Neuropsychotoxicity of Abused Drugs: Involvement of Matrix Metalloproteinase-2 and -9 and Tissue Inhibitor of Matrix Metalloproteinase-2 in Methamphetamine-Induced Behavioral Sensitization and Reward in Rodents

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Abstract. Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) function to remodel the pericellular environment. We have investigated the role of the MMP/TIMP system in methamphetamine (METH) dependence in rodents, in which the remodeling of neural circuits may be crucial. Repeated METH treatment induced behavioral sensitization, which was accompanied by an increase in MMP-2/-9/TIMP-2 activity in the brain. An antisense TIMP-2 oligonucleotide enhanced the sensitization, which was associated with a potentiation of the METH-induced release of dopamine in the nucleus accumbens (NAc). MMP-2/-9 inhibitors blocked the METH-induced behavioral sensitization and conditioned place preference (CPP), a measure of the rewarding effect of a drug, and reduced the METH-increased dopamine release in the NAc. In MMP-2– and MMP-9–deficient mice, METH-induced behavioral sensitization and CPP as well as dopamine release were attenuated. The MMP/TIMP system may be involved in METH-induced sensitization and reward by regulating extracellular dopamine levels.

Keywords: drugs of abuse, matrix metalloproteinase, tissue inhibitor of matrix metalloproteinase, methamphetamine, dopamine

Introduction

Drug dependence is a complex phenomenon with important psychological and social causes and consequences, which may be associated with neural plasticity and the remodeling of specific brain circuits caused by repeated exposure to drugs of abuse (1–3). Methamphetamine (METH), a common drug of abuse, has both acute and long-lasting effects on psychomotor behavior (4–6). These effects of METH are associated with an increase in extracellular dopamine levels in the brain, achieved by facilitating the release of dopamine from presynaptic nerve terminals and inhibiting its reuptake (7). It has been proposed that cellular and molecular mechanisms for drug dependence involve processes similar to those operating in other forms of synaptic plasticity such as learning and memory (1, 8). Although it has been demonstrated that repeated treatment with cocaine and amphetamine produces changes in neural morphology and synaptic connectivity in the mesolimbic system (9, 10), the mechanism underlying psychostimulant-induced remodeling of synaptic structures remains to be determined (11, 12).
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 dependent subjects, we compared the gene expression in the brains of rats that had previously received repeated morphine or METH treatment (12). We hypothesized that those genes whose expression was altered by repeated administration of morphine and METH 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. Since we hypothesized that the development of drug dependence is 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 cytokines/neurotrophic factor and extracellular matrix/proteases. As a result, we have demonstrated that tumor necrosis factor-α (TNF-α) (7, 11, 13), tissue plasminogen activator (tPA) (14 – 16), neuroglycan C (17), and matrix metalloproteinase (MMP) (18, 19) were involved in drug dependence.

Changes in the expression of MMP-2, MMP-9, and TIMP-2 in the brain induced by METH

Gelatinases (MMP-2 and MMP-9) are capable of cleaving collagen IV and V, laminin, and chondroitin sulfate proteoglycan, which are associated with cell adhesion, and have been implicated specifically in cerebral ischemia (29), kainate-induced neuronal injuries (27), and hippocampal long-term potentiation (LTP) and memory (30). For example, MMP-2 plays a principal role in establishing the growth-promoting properties of denervated peripheral nerve (31). MMP-9, but not MMP-2, is particularly involved in dendritic remodeling in the hippocampus of adult rat (27).

We have demonstrated for the first time that repeated administration of METH leads to behavioral sensitization that is accompanied by the induction of MMP-2, MMP-9, and TIMP-2 expression in the brain including the frontal cortex (Fc) and nucleus accumbens (NAc) (Table 1) (18, 19). Our data also showed that MMP-2 and MMP-9 were expressed in neurons as well as glial cells. Repeated, but not single, METH treatment also induced TIMP-2 mRNA and protein expression, and the TIMP-2 was expressed in neurons (19). Some previous

| Table 1. Summary for changes in MMP/TIMP levels in the brain induced by METH |
|-----------------|---|---|
| METH treatment  | Single | Repeated |
| MMP-2 protein   | ±  | ↑  |
| MMP-9 protein   | ±  | ↑  |
| TIMP-2 protein  | ±  | ↑  |

±, No change; ↑, Significant increase.
Indeed, the inhibition of MMPs alters functional and behavioral sensitization, and that the MMP/TIMP system is involved in the learning of a cocaine-associated contextual memory and that reconsolidation of this memory was disrupted by an MMP inhibitor. Furthermore, they showed the ability of an MMP inhibitor to impair the expression of memory for a cocaine-associated cue after extinction. Although they did not examine whether MMP activity and expression were increased by cocaine treatment or a cocaine-associated stimulus, MMPs may also have a crucial role in drug-associated memory.

**Role of the MMP/TIMP system in dopamine neurotransmission**

Behavioral changes induced by METH are linked to its capacity to elevate extracellular dopamine levels through the redistribution of dopamine from synaptic vesicles to the cytosol and promotion of reverse transport (7, 36). METH-induced behavioral sensitization is associated with an enhancement of the METH-induced increase in extracellular dopamine levels in the NAc (7, 15). In the MMP-2/− mice and MMP-9/− mice compared with the wild-type mice, METH-induced dopamine release in the NAc as well as METH-induced behavioral sensitization and CPP were attenuated. TIMP-AS treatment enhanced the sensitization of METH-induced dopamine release in the NAc, while MMP-2/− inhibitors reduced it. In contrast, infusion of purified human MMP-2 into the NAc significantly potentiated the METH-induced dopamine release. The uptake of [3H]dopamine into striatal synaptosomes was reduced in wild-type mice after repeated METH treatment, and the changes in [3H]dopamine uptake were significantly attenuated in MMP-2/− mice and MMP-9/− mice (18). It has been demonstrated that the reverse activation and internalization of plasmalemmal dopamine transporter (DAT) are involved in the METH-induced increase in extracellular dopamine levels (36, 37). These results suggest that both MMP-2 and MMP-9 play a crucial role in METH-induced behavioral sensitization and reward by regulating METH-induced dopamine release and uptake via DAT in the NAc.

The sensitivity of dopamine receptors to endogenous and exogenous ligands is important for dopamine neurotransmission in physiology and pathology. Reduced signaling via Gi-coupled receptors may be an important neuroadaptation in cocaine addiction (38). G protein signaling in the Fc plays a crucial role as a potential pathological change contributing to cocaine sensitization and drug seeking (39). In fact, we also have shown that dopamine receptor agonist–stimulated [35S]GTPγS binding was reduced in the Fc of rats sensitized to METH. TIMP-AS potentiated, while a MMP-2/− inhibitor attenuated, the reduction in dopamine D2 receptor agonist–stimulated [35S]GTPγS binding. Repeated METH treatment also reduced dopamine D2 receptor agonist–stimulated [35S]GTPγS
binding in wild-type mice, but such changes were significantly attenuated in MMP-2(−/−) and MMP-9(−/−) mice (19). These results suggest that the MMP/TIMP system is involved in the METH-induced dysregulation of dopamine release and receptor signaling. As dopamine D2 receptors function in the feedback inhibition of dopamine release (40, 41), the downregulation may contribute to an enhancement of the METH-induced increase in extracellular dopamine levels.

Recently, Kim et al. (42) have demonstrated that MMP-3 has a specific role in dopamine neuronal degeneration. They suggested that the active MMP-3 released from stressed dopamine neurons is a candidate molecule that activates microglia, leads to production of superoxide, and plays a pivotal role in dopamine neuronal death, and they proposed that abrogation of MMP-3 or inhibition of MMP-3 activity in early neuronal degeneration may be an effective means of preventing progressive degeneration of dopamine neurons. This study strongly suggests that MMPs play a crucial role in the regulation of dopaminergic neurons in various diseases.

**Table 2.** Summary for changes in METH-induced behavior and dopamine release in MMP-2(−/−) and MMP-9(−/−) mice

<table>
<thead>
<tr>
<th>Condition</th>
<th>Wild-type</th>
<th>MMP-2(−/−)</th>
<th>MMP-9(−/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conditioned place preference</td>
<td>↑</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>+TIMP-2-AS</td>
<td>N.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+MMP inhibitor</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperlocomotion (single)</td>
<td>↑</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>+TIMP-2-AS</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+MMP inhibitor</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locomotor sensitization (repeated)</td>
<td>↑↑</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>+TIMP-2-AS</td>
<td>↑↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+MMP inhibitor</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine release</td>
<td>↑</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>+TIMP-2-AS</td>
<td>↑↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+MMP inhibitor</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

±, No change; ↑, Significant increase; ↓, Significant decrease; N.D., Not determined.

Conclusion

As reviewed in this article, MMP-2, MMP-9, and TIMP-2 are involved in the rearrangement of the neural network in the mesocorticolimbic dopamine system, which plays a crucial role in the development of behavioral sensitization to METH (Table 2). It is likely that the MMP/TIMP system plays a role in METH-induced behavioral sensitization through modulation of the function of plasma membrane proteins such as dopamine receptors and transporters. These results, together with the fact that MMP acts to degrade components of the ECM such as laminin and collagen IV, suggest that repeated METH-induced overexpression of MMP-2, MMP-9, and TIMP-2 is associated with the structural and functional changes in the mesocorticolimbic dopamine system, leading to METH-induced behavioral sensitization and reward following repeated drug treatment. We have proposed that some cytokines and neurotrophic factors such as basic fibroblast growth factor and brain-derived neurotrophic factor act as pro-addictive cytokines, whereas glia-derived neurotrophic factor and TNF-α act as anti-addictive cytokines, which reduce the rewarding effects of drugs of abuse (11). It appears that MMP-2 and MMP-9 can be classified as pro-addictive, whereas TIMP-2 may be anti-addictive. We propose that the dynamic changes to, and balance of, levels of pro-addictive and anti-addictive factors in the brain are determinants of susceptibility to drug dependence. Furthermore, our findings suggest that inhibitions of pro-addictive factors such as MMP-2 and MMP-9 may be effective in the treatment of drug dependence.

Acknowledgments

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