Current Perspective

Drug Dependence, Synaptic Plasticity, and Tissue Plasminogen Activator

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Abstract. The mesocorticolimbic dopaminergic system plays an important role in the reinforcing effects of drugs of abuse, and the activity-dependent synaptic plasticity of the system is involved in drug dependence. A DNA microarray screening revealed that the expression levels of tissue plasminogen activator (tPA) mRNA in the nucleus accumbens of morphine- or methamphetamine-dependent rats were significantly increased compared with those in control animals. Since tPA plays a role in synaptic plasticity, we hypothesized that tPA may contribute to the development of drug dependence. Single and repeated morphine treatment as well as repeated methamphetamine treatment induced tPA mRNA expression in the nucleus accumbens, which was associated with an increase in the enzyme activity. Conditioned place preference induced by morphine was markedly reduced in mice with a targeted deletion of the tPA gene (tPA−/− mice), being accompanied by a loss of morphine-induced dopamine release. Similarly, methamphetamine-induced conditioned place preference and locomotor sensitization were reduced in tPA−/− mice. The defects of morphine-induced hyperlocomotion as well as methamphetamine-induced locomotor sensitization in tPA−/− mice were reversed by microinjection of exogenous tPA or plasmin into the nucleus accumbens. These results support our hypothesis that tPA plays a role in long-lasting neuronal changes related to drug dependence.

Keywords: dependence, methamphetamine, morphine, plasmin, tissue plasminogen activator

Introduction

Drugs of abuse are chemically divergent molecules with distinct primary mechanisms of action, but they commonly share many resultant features of dependence (1). Activation of the mesocorticolimbic dopamine (DA) system, which originates in the midbrain ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc), prefrontal cortex, and other limbic areas (2), has been implicated in the positive reinforcing (rewarding) effects of drugs of abuse (3–5). Furthermore, our recent study with c-Fos immunohistochemistry has suggested a role for the VTA and NAc as possible neuronal substrates in the discriminative stimulus effects of methamphetamine (6), which are related to aspects of drug actions that result in subjective effects in humans (7).

Once drug dependence is developed, it can be a lifelong condition in which individuals show intense craving and increased risk for relapse after years of abstinence, indicating chronic drug exposure causes stable changes in the structure and function of the brain that may underlie the long-lived behavioral abnormalities in drug dependence. It has been proposed that activity-dependent synaptic plasticity and remodeling of the mesolimbic dopaminergic system may play a crucial role in drug dependence (8, 9).

In order to elucidate the mechanisms by which chronic drug exposure causes stable changes in the brain that may underlie the long-lasting behavioral abnormalities in the dependent subjects, we compared the gene expression in the brains of rats that had previously received repeated morphine or methamphetamine treatment. We hypothesized that those genes whose expression was altered by repeated administration

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of morphine and methamphetamine could be candidates for drug-dependence-related genes. We used DNA microarray technology to profile the gene expression in the brains of drug-dependent animals (10).

**Screening of drug dependence-related genes using a DNA microarray**

For repeated administration of morphine, male Wistar rats were subjected to a 5-day regimen in which increasing doses of morphine (10, 20, 30, 40, and 50 mg /kg, s.c.) were injected twice a day and then challenged with morphine (10 mg /kg, s.c.) on day 6. We confirmed that the anti-nociceptive effect of morphine (10 mg /kg) on day 6 was significantly reduced after the repeated morphine treatment compared with the effect on day 1, suggesting the development of tolerance.

For repeated administration of methamphetamine, animals were subjected to a 5-day regimen in which methamphetamine (2 mg /kg, s.c.) was injected once a day for 5 days. We confirmed the sensitization of the locomotor-stimulating effect of methamphetamine by repeated drug treatment. These animals were decapitated 2 h after the last injection of morphine (day 6) or methamphetamine (day 5), and the brains were quickly removed. Various regions, including the NAc, VTA, frontal cortex, striatum, hippocampus, and amygdala, were rapidly dissected out, frozen, and stored until assayed. Total RNA was extracted from brain tissue and used for the DNA microarray analysis (Atlas Rat 1.2 Array; Clontech, Palo Alto, CA, USA).

Repeated morphine or methamphetamine treatment significantly altered the expression of genes in the rat brain. Repeated morphine treatment altered the expression in the NAc of 213 out of 1,176 genes examined, whereas repeated methamphetamine treatment affected the expression of 260 genes in the same area. The number of genes affected by both drugs was 138 in the NAc, 15 in the striatum, 63 in the frontal cortex, 28 in the hippocampus, and 36 in the amygdala (Fig. 1A). The candidates for the drug-dependence-related genes were functionally divided into various categories including genes related to intracellular signaling, receptor/channel, energy metabolism, extracellular matrix/protease, and cytokine/neurotrophic factor. Since we hypothesized that the development of drug dependence was associated with activity-dependent synaptic plasticity and remodeling of the mesocorticolimbic dopaminergic system, we focused on the role of candidate genes found by the DNA microarray screening, especially in relation to extracellular matrix/proteases and cytokines/neurotrophic factors (9).

**Tissue plasminogen activator and synaptic plasticity**

Tissue plasminogen activator (tPA), a serine protease that catalyzes the conversion of plasminogen to plasmin, is one of the candidate genes found by the DNA microarray screening. This protease plays an important role in fibrinolysis (11), but is abundantly expressed in the central nervous system (12, 13) where it is stored in synaptic vesicles and released into the extracellular space by a depolarization stimulus (14, 15). Then the expression of its mRNA is upregulated (12, 16).

Recent studies have demonstrated that tPA participates in neurite outgrowth and neuronal development by cleaving proteins of the extracellular matrix and potentially forming a path for extending processes (17, 18). Furthermore, tPA is involved in the late phase of long-term potentiation (19, 20), learning and memory (13, 21), excitotoxic neurodegeneration (22–24), and regeneration or recovery from injury in the nervous system.
A role for tPA in morphine dependence

The effects of single and repeated administration of morphine on the expression of tPA mRNA in various regions of the rat brain were measured 2 h after the final morphine treatment by a real-time RT-PCR method. Single morphine treatment remarkably induced the tPA mRNA expression compared with saline treatment in various areas including the NAc. Although the effect of morphine on tPA mRNA expression was significantly reduced after repeated treatment, expression levels in the repeated morphine-treated group were significantly higher than those in the control group in the NAc, frontal cortex, striatum, and hippocampus. The protein levels of tPA determined by Western blotting, as well as the enzyme activity assayed by gel zymography, in the NAc of mice were significantly increased by single morphine treatment. Morphine-induced tPA activity was completely inhibited by pretreatment with naloxone, suggesting the involvement of opioid receptors in the morphine-induced increase in tPA activity.

To determine the cell types in which tPA expression is induced by acute morphine treatment, in situ hybridization with antisense tPA digoxigenin-labeled RNA probes as well as immunohistochemistry with specific tPA antibodies was performed. The tPA mRNA was detected in cells of the NAc, and the signals in morphine-treated animals were apparently more intense than those in saline-treated animals. Immunohistochemistry revealed that tPA-immunoreactivity was localized to cells positive for MAP2, a marker of neuronal cells, indicating that tPA is produced in neuronal cells after morphine treatment (30).

We focused on the role of the tPA-plasmin system in the rewarding effects of morphine, which can be assessed using the conditioned place preference test (31). Morphine induced a dose-dependent conditioned place preference in wild-type mice, while saline treatment had no effect on place preference. The rewarding effects of morphine were markedly reduced in tPA-deficient (tPA−/−) mice. Moreover, morphine failed to induce place preference in plasminogen-deficient (plg−/−) mice, suggesting a role for the tPA-plasmin system in the rewarding effects of morphine. The locomotor-stimulating effects of morphine in tPA−/− mice were compared with those in wild-type mice. Single morphine treatment induced hyperlocomotion in wild-type mice and tPA−/− mice, but the magnitude was significantly reduced in tPA−/− mice compared with wild-type mice. Although repeated morphine treatment significantly potentiated the locomotor-stimulating effects of morphine in wild-type mice, it failed to potentiate the hyperlocomotion in tPA−/− mice.

We examined whether exogenous tPA and plasmin can reverse the defect in the locomotor-stimulating effect of morphine in tPA−/− mice. The attenuation of hyperlocomotion in tPA−/− mice was significantly reversed by microinjections of either exogenous tPA or plasmin into the NAc. By measuring locomotor responses to different doses of apomorphine, a direct dopamine D1/D2 receptor agonist and [35S]GTPγS binding in membrane preparations, we confirmed that the reduction of these behavioral effects of morphine in tPA−/− and plg−/− mice is not due to the alteration of dopamine and/or opioid receptor sensitivity (30).

Since the enhancement of dopamine release in the NAc is an essential process related to the morphine-induced rewarding effect (2, 5, 8), we measured morphine-induced dopamine release in the NAc. In vivo microdialysis revealed that basal levels of dopamine in the NAc were not different between wild-type and tPA−/− mice. The dopamine levels in the NAc were markedly increased by acute morphine treatment in wild-type mice. This morphine-induced dopamine release was markedly diminished in tPA−/− mice and plg−/− mice compared to wild-type mice. Microinjection of tPA or plasmin into the NAc dramatically increased morphine-induced dopamine release in tPA−/− mice as observed in wild-type mice, suggesting that tPA modulates morphine-induced dopamine release probably by converting plasminogen to plasmin in the NAc. These findings suggest that the tPA-plasmin system is involved in the rewarding effects of morphine by acutely regulating morphine-induced dopamine release in the NAc (30).

A role for tPA in methamphetamine dependence

The effects of single and repeated administration of methamphetamine (2 mg/kg per day, s.c. for 5 days) on the expression of tPA mRNA in various regions of the rat brain were measured 2 h after the final injection by a real-time RT-PCR method. Single methamphetamine treatment had no effect on tPA mRNA levels in any regions examined, but the repeated treatment remark-
ably induced the mRNA expression compared with saline treatment in the NAc, striatum, frontal cortex, and hippocampus. The effect of repeated methamphetamine treatment on tPA mRNA expression in the NAc was dose-dependent and was completely blocked by pretreatment with either the dopamine D1 receptor antagonist R(+)-SCH23390 or dopamine-D2-receptor antagonist raclopride. We confirmed that repeated methamphetamine treatment significantly increased tPA activity in the NAc compared with the saline-treated group, although single methamphetamine treatment had no effect (32). On the other hand, Hashimoto et al. (33) demonstrated that a single injection of psychostimulants, including methamphetamine, cocaine, and phencyclidine, induced tPA mRNA expression in the medial and insular prefrontal cortex and the piriform cortex using in situ hybridization histochemistry.

Methamphetamine induced a dose-dependent conditioned place preference in wild-type mice, while saline treatment had no effect on place preference. The rewarding effects of methamphetamine were markedly reduced in tPA−/− mice. We also measured the locomotor-stimulating effects of methamphetamine in tPA−/− mice. Single methamphetamine treatment induced a dose-dependent hyperlocomotion in both wild-type and tPA−/− mice. Single methamphetamine treatment reduced the locomotor-stimulating effects of methamphetamine in tPA−/− mice. Single methamphetamine treatment induced a dose-dependent hyperlocomotion in both wild-type and tPA−/− mice, and there was no difference between wild-type and tPA−/− mice. In both genotypes of animals, the locomotor-stimulating effect of methamphetamine was potentiated by the repeated treatment for 5 days. When the time course of methamphetamine-induced locomotor sensitization in tPA−/− mice was compared with that in wild-type mice, the sensitization was found to be significantly less in tPA−/− mice.

We examined whether exogenous tPA can reverse the defect of methamphetamine-induced locomotor sensitization in tPA−/− mice. The attenuation of repeated methamphetamine-induced locomotor sensitization in tPA−/− mice was significantly reversed by microinjections of exogenous tPA into the NAc, although the microinjection itself had no effect on locomotor activity in either saline- or single methamphetamine-treated tPA−/− mice. These results suggest that tPA is involved in the rewarding effects as well as the sensitization of the locomotor-stimulating effect of methamphetamine (32).

Conclusions and perspectives

As reviewed in this article, tPA plays an important role, through the formation of plasmin, in the rewarding effects of both morphine and methamphetamine (Table 1). Since the deletion of tPA genes results in a reduction of the rewarding effects, tPA may act to potentiator and/or promote drug dependence.

It has been demonstrated that some cytokines and neurotrophic factors including basic fibroblast growth factor and brain-derived neurotrophic factor act as pro-addictive cytokines as tPA does, whereas others act as anti-addictive cytokines, which reduce the rewarding effects of drugs of abuse (9). TNF-α, for instance, is induced by repeated methamphetamine treatment and inhibits the rewarding and discriminative stimulus effects of methamphetamine by activating plasmalemmal and vesicular dopamine transporter as well as inhibiting the methamphetamine-induced increase in extracellular dopamine levels (34). This cytokine is also induced by acute morphine treatment and reduces the rewarding effects of morphine (T. Nabeshima et al., unpublished observations). We propose that the dynamic changes and balance of pro-addictive and anti-addictive cytokine levels in the brain are one of the determinants of the susceptibility to drug dependence.

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