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Molecular Mechanism of Drug Dependence:

A role of tissue plasminogen activator in drug dependence

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Drugs of abuse are chemically divergent molecules with distinct primary mechanism of action, but addictive drugs commonly share many resultant features of addiction. Activation of 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, has been implicated in the positive reinforcing (rewarding) effects of drugs of abuse. It has been proposed that activity-dependent synaptic plasticity and remodeling of the mesolimbic dopaminergic system may play a crucial role in drug dependence.

In order to elucidate the mechanisms by which chronic drug exposure causes stable changes in the brain which 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, by using DNA microarray technology. We hypothesized that if the expression of genes were altered commonly by repeated administration of morphine and methamphetamine, they could be candidates of drug addiction-related genes.

Screening of drug addiction-related genes by DNA microarray

Repeated morphine or methamphetamine treatment significantly altered the expression of many genes in the rat brain. The number of genes commonly 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. The candidates for the drug dependence-related genes were functionally divided into various categories including intracellular signaling-, receptor/channel-, energy metabolism-, extracellular matrix/protease- and cytokine/neurotrophic factor-related genes.

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 that we have been exploring the role in drug dependence. This protease plays an important role in fibrinolysis, but it is abundantly expressed in the central nervous system where tPA is stored in synaptic vesicles and released into the extracellular space by a depolarization stimulus and then the expression of its mRNA is upregulated. 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 process. Furthermore, tPA is involved in the late phase of long-term

potentiation and learning and memory. These findings indicate that tPA contributes to the regulation of numerous aspects of synaptic plasticity and remodeling.

A role of tPA in morphine dependence

A single morphine treatment induced tPA mRNA and protein expression in a naloxone-sensitive manner, which was associated with an increase in the enzyme activity in the NAc. The acute effect of morphine in inducing tPA expression was decreased after repeated administration. Morphine-induced conditioned place preference and hyperlocomotion were significantly reduced in tPA deficient (tPA-/-) and plasminogen deficient (plg-/-) mice being accompanied by a loss of morphine-induced dopamine release in the NAc. The defect of morphine-induced dopamine release and hyperlocomotion in tPA-/- mice was reversed by microinjections of either exogenous tPA or plasmin into the NAc. Our findings demonstrate a novel function of the tPA-plasmin system in regulating dopamine release, which is involved in the rewarding effects of morphine (Nagai et al., 2004a).

A role of tPA in methamphetamine dependence

Repeated methamphetamine treatment dose-dependently induced tPA mRNA expression in the NAc, frontal cortex, striatum and hippocampus, whereas single treatment did not affect the expression of tPA mRNA. The methamphetamine-induced increase in tPA mRNA expression in the NAc was completely inhibited by pretreatment with R(+)-SCH23390 and raclopride, dopamine D1 and D2 receptor antagonists, respectively. In addition, repeated methamphetamine treatment increased tPA activity in the NAc. Methamphetamine-induced conditioned place preference and behavioral

sensitization after repeated treatment were significantly reduced in tPA-/- mice compared with wild-type mice. The defect of behavioral sensitization in tPA-/- mice was reversed by microinjections of exogenous tPA into the NAc. These results suggest that tPA is involved in the rewarding effects as well as the sensitization of the locomotor-stimulating effect of methamphetamine (Nagai et al., 2004b).

Conclusions and perspectives

Since the deletion of tPA genes resulted in a reduction of the rewarding effects of morphine and methamphetamine (Table 1), tPA may act to potentiate and/or promote the addictive effects of drugs.

Table 1. Summary for the changes in biomarkers and behavior induced by morphine and methamphetamine in tPA-/- Mice

	Methamphetamine	Morphine
tPA mRNA		
Single	±	↑↑
Repeated	↑↑	↑
Dopamine release		
Wild	↑	↑
tPA-/-	N.D.	↓
tPA-/- + tPA	N.D.	↑
Conditioned place preference		
Wild	↑	↑
tPA-/-	↓	↓
Hyperlocomotion		
Wild	↑	↑
tPA-/-	±	↓
tPA-/- + tPA	±	↑
Sensitization		
Wild	↑	↑
tPA-/-	↓	↓
tPA-/- + tPA	↑	N.D.

±, No Change ; ↑, Significant Increase; ↓, Significant Decrease; N.D., Not Determined.

It has been demonstrated that some cytokines and neurotrophic factors such as 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 (Yamada and Nabeshima, 2004). TNF-α, for instance, is induced by repeated methamphetamine treatment, and inhibits the rewarding and discriminative stimulus effects of methmphetamine, by activating plasmalemmal and vesicular dopamine transporter as well as inhibiting methamphetamine-induced increase in extracellular dopamine levels (Nakajima et al., 2004). This cytokine also induced by acute morphine treatment, and reduces the rewarding effects of morphine. We propose that the dynamic changes and balance of pro-addictive and anti-addictive cytokine levels in the brain are one of determinants of the susceptibility to drug dependence.

Acknowledgments

This study was supported in part by Special Coordination Funds for Promoting Science and Technology, Target-Oriented Brain Science Research Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a grant-in-aid for Health Sciences Research from the Ministry of Health, Labour and Welfare of Japan.

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