Full Paper

CC-Chemokine Ligand 2 Facilitates Conditioned Place Preference to Methamphetamine Through the Activation of Dopamine Systems

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Abstract. Methamphetamine addiction is characterized by drug craving caused by stimulation of the reward system. Because neuroinflammation underlies several neurological disorders, we investigated whether CC-chemokine ligand 2 (CCL2) participates in the methamphetamine dependence using mice. Upregulation of CCL2 but not CC-chemokine receptor 2 (CCR2), a dominant receptor for CCL2, mRNA in both the prefrontal cortex (PFC) and nucleus accumbens (NAC) was observed after methamphetamine (3 mg/kg, s.c.) administration. Using immunohistochemistry, high CCL2 protein levels localized to neurons in the PFC and NAC. In the conditioned place preference (CPP) test, methamphetamine (0.3 – 3 mg/kg, s.c.) induced a CPP, reflecting psychic dependence on methamphetamine, in a dose-dependent manner. The CPP to methamphetamine was attenuated by RS504393 (1 mg/kg, s.c.), a CCR2 antagonist. Moreover, methamphetamine increased phosphorylated tyrosine hydroxylase (pTH) levels in the ventral tegmental area (VTA). Increased levels of pTH in the VTA by methamphetamine was also suppressed by RS504393. Furthermore, intracerebroventricular injection of recombinant CCL2 increased pTH levels in the VTA. Taken together, we demonstrate that activation of dopamine neurons, which enhances reward-system activity, via the CCL2-CCR2 axis plays a crucial role in psychic dependence on methamphetamine. Novel treatments targeting this machinery may be effective for drug addiction.

Keywords: CC-chemokine ligand 2 (CCL2), monocyte chemoattractant protein-1 (MCP-1), chemokine, inflammation, addiction

Introduction

Methamphetamine is a potent psychostimulant, and economic loss based on drug dependence is considered to be a severe social problem. The notion of drug dependence includes both physical dependence and psychic dependence (addiction) (1). Physical dependence to opioids or alcohol is characterized by withdrawal symptoms. On the other hand, addiction to methamphetamine or cocaine is determined by craving for these addictive drugs in humans (2). Addiction is described as a chronic neurological disorder associated with plasticity in the mesolimbic system (3). Methamphetamine acts on catecholaminergic nerve endings and increases dopamine and noradrenaline in the synaptic cleft (4). Generally, mesolimbic dopaminergic projections from the ventral tegmental area (VTA) to the prefrontal cortex (PFC) and nucleus accumbens (NAC) are defined as the reward system, and the activation of this projection is a key component in the development of psychic dependence (5). Tyrosine hydroxylase (TH), which is a rate-limiting enzyme for dopamine synthesis, is activated by its phosphorylation, and phosphorylated TH (pTH) may be involved in psychic dependence (6). However, the underlying mechanisms for the long-term potentiation of dopamine release, which leads to the development of psychic dependence on addictive drugs, has not been clarified.

Recently, chronic neuroinflammation has been examined as a basic molecular process in several chronic neuro...
logical disorders such as Parkinson’s disease, multiple sclerosis, and schizophrenia (7 – 9). Crosstalk between neurons and inflammatory glial cells because of the cytokine–chemokine network plays a pivotal role in the pathogenesis of chronic inflammation in the central nervous system (CNS) (9 – 11). Among several chemokines, CC-chemokine ligand 2 (CCL2), also named as monocyte chemoattractant protein-1 (MCP-1), is derived from neurons and glial cells and has been the focus of attention because of its facilitative effects on neuro-transmission. CCL2 acts on dopamine neurons in the substantia nigra and enhances dopamine release into the striatum. Thus, it has been considered that this machinery contributes to Parkinson’s disease (12, 13). There are several reports suggesting that CCL2-mediated neuro-inflammation also underlies the pathogenesis of drug abuse (14). Moreover, upregulation of CC-chemokine receptor 2 (CCR2), a dominant receptor for CCL2, mediates behavioral sensitization to methamphetamine, which reflects a psychosis (15). Despite these lines of evidence, the pathophysiological roles of CCL2 in drug addiction remain unclear. Herein, we highlight the involvement of CCL2 in the development of psychic dependence on methamphetamine in a murine model.

Materials and Methods

Animals and drug administration

Male C57BL/6 mice were (SLC, Osaka) the subjects of all experiments. The animals were kept in plastic cages in an air-conditioned (23°C – 24°C, 60% humidity) and light-controlled (light on AM 8:00 – PM 8:00) room and given water and food ad libitum. Methamphetamine (DS Pharma, Osaka) and RS504393 (Tocris Biosciences, Bristol, UK) were dissolved in physiological saline and dimethylsulfoxide, respectively. These drugs were purchased from Hokkaido System Science (Hokkaido). PCR was performed under the following conditions: 95°C for 10 min, followed by 50 cycles of 95°C for 15 s and 60°C for 60 s. The fluorescent intensity of the intercalated SYBR Green was measured and normalized to GAPDH.

Immunohistochemistry

Mice were deeply anesthetized with pentobarbital (80 mg/kg, i.p.) and perfused transcardially with PBS and fixed with 4% PFA. Whole brains were collected, post-fixed, and dehydrated in 25% sucrose at 4°C overnight. Frozen brains were embedded in freezing O.C.T. compound (Sakura, Tokyo) and sliced into 12-μm-thick sections using a cryostat (Leica Microsystems, Wetzlar, Germany). Coronal sections of the forebrain or midbrain were mounted on glass slides. The sections were blocked with 4% BSA for 2 h and incubated overnight in primary antibodies against CCL2 (Santa Cruz Biotechnology, Dallas, TX, USA), NeuN (Merck Millipore, Billerica, MA, USA), and pTH (Ser 40, Santa Cruz Biotechnology). The sections were washed and incubated with secondary antibodies conjugated with fluorescent markers (Alexa fluor 488 or Alexa fluor 594, Invitrogen) for 2 h. Fluorescence was detected using an all-in-one BIORÉVO fluorescent microscope (Keyence, Tokyo).

Conditioned place preference (CPP) test

CPP paradigm was conducted within a conditioning-chamber consisting of two equal-sized (160 × 160 × 160 mm³) compartments, which was constructed using acrylic resin board. One side of chamber was white with a rough-surfaced acrylic floor, and the other side of the chamber was black with a smooth-surfaced acrylic floor. The two compartments were separated by a sliding plate within the gateway. The experimental schedule was conducted over 9 days and was divided into three sections, which were pre-conditioning on days 1 – 2, conditioning on days 3 – 8, and post-conditioning on day 9.

Pre-conditioning: On day 1, mice were put into the chamber with both compartments open and allowed to freely move within the chamber for 15 min. On day 2, mice were given the same treatment as the day before, and the time spent in each compartment was measured
over 15 min (900 s). The compartment in which the subject spent a longer duration was designated the preferred compartment.

**Conditioning:** On day 3, mice were administered methamphetamine (1 mg/kg, s.c.), and were kept in the non-preferred compartment for 60 min. The next day, mice were administered saline and were kept in the preferred compartment for 60 min. A conditioning session was conducted once a day, and these sessions were repeated three times over 6 days (on days 3 – 8).

**Post-conditioning:** On day 9, conditioned mice were put in the chamber with both compartments open and allowed to freely move within the chamber for 15 min, just as during the pre-conditioning. The CPP was evaluated by measuring the time spent in the drug-conditioned compartment over 15 min (900 s). The CPP score reflects the degree of conditioning and was calculated as follows: CPP score (s) = (time spent in the drug-paired compartment during the post-conditioning test) – (time spent in the same compartment during the pre-conditioning evaluation session).

**Statistical analyses**

Data are presented as the mean ± S.E.M. Statistical analysis was performed by one-way analysis of variance followed by Tukey’s multiple comparisons test. Significance was established at $P < 0.05$.

**Results**

**Upregulation of CCL2 in the reward system after methamphetamine administration**

The mRNA expression of CCL2 in the PFC and NAC after methamphetamine (3 mg/kg, s.c.) administration was evaluated by quantitative RT-PCR. In both the PFC and NAC, CCL2 mRNA levels at 1 h after methamphetamine administration were significantly higher than those in saline-treated mice. On the other hand, CCR2, a dominant receptor for CCL2, mRNA levels were unchanged after methamphetamine administration (Fig. 1: A – D). Using immunohistochemistry, we found that protein expression of CCL2 in both the PFC and NAC was markedly increased at 1 h after the last administration of methamphetamine (3 mg/kg, s.c., once a day).

![Fig. 1. Upregulation of CC-chemokine ligand 2 (CCL2) in the reward system after methamphetamine administration.](image)
CCL2 and Methamphetamine Dependence

for 3 days) in comparison with the vehicle control. Moreover, increased CCL2 was localized on NeuN⁺ neurons in both regions (Fig. 1: E, F).

Facilitative role of CCL2 in methamphetamine CPP

To examine the role of upregulated CCL2 in methamphetamine addiction, the CCR2 antagonist RS504393 (1 mg/kg, s.c.) was administered to mice 15 min before methamphetamine administration. In the behavioral test, CPP to methamphetamine (0.3 – 3 mg/kg, s.c.) was observed in a dose-dependent manner, indicating the development of psychic dependence (addiction) to methamphetamine (Fig. 2A). The CPP to methamphetamine (1 mg/kg, s.c.) was significantly attenuated by RS504393, even though RS504393 did not induce a conditioned place aversion by itself (Fig. 2B).

Activation of dopamine neurons in the reward system by CCL2

To determine the effects of upregulated CCL2 on dopaminergic projections, the activities of dopamine neurons in the VTA were evaluated by immunohistochemistry. Phosphorylated tyrosine hydroxylase (pTH), an active form of TH, was increased at 1 h after the last administration of methamphetamine (3 mg/kg, s.c., once a day for 3 days), compared with vehicle. Increased pTH by methamphetamine was attenuated by co-treatment of RS504393 (1 mg/kg, s.c.) (Fig. 3A). Finally, to investigate the direct effects of CCL2 on dopamine neurons, recombinant CCL2 (50 ng, i.c.v.) was locally injected into mice. The expression level of pTH in the VTA at 24 h after CCL2 injection was clearly higher than that after PBS injection (Fig. 3B).

Discussion

There are few previous reports showing the pathophysiological roles of chemokines in psychic dependence to addictive drugs. We focused on CCL2 upregulation by methamphetamine and found that CCL2 contributes to psychic dependence on methamphetamine through the activation of a reward system in the brain. In our experiments, the sources of CCL2 were neurons in both the PFC and NAC (Fig. 1), which are key regions for the development and maintenance of psychic dependence on addictive drugs. Thus, a large number of studies targeting these regions were performed (2, 5). Indeed, neurogenesis/gliogenesis and structural reorganization leading to plasticity occur in the PFC and NAC following exposure to addictive drugs (16, 17), elucidating the dynamics of the neural network that underlies psychic dependence. Synaptic plasticity is closely related to crosstalk between neurons and glial cells through inflammatory mediators including cytokines and chemokines (3, 18). CCL2 is a well-characterized chemokine, and
it is a key factor in inflammatory diseases in the CNS (9, 11, 19). As CCL2 and CCR2 are normally expressed in neurons, astrocytes, and microglia (20 – 22), the CCL2-CCR2 axis may modulate complex cellular crosstalk in the CNS and elicit synaptic plasticity accompanied by neuroinflammation.

It is believed that dopaminergic projections from the VTA to PFC and NAC underlie the development of psychic dependence on methamphetamine, cocaine, opioids, and alcohol (1, 23). Augmentation of dopamine signaling may facilitate the rewarding effects of and psychic dependence on methamphetamine. Regarding the upregulation of pTH by methamphetamine in the VTA, compensatory mechanisms to restore catecholamines were at least partial contributors. However, it was surprising that the increase in pTH by methamphetamine was suppressed by the CCR2 antagonist RS504393. Furthermore, we also clarified that i.c.v. injection of recombinant CCL2 increased pTH in the VTA (Fig. 3). According to these facts, we hypothesized that CCL2 enhances and maintains the activity of dopamine neurons in the reward system. As mentioned above, previous reports showed that CCL2 activated dopamine neurons in the substantia nigra in vitro and in vivo, and it enhanced dopamine release into the striatum (12, 13). Reduction of pTH by RS504393 might be based on its property of CCL2 antagonist. Although these reports revealed the possible involvement of CCL2 in Parkinson’s disease, detailed mechanisms for these phenomena remain unclear. Nevertheless, our findings in this study suggest that CCL2 may exacerbate the development of psychic dependence because of the enhancement of the reward system. Because of its utility for the assessment of drug craving in rodents, we evaluated psychotic dependence on methamphetamine using the CPP test (24, 25). CPP was elicited by methamphetamine, and the methamphetamine-induced CPP was prevented by RS504393, confirming our hypothesis.

Recently, a relationship between CCL2 expression and addictive drugs was revealed in other reports. CCL2 is upregulated in the postmortem brain of alcoholic patients, and chronic exposure to alcohol increases CCL2 levels in the brain of the mouse (14, 26). These findings may suggest a common mechanism underlying the development of psychic dependence on different addictive drugs. CCL2 is normally characterized as a stimulating factor for microglia and astrocytes, and plays crucial roles in several neurological disorders in the CNS (18, 20). In light of the phenomena caused by CCL2, neuroinflammation because of the crosstalk between neurons and glial cells is considered to be a key common element in drug addiction. Activation of microglia and astrocytes in the brain following exposure to addictive drugs including methamphetamine has been reported (27 – 29). Morphine also upregulates chemokines and activates glial cells in the brain (24), and these activations might be based on CCL2. Psychic dependence on methamphetamine or morphine was attenuated by agents that suppress the activity of microglia and astrocytes, such as minocycline or ibudilast (30, 31). We previously reported that behavioral sensitization to methamphetamine was improved by peroxisome proliferator-activated receptor-γ agonists, being suppressive agents for inflammatory macrophages and microglia (32). Therefore, we speculate that comprehensive plasticity of neuron-glia communication in the brain associated with chronic neuroinflammation may underlie the pathophysiology of psychic dependence on addictive drugs. However, we need to clarify the detailed roles of glial cells in psychic dependence in future studies.

In conclusion, activation of dopamine neurons, which enhances activity within the reward system, via the CCL2-CCR2 axis plays a pivotal role in the development of psychic dependence on methamphetamine. Novel therapeutic approaches targeting this machinery may be effective in the treatment of psychic dependence on methamphetamine.

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Conflicts of Interest

There is no conflict of interest in this study.

References


