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Research article

Upregulation of CCL7 and CCL2 in reward system mediated through dopamine D1 receptor signaling underlies methamphetamine-induced place preference in mice

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ABSTRACT

We previously showed that the CC-chemokine ligand 2 (CCL2)–CC-chemokine receptor 2 (CCR2) system is responsible for conditioned place preference (CPP) by methamphetamine (Meth). In this study, we investigated the roles for other chemokines mediating Meth-induced CPP and the upstream factors upregulating chemokines in mice. We found that CCL7 mRNA level was upregulated in the prefrontal cortex (PFC) after Meth administration (3 mg/kg, subcutaneous), and increased CCL7 immunoreactivity was localized to the PFC NeuN-positive neurons. Meth-induced CPP was blocked by the dopamine D1 receptor antagonist SCH 23390 but not by the D2 receptor antagonists raclopride or haloperidol. The D1 receptor agonist SKF 81297 alone elicited CPP, suggesting a critical role of D1 receptor signaling in Meth-induced reward. Consistent with these results, the Meth-induced upregulation of CCL7 and CCL2 were attenuated by SCH 23390, and a single administration of SKF 81297 upregulated mRNA expression levels of CCL7 and CCL2 in the PFC. Furthermore, Meth-induced CPP was prevented by INCB 3284, a selective antagonist of CCR2, a receptor that binds both CCL7 and CCL2. Collectively, we identified two CC-chemokines (i.e., CCL7 and CCL2) as key regulatory factors in Meth-induced reward. Pharmacological inhibitors of these chemokines may warrant development as novel therapeutics for ameliorating Meth addiction.

1. Introduction

Methamphetamine (Meth) is a highly addictive psychostimulant with a strong reinforcing effect that leads to both drug dependence and economic loss. Meth addiction falls under the classification of psychological dependence characterized by craving for addictive drugs [1]. Meth acts on dopaminergic nerve endings to increase the dopamine concentration in the synaptic cleft [2]. Generally, enhancement within the reward system consisting of mesolimbic dopaminergic projections from the ventral tegmental area (VTA) to the prefrontal cortex (PFC) and nucleus accumbens (NAC) plays a fundamental role in reward [3]. Regarding the control of synaptic plasticity, several lines of evidence suggest that chronic neuroinflammation is a critical component for chronic diseases, such as drug dependence, in the central nervous system (CNS) [4].

Chemokines were originally identified as small chemotactic cytokines regulating inflammatory responses. Recent studies have shown that chemokine ligands and receptors are widely expressed in several cell types, and communication between neurons and glial cells mediated by chemokines leads to the expression of multiple functions under both physiological and pathological conditions [5]. Indeed, CC-chemokine ligand 2 (CCL2), also known as monocyte chemoattractant protein-1 (MCP-1), is a well-characterized chemokine contributing to various diseases in both the peripheral and CNS [6]. A previous report showed that CCL2 directly activates dopamine neurons in the substantia nigra and facilitates dopamine release in the striatum [7], and that enhancing the CCL2–CC-chemokine receptor 2 (CCR2) system may contribute to abuse of addictive drugs [8]. Importantly, we previously demonstrated that CCL2 is upregulated in both the PFC and NAC after exposure to Meth and that treatment with a CCR2 antagonist significantly suppresses Meth-induced conditioned place preference (CPP) [9]. These lines of evidence strongly indicate that the CCL2–CCR2 system is crucial for Meth-induced reward.

However, the roles of other inflammatory chemokines and upstream factors upregulating chemokines are still unknown. Given that chemokine systems play a pivotal role in regulating Meth-induced reward,

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the mechanisms underpinning the chemokine-mediated regulation of Meth-induced CPP warrant further investigation. In the present study, we found the upregulation of CCL7 in the reward system following Meth exposure and determined the contribution of dopamine receptor signaling to upregulations of CCL7 and CCL2. Our findings not only emphasize the importance of the chemokine system but also offer insight into the development of novel therapeutics for Meth-induced reward.

2. Materials and methods

2.1. Animals and drug administration

All experimental procedures were approved by the Animal Research Committee of Wakayama Medical University. Male C57BL/6 mice aged 8–10 weeks (SLC, Hamamatsu, Japan) were housed five per plastic cage in a temperature-controlled environment (23 °C–24 °C, with 60%–70% humidity) a 12-h dark/light cycle (light on at 8:00 a.m.) and allowed access to water and food *ad libitum*. Meth (DS Pharma, Osaka, Japan), INCB 3284 dimesylate (Tocris Bioscience, Bristol, UK), SCH 23390 hydrochloride (Tocris Bioscience), raclopride (Tocris Bioscience), haloperidol (DS Pharma), SKF 81297 hydrobromide (Tocris Bioscience), and sumanirole maleate (Tocris Bioscience) were dissolved in sterile physiological saline, and were administered to mice by dorsal subcutaneous (s.c.) or intraperitoneal (i.p.) injection.

2.2. Reverse transcription-quantitative polymerase chain reaction

Mice were euthanized by decapitation, and fresh PFC samples were dissected from 1-mm-thick forebrain sections. Tissues were homogenized with TRIzol reagent (Invitrogen, Carlsbad, CA). Chloroform was added, and the homogenate was centrifuged at 4 °C for 15 min. The aqueous phase containing RNA was transferred to a fresh tube, and the RNA was isolated by 2-propanol precipitation. Total RNA (1 μg) was used for the synthesis of cDNA by reverse transcription (RT) as follows. Total RNA was incubated with Random Primers (Invitrogen) at 70 °C for 10 min and then cooled on ice. Samples were converted to cDNA by incubation with M-MLV Reverse Transcriptase (Promega, Madison, WI) and dNTP Mix (Promega) at 37 °C for 60 min. The synthesized cDNA (10 ng) was used as a template for quantitative polymerase chain reaction (qPCR) with a KAPA SYBR FAST qPCR Kit (KAPA Biosystems, Boston, MA) using an ECO Real-Time PCR System (AsOne, Osaka, Japan). Primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 5′-GGG TGT GAA CCA CGA GAA AT-3′, 5′-ACT GTG GTC ATG AGC CCT TC-3′), CCL2 (5′-AGG TCC CTG TCA TGC TTC TG − 3′, 5′-TCA TTG GGA TCA TCT TGC TG-3′), and CCL7 (5′- ATC TCT GCC ACG CTT CTG TG − 3′, 5′- CCT CTT GGG GAT CTT TTG TTT C − 3′) were purchased from Hokkaido System Science (Hokkaido, Japan). Reactions were performed at 95 °C for 15 s followed by 60 °C for 60 s. The fluorescence intensity of the intercalated SYBR Green was measured and normalized to GAPDH.

2.3. Immunohistochemistry

Mice were deeply anesthetized with pentobarbital and perfused transcardially with PBS followed by 4% paraformaldehyde. Whole brains were collected, post-fixed in 4% paraformaldehyde, and dehydrated in 25% sucrose at 4 °C overnight. Frozen tissues embedded in freezing optimal cutting temperature compound (Sakura, Tokyo, Japan) were sliced into 12-μm-thick sections using a cryostat (Leica Microsystems, Welzlar, Germany). Coronal sections of the forebrain and midbrain were mounted onto glass slides. The sections were washed and incubated with PBS containing 0.1% Triton X-100 at room temperature for 30 min. The sections were blocked with 4% bovine serum albumin at room temperature for 2 h and incubated at 4 °C overnight with primary antibodies against CCL7 (1:100; rabbit polyclonal; Gene Tex, Irvine, CA) and NeuN (1:100; mouse monoclonal; Millipore, Billerica, MA). The next day, sections were washed and incubated with secondary antibodies conjugated with fluorescent dyes (Alexa Fluor 488 or Alexa Fluor 594, 1:200; Invitrogen) at room temperature for 2 h, followed by nuclear staining with Hoechst 33342 (Invitrogen) for 10 min. Finally, the sections were washed, and a cover glass with mounting medium was placed over the sections. Fluorescence was detected using an all-in-one Biorevo fluorescence microscope (Keyence, Tokyo, Japan).

2.4. Conditioned place preference (CPP) test

The CPP test was conducted as previously described [9] within a conditioning chamber consisting of two equal sized (160 × 160 × 160 mm) compartments made of acrylic resin board. One compartment consisted of white walls and a floor with a rough surface, and the other compartment had black walls with a smooth floor surface. The two compartments were separated by a sliding plate door. The experimental schedule was conducted over 10 days and was divided into 3 periods (i.e., pre-conditioning on days 1–3, Meth or SKF 81297 conditioning on days 4–9, and post-conditioning on day 10). The dose of Meth used for CPP test was determined in accordance with our previous report [9].

2.4.1. Pre-conditioning

On days 1–2, mice were placed in the chamber with the door open and allowed to freely move between the two compartments for 15 min. On day 3, mice were exposed to the same conditions as the previous day, and the time spent in each compartment was measured over 15 min (900 s). The compartment that mice spent more time in was designated the preferred compartment.

2.4.2. Conditioning

On day 4, mice were administered Meth (1 mg/kg, s.c.) or SKF 81297 (10 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 treatment was conducted once daily, and these treatments were repeated three times over 6 days.

2.4.3. Post-conditioning

On day 10, conditioned mice were placed in a chamber with the door open and allowed to freely move between the two compartments for 15 min, as in the pre-conditioning period. The CPP caused by Meth or SKF 81297 was evaluated by measuring the time spent in each compartment over 15 min (900 s). The CPP score reflecting the magnitude of reward was calculated as follows: CPP score (s) = (time spent in the Meth- or SKF 81297-paired compartment during the post-conditioning test) – (time spent in the same compartment during the pre-conditioning test).

2.4.4. Statistical analysis

Data are presented as the mean ± standard error of the mean (S.E.M.). Student's *t* tests or one-way analysis of variance followed by Tukey's multiple comparisons tests were performed using GraphPad Prism5. Values of *P* less than 0.05 were considered statistically significant.

3. Results

We evaluated mRNA expression of CCL7 in the PFC using reverse transcription (RT)-qPCR. The mRNA expression level of CCL7 60 min after a single administration of Meth (3 mg/kg, s.c.) was significantly higher than that after saline, and the upregulation persisted for at least 120 min (Fig. 1A, [F(4, 23) = 10.37]). Using immunohistochemistry, we found that compared with that following saline, the protein