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Involvement of Tissue Plasminogen Activator-Plasmin System in Depolarization-Evoked Dopamine Release in the Nucleus Accumbens of Mice

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ABSTRACT

Tissue plasminogen activator (tPA), a serine protease, catalyzes the conversion of plasminogen to plasmin. In the present study, we investigated the role of the tPA-plasmin system in depolarization-evoked dopamine (DA) and acetylcholine (ACh) release in the nucleus accumbens (NAc) and hippocampus, respectively, of mice, by using in vivo microdialysis. Microinjection of either tPA or plasmin significantly potentiated 40 mM KCl-induced DA release without affecting basal DA levels. In contrast, plasminogen activator inhibitor-1 dose-dependently reduced 60 mM KCl-induced DA release. The 60 mM KCl-evoked DA release in the NAc was markedly diminished in tPA-deficient (tPA−/−) mice compared with wild-type mice, although basal DA levels did not differ between the two groups. Microinjections of either exogenous tPA (100 ng) or plasmin (100 ng) into the NAc of tPA−/− mice restored 60 mM KCl-induced DA release, as observed in wild-type mice. In contrast, there was no difference in either basal or 60 mM KCl-induced ACh release in the hippocampus between wild-type and tPA−/− mice. Our findings suggest that the tPA-plasmin system is involved in the regulation of depolarization-evoked DA release in the NAc.

Dopamine (DA) is a major neurotransmitter within the mammalian central nervous system. DA-containing neurons arise mainly from DA cell bodies in the substantia nigra and ventral tegmental area in the midbrain. The mesolimbic DAergic system that originates in the midbrain tegmentum and projects to the nucleus accumbens (NAc) and lateral septal nuclei of the basal forebrain and the amygdala, hippocampus, and entorhinal cortex, all of which are considered components of the limbic system, are of particular interest for the pathophysiology of idiopathic psychiatric disorders, such as schizophrenia, Parkinson's disease, and attention-deficit/hyperactivity disorder (Tarazi, 2001). It is well-established that drugs of abuse, such as alcohol, nicotine, and cocaine share the properties of activating the mesolimbic DAergic neurons and elevating DA levels at their terminals in the NAc (Koob and Weiss, 1992; Pontieri et al., 1996). In schizophrenia, positive symptoms (such as hallucinations) are associated with increased subcortical DA neurotransmission, whereas negative and cognitive symptoms may be related to impaired mesocortical DA function (Seeman et al., 1976; Abi-Dargham and Moore, 2003). Parkinson's disease is a neurodegenerative disorder in which the most predominant pathological feature is the progressive loss of mesencephalic DAergic neurons (Goldberg et al., 2005; Jin et al., 2005). Malfunction of the DAergic system may represent a central factor in the etiology of attention-deficit/hyperactivity disorder (Volkow et al., 2001; Johansen and Sagvolden, 2005). Therefore, it is important to clarify the molecular mechanism behind the regulation of DAergic neurotransmission to de-

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ABBREVIATIONS: DA, dopamine; ACh, acetylcholine; BDNF, brain-derived neurotrophic factor; DA dopamine; NAc, nucleus accumbens; PAI-1, plasminogen activator inhibitor-1; PAR1, protease activated receptor-1; tPA, tissue plasminogen activator; aCSF, artificial cerebrospinal fluid; HPLC, high-performance liquid chromatography; ANOVA, analysis of variance; BSA, bovine serum albumin; tPA−/−, tissue plasminogen activator-deficient.

velop novel approaches to the treatment of these psychiatric disorders.

Tissue plasminogen activator (tPA) is a serine protease that catalyzes the conversion of plasminogen to plasmin and plays an important role in fibriolysis. It has been demonstrated that tPA is stored in synaptic vesicles in the central nervous system (Gualandris et al., 1996) and released into the extracellular space by depolarization from catecholaminergic vesicles in PC12 cells (Parmer et al., 1997). Accumulating evidence suggests that tPA plays a crucial role in cell migration, neuronal development and death, learning and memory, and anxiety-related behavioral and hormonal responses (Baranes et al., 1998; Seeds et al., 1999; Calabresi et al., 2000; Chen et al., 2003; Matys et al., 2004).

We have demonstrated that the tPA-plasmin system participates in the development of drug dependence (Yamada et al., 2005). Methamphetamine and morphine increased the expression of tPA and its enzyme activity in the NAc (Nagai et al., 2004, 2005b). The rewarding and locomotor-sensitizing effects of methamphetamine and morphine were markedly reduced in tPA-deficient (tPA−/−) mice. In tPA−/− mice, morphine-induced DA release in the NAc was significantly reduced compared with that in wild-type mice. Furthermore, microinjections of either exogenous tPA or plasmin potentiated morphine-induced DA release in the NAc, whereas plasminogen activator inhibitor-1 (PAI-1) inhibited morphine-induced DA release in the NAc (Nagai et al., 2005a). Our previous findings suggest that the tPA-plasmin system is involved in the modulation of DAergic neuronal function in the NAc induced by drugs of abuse. It is noteworthy that depolarization-evoked DA release in the NAc was markedly reduced in tPA−/− mice compared with wild-type mice (Nagai et al., 2004), suggesting that the modulation of DA release induced by the tPA-plasmin system is physiologically important. In the present study, we investigated the role of the tPA-plasmin system in the depolarization-evoked release of DA in the NAc of mice by using in vivo microdialysis.

Materials and Methods

Animals. Male ICR mice (7–8 weeks old) were obtained from Japan SLC Inc. (Shizuoka, Japan). Wild-type (C57BL/6J) and tPA−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME). tPA−/− mice used in this study were obtained by crossing F12 heterozygous tPA+/− mice having a 99.99% pure C57BL/6J genetic background. All animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of Kanazawa University.

In Vivo Microdialysis. For the analysis of DA release, animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and a guide cannula (MI-AG-6; Eicom Corporation, Kyoto, Japan) was implanted in the NAc (+1.5 mm anteroposterior, +0.8 mm mediolateral from the bregma, −4.0 mm dorsoventral from the skull) according to the atlas of Franklin and Paxinos (1997). On recovery from the surgery, a dialysis probe equipped with a microinjection tube (MIA-6-1, 1 mm membrane length; Eicom) was inserted through the guide cannula and was perfused with an artificial cerebrospinal fluid (aCSF; 147 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) at a flow rate of 1.0 μl/min. The microdialysis probes were constructed of three stainless steel tubes, two silica tubes (an inlet and an outlet) for microdialysis with a 75 μm o.d., and a microinjection silica tube with a 75 μm o.d. The microinjection tube was placed in parallel with the tubes for microdialysis. The microinjection tube was half the length of the dialysis membrane. These three silica tubes were sealed together with epoxy resin, and each one was secured with stainless steel tubing at the top of the probe. Outflow fractions were collected every 20 min. After the collection of three baseline fractions, PAI-1 (0.3–3 ng; Calbiochem, Darmstadt, Germany), human recombinant tPA (30–100 ng; provided by Eisai Co. Ltd., Tokyo, Japan), or human plasmin (30–100 ng; Chromogenix, Milan, Italy) dissolved in 1 μl of 0.1% BSA-containing aCSF solution was injected during a 10-min period through the microinjection tube into the NAc (Nagai et al., 2004). Ten minutes after the microinjection, high potassium-containing aCSF (40 or 60 mM; isomolar replacement of NaCl with KCl) was perfused for 20 min through the dialysis probe. DA levels in the dialysates were analyzed using an HPLC system equipped with an electrochemical detector (Nagai et al., 2004).

For the analysis of acetylcholine (ACh) release, a guide cannula (AG-4; Eicom Corporation) was implanted in the hippocampus (−3.3 mm anteroposterior, +3.2 mm mediolateral from the bregma, −2.5 mm dorsoventral from the skull). On recovery from the surgery, a dialysis probe (AI-4-2, 2 mm membrane length; Eicom Corporation) was inserted through the guide cannula, and perfused with an aCSF containing 10 μM eserin at a flow rate of 1.0 μl/min. Outflow fractions were collected every 15 min. After the collection of three baseline fractions, 60 mM KCl-containing aCSF was perfused for 30 min. ACh levels in the dialysates were analyzed using an HPLC system equipped with an electrochemical detector (Tran et al., 2001).

Statistical Analysis. All data were expressed as the mean ± S.E. In the analysis of the time course for the microdialysis, an analysis of variance (ANOVA) with repeated measures was used followed by the Bonferroni test when F ratios were significant ($p < 0.05$).

Results

Effect of tPA on Depolarization-Evoked DA Release in the NAc of ICR Mice. First, we studied the effect of depolarization stimulus on DA release in the NAc of ICR mice by in vivo dialysis. Perfusion of 40 or 60 mM KCl-containing aCSF for 20 min through the dialysis probe resulted in a concentration-dependent increase in extracellular DA levels ($p < 0.01$, Fig. 1). KCl (60 mM) significantly increased extra-

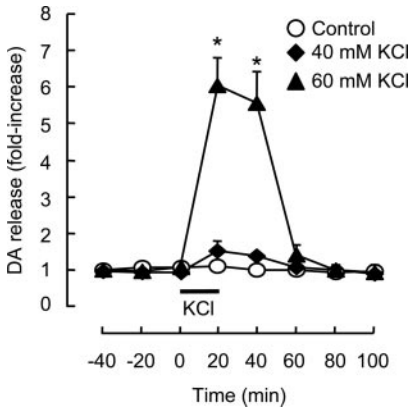

Fig. 1. Depolarization-evoked DA release in the NAc of ICR mice measured by in vivo dialysis. A dialysis probe was inserted through the guide cannula and was perfused with an aCSF at a flow rate of 1.0 μl/min. After the collection of three baseline fractions, either aCSF (control) or high potassium-containing aCSF (40 or 60 mM KCl) was perfused for 20 min through the dialysis probe. There was no difference in basal levels of DA (nanomolar levels) before the stimulation among three groups: control (4 mM KCl), 0.47 ± 0.02 ($n = 3$); 40 mM KCl, 0.54 ± 0.15 ($n = 4$); and 60 mM KCl, 0.41 ± 0.10 ($n = 6$). An ANOVA with repeated measures was conducted: group [$F(2, 12) = 46.763$, $p < 0.01$]; time [$F(4, 48) = 16.267$, $p < 0.01$]; and group-by-time interaction [$F(8, 48) = 13.522$, $p < 0.01$]. Values represent the mean ± S.E. *, $p < 0.05$ compared with the corresponding control (4 mM KCl) group.

cellular DA levels in the NAc at 20 and 40 min after the depolarization treatment ($p < 0.05$, Fig. 1).

To investigate the role of tPA-plasmin system in depolarization-evoked DA release, we studied the effect of the microinjection of recombinant tPA into the NAc on KCl-evoked DA release in the NAc of ICR mice. Microinjection of tPA (30 and 100 ng/site) dose-dependently increased the 40 mM KCl-evoked release of DA ($p < 0.01$; Fig. 2A). The microinjection of tPA (100 ng/site) into the NAc significantly potentiated the depolarization-evoked increase in extracellular DA levels in the NAc at 20 and 40 min after depolarization treatment ($p < 0.05$, Fig. 2A). However, microinjection of tPA failed to potentiate DA release in the NAc when a strong depolarization stimulus (60 mM KCl) was applied (Fig. 2B). Basal extracellular DA levels in the NAc were not affected by the microinjection of tPA (100 ng/site) (data not shown).

Effect of Plasmin on Depolarization-Evoked DA Release in the NAc of ICR Mice. We also investigated the effect of plasmin on the 40 mM KCl-evoked DA release (Fig. 3). The microinjection of plasmin (30 and 100 ng/site) into the NAc significantly potentiated the 40 mM KCl-evoked increase in extracellular DA levels in the NAc at 20 and 40 min after depolarization treatment ($p < 0.05$, Fig. 3). Basal extracellular DA levels in the NAc were not affected by plasmin (100 ng/site) (data not shown).

Effect of PAI-1 on Depolarization-Evoked DA Release in the NAc of ICR Mice. The effect of PAI-1, an endogenous inhibitor of tPA, on depolarization-evoked DA release is shown in Fig. 4. The microinjection of PAI-1 (0.3–3 ng/site) into the NAc dose-dependently inhibited the 60 mM KCl-evoked increase in extracellular DA levels ($p < 0.01$). The microinjection of PAI-1 (1 and 3 ng/site) into the NAc significantly inhibited the 60 mM KCl-evoked increase in extracellular DA levels in the NAc at 40 min after depolarization treatment ($p < 0.05$, Fig. 4). The microinjection of PAI-1 at a dose of 3 ng/site into the NAc significantly decreased basal extracellular DA levels to approximately 55.5% of control levels at 60 to 120 min after PAI-1 microinjection (data not shown).

Defect of Depolarization-Evoked DA Release in the NAc of tPA−/− Mice and Its Rescue by Exogenous tPA and Plasmin. We have demonstrated previously that 60 mM KCl-evoked DA release is significantly attenuated in the tPA−/− mice (Nagai et al., 2004). As shown in Fig. 5A, extracellular DA levels in the NAc of wild-type mice were significantly increased by 60 mM KCl stimulation, and the 60 mM KCl-evoked DA release was markedly diminished in tPA−/− mice ($p < 0.01$). The 60 mM KCl-evoked DA release was markedly diminished in tPA−/− mice compared with wild-type mice at 20 and 40 min after depolarization treatment ($p < 0.05$ and <0.01, respectively; Fig. 5A). Basal levels of DA in the NAc did not differ between wild-type and tPA−/− mice.

We investigated whether the defect of depolarization-evoked DA release in tPA−/− mice is rescued by exogenous tPA or plasmin. Microinjection of either tPA (100 ng) or plasmin (100 ng) into the NAc significantly increased the 60 mM KCl-evoked DA release in tPA−/− mice as observed in wild-type mice ($p < 0.05$). The microinjection of tPA (100 ng/site) and plasmin (100 ng/site) into the NAc significantly increased the 60 mM KCl-evoked DA release at 20 min ($p < 0.05$ for tPA, $p < 0.01$ for plasmin) and 40 min ($p < 0.01$ for tPA, $p < 0.05$ for plasmin) after depolarization treatment (Fig. 5, B and C).

Depolarization-Evoked ACh Release in the Hippocampus of tPA−/− Mice. It is well-recognized that depolarization increases the release of not only DA but also other neurotransmitters such as ACh (Nilsson et al., 1990). Because it is possible that the release of ACh may also be modulated by the tPA-plasmin system, we examined the 60 mM KCl-evoked ACh release in the hippocampus of tPA−/− mice. Basal levels of ACh in the hippocampus did not differ

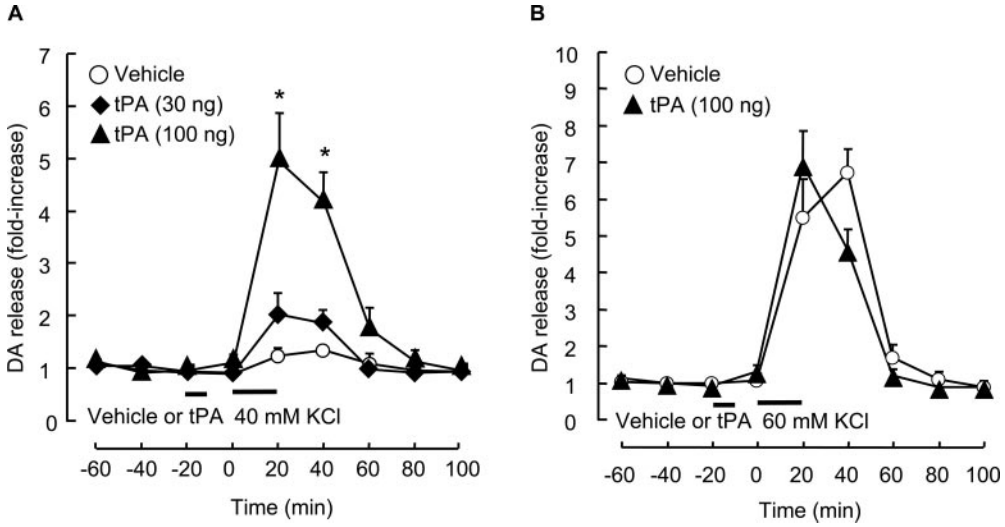

Fig. 2. Effect of tPA on 40 (A) or 60 mM (B) KCl-evoked DA release in the NAc of ICR mice. A dialysis probe equipped with a microinjection tube was inserted through the guide cannula and was perfused with aCSF at a flow rate of 1.0 μl/min. After the collection of three baseline fractions, 1 μl of either tPA (30 or 100 ng) or vehicle (0.1% BSA)-containing aCSF solution was injected during a 10-min period through the microinjection tube into the NAc. Ten minutes after the microinjection, 40 (A) or 60 mM (B) KCl-containing aCSF was perfused for 20 min through the dialysis probe. There was no difference in basal levels of DA (nanomolar levels) before the drug treatment among three groups: A, vehicle + 40 mM KCl, 0.41 ± 0.07 (n = 4); tPA (30 ng) + 40 mM KCl, 0.45 ± 0.07 (n = 6); and tPA (100 ng) + 40 mM KCl, 0.43 ± 0.05 (n = 4). B, vehicle + 60 mM KCl, 0.42 ± 0.13 (n = 4); and tPA (100 ng) + 60 mM KCl, 0.43 ± 0.06 (n = 4). Values represent the mean ± S.E. An ANOVA with repeated measures for Fig. 2A was conducted: group [$F(2,12) = 53.307$, $p < 0.01$]; time [$F(4,48) = 25.005$, $p < 0.01$]; and group-by-time interaction [$F(8,48) = 7.873$, $p < 0.01$]. *, $p < 0.05$ compared with the corresponding vehicle-treated group.

between wild-type and tPA−/− mice. Moreover, there was no difference in the 60 mM KCl-evoked hippocampal ACh release between the two groups (Fig. 6).

Discussion

In this study, we investigated the role of the tPA-plasmin system in depolarization-evoked release of DA in the NAc of mice by using a microdialysis technique. We demonstrated that a weak (40 mM KCl) depolarization-evoked release in the NAc was potentiated by either tPA or plasmin, whereas a strong (60 mM KCl) depolarization occluded the effect of tPA on DA release. Therefore, it is plausible that the stimulating effect of exogenous tPA on depolarization-evoked DA release may depend on the level of endogenous tPA released into the extracellular space. A strong depolarization (60 mM KCl) may cause a large amount of tPA to be released, resulting in the saturation of the stimulating effect of endogenous tPA. Thus, exogenous tPA microinjected into the NAc failed to potentiate the strong depolarization-evoked DA release. In contrast, a weak (40 mM KCl) depolarization-evoked DA release was potentiated by exogenous tPA because the effect of the endogenous tPA released by such a weak depolarization may be minimal. Therefore, it is likely that the modulation of depolarization-evoked DA release in the NAc by tPA may be physiologically relevant.

In contrast to the stimulating effect of tPA and plasmin on DA release, PAI-1, a primary physiological inhibitor of tPA and urokinase plasminogen activator (Loskutoff et al., 1989), significantly attenuated the basal and 60 mM KCl-evoked DA release in the NAc, suggesting the involvement of endogenous tPA in the modulation of DA release. To further confirm the role of endogenous tPA for DA release, we measured depolarization-evoked DA release in tPA−/− mice. The 60 mM KCl-induced DA release was significantly reduced in the NAc of tPA−/− mice compared with wild-type mice. Taken together, it is suggested that tPA acts as a modulator of DA release under pathological (addictive drug-induced DA release, Nagai et al., 2004) and physiological (depolarization-evoked DA release, present study) conditions.

The defect of depolarization-evoked DA release in tPA−/− mice was restored by microinjection of either tPA or plasmin into the NAc. These results suggest that the defect in depolarization-evoked DA release in tPA−/− mice is due to a deficiency of tPA in the NAc and not to a developmental malfunction. Furthermore, the effect of tPA on the release may be mediated by plasmin. We have demonstrated previously that there are no differences in the protein levels of tyrosine hydroxylase, a rate-limiting enzyme of DA synthesis, between wild-type and tPA−/− mice (Nagai et al., 2004). Therefore, it is unlikely that the impairment of depolarization-evoked DA release in tPA−/− mice is due to a disruption of DA synthesis.

No change in depolarization-evoked ACh release was observed in the hippocampus of tPA−/− mice compared with wild-type mice, although both tPA and plasminogen are expressed in the hippocampus (Salles and Strickland, 2002). In contrast, a relatively small but significant reduction of nicotine-induced ACh release was observed in the striatum and hippocampus of tPA−/− mice compared with the change in nicotine-induced dopamine release in the NAc of the mutant mice (T. Nagai, unpublished data). Therefore, it is likely that the release of ACh may also be regulated, at least in part, by the tPA-plasmin system under certain conditions, although the contribution may be minimal. Although it is unclear as to

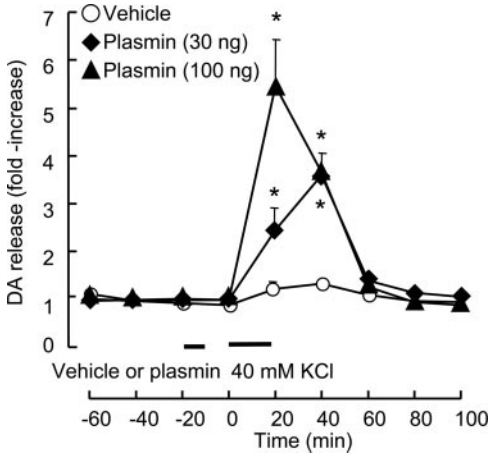

Fig. 3. Effect of plasmin on 40 mM KCl-evoked DA release in the NAc of ICR mice. A dialysis probe equipped with a microinjection tube was inserted through the guide cannula and was perfused with an aCSF at a flow rate of 1.0 μl/min. After the collection of three baseline fractions, 1 μl of either plasmin (30 or 100 ng) or vehicle (0.1% BSA)-containing aCSF solution was injected during a 10-min period through the microinjection tube into the NAc. Ten minutes after the microinjection, 40 mM KCl-containing aCSF was perfused for 20 min through the dialysis probe. There was no difference in basal levels of DA (nanomolar levels) before the drug treatment among three groups: vehicle + 40 mM KCl, 0.41 ± 0.07 (n = 5); plasmin (30 ng) + 40 mM KCl, 0.41 ± 0.01 (n = 4); and plasmin (100 ng) + 40 mM KCl, 0.43 ± 0.07 (n = 5). Values represent the mean ± S.E. An ANOVA with repeated measures was conducted: group [$F(2,11) = 10.164$, $p < 0.01$]; time [$F(4,44) = 28.490$, $p < 0.01$]; and group-by-time interaction [$F(8,44) = 10.899$, $p < 0.01$]. *, $p < 0.05$ compared with the corresponding vehicle-treated group.

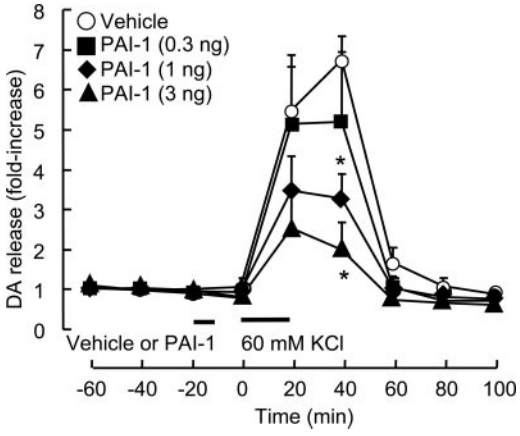

Fig. 4. Effect of PAI-1 on 60 mM KCl-evoked DA release in the NAc of ICR mice. A dialysis probe equipped with a microinjection tube was inserted through the guide cannula and was perfused with an aCSF at a flow rate of 1.0 μl/min. After the collection of three baseline fractions, 1 μl of either PAI-1 (0.3, 1, or 3 ng) or vehicle (0.1% BSA)-containing aCSF solution was injected during a 10-min period through the microinjection tube into the NAc. Ten minutes after the microinjection, 60 mM KCl-containing aCSF was perfused for 20 min through the dialysis probe. DA levels in the dialysates were analyzed using an HPLC system. There was no difference in basal levels of DA (nanomolar levels) before the drug treatment among four groups: vehicle + 60 mM KCl, 0.42 ± 0.25 (n = 4); PAI-1 (0.3 ng) + 60 mM KCl, 0.48 ± 0.05 (n = 4); PAI-1 (1.0 ng) + 60 mM KCl, 0.49 ± 0.03 (n = 5); and PAI-1 (3.0 ng) + 60 mM KCl, 0.43 ± 0.06 (n = 4). Values represent the mean ± S.E. An ANOVA with repeated measures was conducted: group [$F(2,9) = 10.962$, $p < 0.01$]; time [$F(4,36) = 30.661$, $p < 0.01$]; and group-by-time interaction [$F(8,36) = 3.771$, $p < 0.01$]. *, $p < 0.05$ compared with the corresponding vehicle-treated group.

whether the specificity lies with DA or with the NAc, we assume that it may be determined by the target proteins of the tPA-plasmin system in the brain. This possibility should be the subject of further research.

Although the mechanism by which the tPA-plasmin system regulates depolarization-evoked DA release remains to be determined, there are several possible explanations. First, it has been demonstrated that tPA, through the formation of plasmin, converts the precursor pro-brain-derived neurotrophic factor (BDNF) to mature BDNF in vitro and that this conversion is critical for the expression of late-phase long-term potentiation in the mouse hippocampus (Pang et al., 2004). Most of the BDNF secreted by neurons seems to be in the precursor form, and the secretion of pro-BDNF is activity-dependent (Chen et al., 2004). BDNF is released upon neuronal depolarization and triggers rapid intracellular signaling and action potentials in neurons (Poo, 2001). Because BDNF promotes the depolarization-evoked release of DA from mesencephalic neurons through the activation of BDNF receptor tyrosine receptor kinase-B (Blochl and Sirrenberg, 1996), it is possible that the tPA-plasmin system regulates depolarization-evoked DA release by activating tyrosine receptor kinase-B signaling through the maturation of BDNF.

Second, it was reported that tPA and plasmin bind to laminin in vitro (Goldfinger et al., 2000), and plasmin degrades several extracellular matrix components such as laminin (Nakagami et al., 2000). Laminin in the synaptic cleft causes calcium channels to localize to the active zones (Sunderland et al., 2000) and induces a small but significant increase in the level of calcium in ciliary ganglion neurons when added in soluble form to the culture medium (Bixby et al., 1994). Thus, the tPA-plasmin system may modulate depolarization-evoked DA release by degrading laminin.

Finally, plasmin was recently demonstrated to activate protease activated receptor-1 (PAR1) (Kuliopulos et al., 1999). PAR1 belongs to the cell surface G-protein-coupled receptor family and has seven transmembrane domains and an extracellular N terminus (Vu et al., 1991). Proteolytic activation of the receptor by serine proteases, including thrombin and plasmin, at the N terminus results in unmasking of the tethered ligand sequence, which then binds to a specific binding site for the tethered ligand on extracellular loop 2 and causes receptor activation (Grand et al., 1996; Dery et al., 1998). PAR1 signaling is mediated through $G\alpha_q$ protein, resulting in an activation of phospholipase C, hydrolysis of phosphoinositide, and the formation of inositol triphosphate and diacylglycerol, leading to the mobilization of Ca^{2+} (Dery et al., 1998). It is interesting that a previous

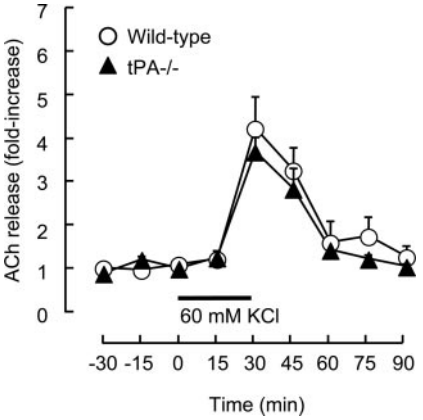

Fig. 6. Depolarization (60 mM KCl)-evoked ACh release in the hippocampus of tPA−/− mice. A dialysis probe was inserted through the guide cannula and was perfused with an aCSF containing 10 μM eserin at a flow rate of 1.0 μl/min. After the collection of three baseline fractions, 60 mM KCl-containing aCSF was perfused for 30 min. There was no difference in basal levels of ACh (nanomolar levels) before the stimulation between wild-type and tPA−/− mice: wild-type, 64.1 ± 8.5 (n = 6); and tPA−/−, 74.4 ± 10.2 (n = 6).

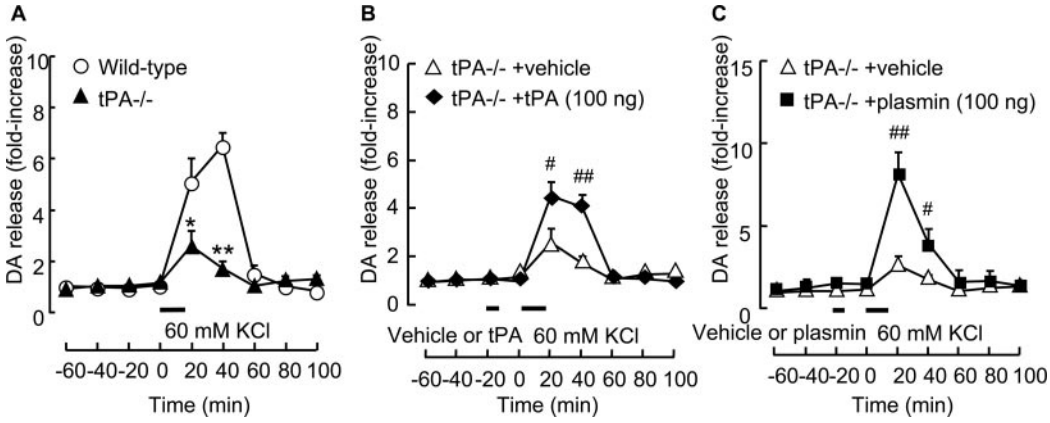

Fig. 5. Depolarization (60 mM KCl)-evoked DA release in the NAc of tPA−/− mice. A, effect of depolarization (60 mM KCl) on extracellular DA levels in the NAc of tPA−/− and wild-type mice. A dialysis probe was inserted through the guide cannula and was perfused with an aCSF at a flow rate of 1.0 μl/min. After the collection of three baseline fractions, 60 mM KCl-containing aCSF was perfused for 20 min through the dialysis probe. There was no difference in basal levels of DA (nanomolar levels) before the stimulation between wild-type and tPA−/− mice: wild-type, 0.56 ± 0.05 (n = 5); and tPA−/−, 0.53 ± 0.08 (n = 5). An ANOVA with repeated measures was conducted: group [$F(1,8) = 19.864$, $p < 0.01$]; time [$F(4,32) = 38.137$, $p < 0.01$]; and group-by-time interaction [$F(4,32) = 12.293$, $p < 0.01$]. *, $p < 0.05$, and **, $p < 0.01$ compared with wild-type mice. B and C, effects of tPA (B) and plasmin (C) on 60 mM KCl-evoked DA release in the NAc of tPA−/− mice. A dialysis probe equipped with a microinjection tube was inserted through the guide cannula and was perfused with an aCSF at a flow rate of 1.0 μl/min. After the collection of three baseline fractions, 1 μl of either tPA (100 ng), plasmin, or vehicle (0.1% BSA)-containing aCSF solution was injected during a 10-min period through the microinjection tube into the NAc of tPA−/− mice. Ten minutes after the microinjection, 60 mM KCl-containing aCSF was perfused for 20 min through the dialysis probe. There was no difference in basal levels of DA (nanomolar levels) before the drug treatment: tPA−/− + vehicle, 0.53 ± 0.08 (n = 5); tPA−/− + tPA (100 ng), 0.42 ± 0.01 (n = 4); and tPA−/− + plasmin (100 ng), 0.48 ± 0.04 (n = 5). Values represent the mean ± S.E. An ANOVA with repeated measures was conducted: group [tPA: $F(1,7) = 8.520$, $p < 0.05$, plasmin: $F(1,8) = 7.423$, $p < 0.05$]; time [tPA: $F(4,28) = 41.215$, $p < 0.01$, plasmin: $F(4,32) = 27.429$, $p < 0.01$]; and group-by-time interaction [tPA: $F(4,28) = 12.408$, $p < 0.01$, plasmin: $F(4,32) = 12.030$, $p < 0.01$]. #, $p < 0.05$, and ##, $p < 0.01$ compared with the corresponding vehicle-treated tPA−/− mice.

study demonstrated high levels of PAR1 mRNA expression in the DAergic neurons in the substantia nigra and ventral tegmental area (Weinstein et al., 1995). Thus, it is possible that the tPA-plasmin system stimulates PAR1, which in turn would increase the intracellular mobilization of Ca^{2+}, leading to a potentiation of depolarization-evoked DA release in the NAc. However, we observed that microinjection of thrombin (30–100 ng) had no effect on 40 mM KCl-evoked DA release in the NAc of ICR mice (data not shown).

In conclusion, we demonstrated that tPA and plasmin potentiated 40 mM KCl-evoked DA release, whereas PAI-1 reduced 60 mM KCl-evoked DA release. The 60 mM KCl-evoked DA release was markedly diminished in tPA−/− mice, and the microinjection of either exogenous tPA or plasmin into the NAc restored the defect of DA release in tPA−/− mice. Our findings suggest that depolarization-evoked DA release in the NAc is under the control of the tPA-plasmin system. The molecular mechanism behind the regulation of dopaminergic neurotransmission by the tPA-plasmin system would be a novel target for the treatment of dopamine-related psychiatric disorders.

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