitotoxin-induced neuronal death.

The extracellular signal regulated kinases 1 and 2 (ERK 1/2) are members of the mitogen-activated protein kinase family that regulate cellular responses to a variety of extracellular stimuli, and mediate several effects of tPA in the CNS (Pawlak et al., 2003). The role of ERK 1/2 activation on cell survival is still controversial. Indeed, whereas some studies indicate that sustained ERK 1/2 activation leads to neuronal death (Lesuisse and Martin, 2002), others have shown that transient activation of ERK 1/2 turns on several neuroprotective signaling pathways in the CNS (Luo and DeFranco, 2006).

The cAMP response element binding protein (CREB) is a multifunctional transcriptional regulator that plays an important role in neuronal survival (Hardingham et al., 2002). Recent evidence indicates that CREB-mediated induction of the activating transcription factor 3 (Atf3) protects neurons from the harmful effects of

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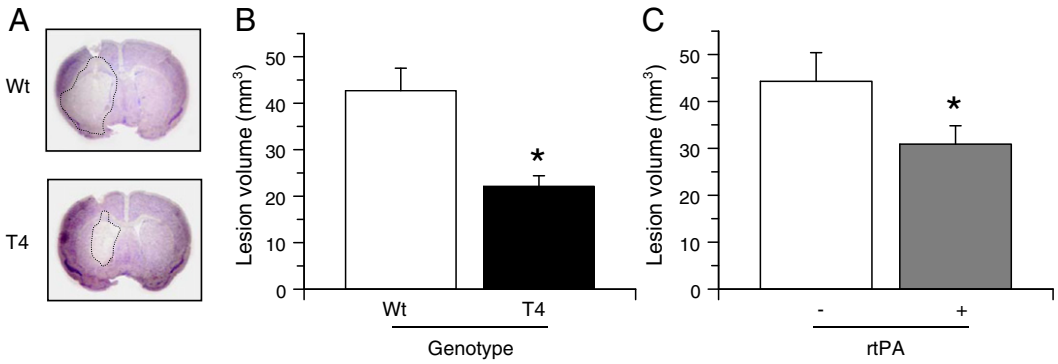

Fig. 1. tPA protects the brain from excitotoxin-induced cell death. (A) Representative thionin-stained brain sections of T4 mice and their wild-type (Wt) littermate controls 24 h after the intrastriatal injection of NMDA. (B) and (C) mean volume of the lesion 24 h after the intrastriatal injection of NMDA in T4 mice and their wild-type (Wt) littermate controls (B) and in Wt mice treated with rtPA 1 mg/kg/IV (+) or a comparable volume of saline solution (−) after the injection of NMDA (C). n = 12 per experimental group in B and 11 in C. * in B: $p<0.05$ compared to Wt littermate controls. * in C: $p<0.05$ compared to Wt mice treated with saline solution. Lines denote SD.

extrasynaptic NMDARs activation. Indeed, CREB–Atf3 signaling is controlled by synaptic NMDARs and is the central component of a neuroprotective response in the brain (Zhang et al., 2011). In the work presented here, we show that either the release of neuronal tPA, treatment of neuronal cultures with 5 nM of tPA, or the intravenous administration of rtPA in an in vivo model of excitotoxic injury, protects the brain from excitotoxin-induced neuronal death, and that this effect is independent of tPA's ability to cleave plasminogen into plasmin. We report that tPA activates synaptic NR2A-containing NMDARs and turns on the ERK 1/2–CREB–Atf3 pro-survival pathway. Our data describe a novel neuroprotective pathway for tPA in the CNS with clinical implications for the potential development of a therapeutic strategy to promote cell survival in patients with neurological conditions associated with excitotoxin-induced neuronal death.

Results

Effect of tPA on excitotoxin-induced neuronal death

To study the role of tPA on excitotoxin-induced neuronal death, T4 mice and their Wt littermate controls were injected with NMDA into the striatum followed by determination of the volume of the lesion as described in the Experimental methods section. We found that T4 mice have a 48.26% decrease in the volume of NMDA-induced lesion (42.72 ± 4.8 mm^3 in Wt and 22.10 ± 2.3 mm^3 in T4 mice; Fig. 1, $p<0.05$), suggesting that neuronal tPA has a protective effect against excitotoxin-induced cell death. Then we performed similar observations in Wt mice treated with 1 mg/kg/IV of rtPA or a comparable volume of saline solution immediately after the intrastriatal injection of NMDA. In agreement with our observations in T4 mice, we found that treatment with rtPA induces a 27.62% decrease in the volume of NMDA-induced lesion (Fig. 1, $p<0.05$).

Effect on cell survival of co-treatment with tPA and NMDA

Because it has been reported that tPA potentiates NMDA-induced neuronal death (Reddrop et al., 2005), we used the MTT and LDH release assays to study cell survival and death in neurons incubated with either 50 μM of NMDA, 5–500 nM of either proteolytically active tPA (atPA) or with tPA with an alanine for serine substitution at the active site Ser481 (proteolytically inactive tPA; itPA), or with a combination of 50 μM of NMDA and 5–500 nM of either atPA or itPA. Our results indicate that, as previously described (Haile et al., 2012), treatment with tPA alone does not induce neuronal death. In contrast, neuronal survival decreased from $100 \pm 1.9\%$ in control cells to $53.63 \pm 2.86\%$ in cells treated with NMDA alone. Surprisingly, co-treatment with 5 or 10 nM of tPA increased cell survival from $53.63 \pm 2.86\%$ (in

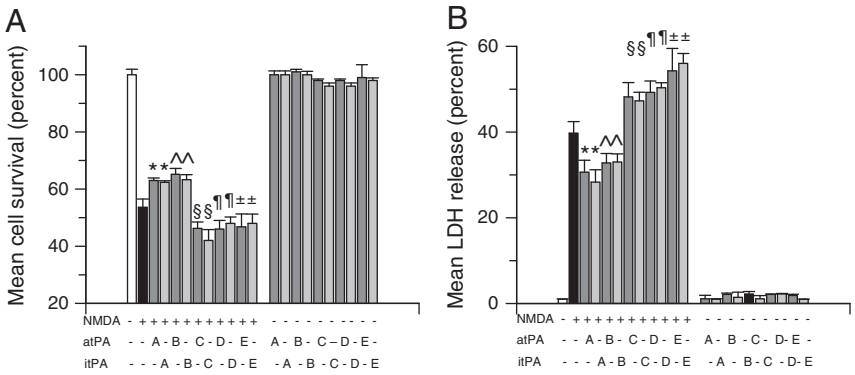

Fig. 2. Dose-dependent effect of tPA on NMDA-induced neuronal death. Mean neuronal survival (A) and LDH release (B) in wild-type cerebral cortical neurons incubated with NMDA alone, or with 0–500 nM of proteolytically active (atPA; dark gray bars) or inactive (itPA; light gray bars) tPA alone, or with a combination of NMDA and 0–500 nM of atPA or itPA. Lines denote SD. n = 22 per experimental group in A and B, respectively. * and ^ in A and B: $p<0.05$ compared to neurons incubated with NMDA alone or with neurons co-incubated with NMDA and 100 nM, or 200 nM or 500 nM of tPA. §, ¶ and +/− in A and B: $p<0.05$ compared to neurons incubated with NMDA alone or with neurons co-incubated with NMDA and 5 or 10 nM of tPA. A–E denotes doses of tPA used in each observation.