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Chemokines and cocaine: CXCR4 receptor antagonist AMD3100 attenuates cocaine place preference and locomotor stimulation in rats

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Abstract

Plasma levels of the chemokine CXCL12 are elevated in mice following acute cocaine exposure and decreased in human cocaine abusers during withdrawal. CXCL12 is also one of the few chemokines located in the brain and can modulate dopamine transmission through activation of its receptor CXCR4. To assess a role for the CXCL12/CXCR4 system in behavioral effects of cocaine, we tested the hypothesis that AMD 3100 (Plerixafor), a CXCR4 antagonist, would inhibit conditioned place preference (CPP) and locomotor activation produced by cocaine. Rats injected with cocaine (10 mg/kg) displayed CPP relative to saline-injected controls following 4 conditioning sessions. AMD 3100 (1, 2.5, 5 mg/kg) administered prior to cocaine conditioning reduced development of cocaine CPP. AMD 3100 (5 mg/kg) also inhibited expression of cocaine-induced CPP in a paradigm in which it was injected once (following cocaine conditioning and just prior to CPP testing). In addition, AMD 3100 (5, 10 mg/kg) pretreatment reduced locomotor activation produced by an acute cocaine injection (15 mg/kg) but did not affect basal locomotor activity relative to saline-injected controls. Repeated cocaine exposure produced a significant increase (1.49-fold) in CXCL12 mRNA expression in the ventral tegmental area (VTA). Our results suggest that the CXCL12/CXCR4 system in the brain reward circuit is impacted by cocaine exposure and influences behavioral effects related to the abuse liability of cocaine.

Keywords

chemokine; CXCL12; SDF-1 α ; CXCR4; AMD 3100; Plerixafor; cocaine; psychostimulant; conditioned place preference; addiction

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1. Introduction

Central functions of chemokines are not restricted to neuroinflammation and chemotaxis but extend to neuromodulatory actions on physiological circuits underlying central nervous system (CNS) diseases (Cui et al., 2014; Adler et al., 2005). CXCL12, formerly known as stromal cell derived factor one alpha (SDF-1 α), is one of the few chemokines found in the brain and is secreted by neuronal and non-neuronal populations (Banisadr et al., 2003; Tissir et al., 2004; Stumm et al., 2007; Guyon et al., 2014). CXCL12 binds to and activates two receptors, CXCR4 and CXCR7, the former being the major receptor for CXCL12 in the brain that is expressed by astrocytes, microglia, and neurons (Bleul et al., 1996; Bajetto et al., 2001). CXCR4 also has a commercially-available antagonist (AMD3100) available to probe its receptor mechanism. AMD3100 (Plerixafor) displays selectivity for CXCR4 (*e.g.* calcium flux assays revealed no interaction of AMD3100 with the chemokine receptors CXCR1 through CXCR3, or CCR1 through CCR9) (Hatse et al., 2002; Wilson et al., 2011). AMD3100 is also FDA-approved as an immunostimulant to mobilize stem cells in cancer patients (Khan et al., 2007); therefore, human safety data would facilitate its clinical use for the treatment of addiction.

Chemokine systems are altered by cocaine exposure and can influence behavioral effects of cocaine. Plasma chemokine levels are reduced in cocaine abusers during withdrawal and elevated in mice following acute cocaine exposure (Araos et al., 2015). The administration of CXCL12 intracerebroventricularly (icv) or into the ventral tegmental area (VTA) enhances locomotor activation produced by cocaine (Trecki and Unterwald, 2009). CXCL12 injected into the substantia nigra enhances extracellular dopamine in the dorsal striatum in a CXCR4 receptor-dependent manner (Skrzydelski et al. 2007; Guyon et al., 2014). Immunohistochemical data show that CXCR4 receptors are expressed by dopamine neurons in the substantia nigra (Banisadr et al., 2002) and GABAergic medium spiny neurons in the lateral shell of the nucleus accumbens (Trecki et al., 2010). Here, we tested the hypothesis that the CXCR4 receptor system influences rewarding and locomotor stimulant effects of cocaine.

2. Materials and Methods

2.1. Animals and Chemicals

Male Sprague-Dawley rats (250–275 g) from Taconic Biosciences (Hudson, NY) were used. All animal use procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animal and approved by the Temple University Institutional Animal Care and Use Committee. Rats were housed in a controlled environment (21–23 °C) on a 12-h light/dark cycle and provided food and water ad libitum. Cocaine hydrochloride was purchased from Sigma-Aldrich (St Louis, MO). AMD3100 was purchased from AstaTech (Bristol, PA). Drugs were dissolved in physiological saline and injected intraperitoneally (ip) in a volume of 1 ml/kg.

2.3. Conditioned place preference (CPP)

CPP experiments were conducted as described (Gregg et al., 2015). CPP chambers (45 cm × 20 cm × 20 cm) consisted of two compartments separated by a removable door. A 6-day biased design consisting of three phases was used. A 30-min pre-test was conducted on day 1 to determine the initial compartment preference. The compartment in which a rat spent the least amount of time was designated as the drug-paired (least-preferred) compartment. A 4-day conditioning session was initiated the day began after the pre-test.

For experiments investigating effects of AMD 3100 on development of cocaine CPP, rats were pretreated in the morning with saline or AMD3100 (1, 2.5 or 5 mg/kg) 15 min before cocaine (10 mg/kg) and then confined to the drug-paired compartment for 30 min. In the afternoon, rats were injected with saline and placed in the opposite compartment for 30 min. Control animals were conditioned with saline in each compartment for 30 min. On day 6, rats were post-tested by being placed into the chamber with free access to both compartments for 30 min. For each rat, a difference score was calculated as the difference in time spent on the drug-paired side between post-test and pre-test days.

Experiments investigating effects of AMD 3100 on expression of cocaine CPP followed a similar procedure. During conditioning, rats were injected in the morning with cocaine (10 mg/kg) and confined to the drug-paired compartment for 30 min. In the afternoon rats were injected with saline and confined to the opposite compartment for 30 min. On the day after the 4-day conditioning phase, rats were injected with AMD 3100 (5 mg/kg) or saline, placed into the chambers and allowed free access to both compartments for 30 min.

2.4. Locomotor activity

Measurements of locomotor activity were conducted as previously described using a Digiscan DMicro system (Accuscan Inc.) consisting of transparent plastic chambers (45 cm × 20 cm × 20 cm) set inside metal frames equipped with 16 infrared light emitters and detectors (Barr et al., 2015). Following a 60-min habituation in activity chambers, rats were pretreated with AMD 3100 (5, 10 mg/kg) or saline 15 min before cocaine (15 mg/kg) or saline and activity was measured for 90 min.

2.5. Gene expression

Rats were injected with cocaine (10 mg/kg) or saline once daily for 4 days to achieve the same cocaine exposure as in our CPP studies. Thirty min following the last injection, rats were euthanized and brains were flash frozen. The VTA and nucleus accumbens were dissected from frozen slices using 1 and 2 mm round punches respectively. RNA was isolated using the Quick-RNA Miniprep kit (Zymo Research, Irvine, CA, USA), and cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time PCR was carried out with TaqMan Fast Advanced Master Mix and the TaqMan Gene Expression Assays for CXCL12 (Rn00573260_m1) and the internal control gene 18S rRNA (Hs99999901_s1) using the StepOnePlus Real-Time PCR System (Applied Biosystems). Relative gene expression was measured according to the 2^{-CT} method.

2.6. Statistical analysis

CPP data were analyzed using either a two-way ANOVA (pretreatment x treatment) or a Student's t-test. Two-way ANOVA was used to analyze time-course locomotor data (treatment x time) and cumulative locomotor data (pretreatment x treatment). Gene expression data were analyzed by a Student's t-test. For significant main effects with ANOVA, differences between individual groups were determined with a Bonferroni *post-hoc* test. Statistical significance was set at $p < 0.05$ in all cases.

3. Results

3.1. AMD3100 inhibited development and expression of cocaine CPP (Figs. 1 and 2)

Two-way ANOVA revealed pretreatment [$F(1, 26) = 5.03, p < 0.05$] and treatment [$F(1, 26) = 15.16, p < 0.001$] effects on cocaine CPP (Fig 1). Cocaine (SAL + COC) produced greater preference than saline (SAL + SAL; $p < 0.01$). AMD3100 5 mg/kg pretreatment reduced cocaine CPP (AMD + COC vs SAL + COC; $p < 0.05$). Two lower doses of AMD3100 (1, 2.5 mg/kg, ip) were tested against cocaine, and dose-effect data were graphed as % cocaine (alone) CPP (Fig. 1, box). One-way ANOVA revealed a main effect [$F(3, 36) = 3.688, p < 0.05$] with 2.5 and 5 mg/kg AMD3100 reducing cocaine CPP ($p < 0.05$).

Effects of AMD3100 (5 mg/kg) on the expression of cocaine (10 mg/kg) CPP are presented in Fig. 2. All rats were conditioned with cocaine and then challenged with either AMD 3100 or saline on the day of CPP testing. CPP was reduced in rats challenged with AMD 3100 (AMD) compared to those challenged with saline (SAL) ($p < 0.01$, Student's t-test).

3.2. AMD3100 reduced locomotor activity produced by acute cocaine exposure (Fig. 3)

Two-way ANOVA revealed treatment [$F(5, 494) = 134.39, p < 0.0001$] and time [$F(18, 494) = 7.59, p < 0.0001$] effects and a significant interaction [$F(90, 494) = 2.27, p < 0.0001$] for time-course data. Cumulative activity counts (0–60 min) are shown in the Fig. 3 box. Two-way ANOVA revealed effects of pretreatment [$F(2, 25) = 7.67, p < 0.01$] and treatment [$F(1, 25) = 51.92, p < 0.0001$]. Rats injected with cocaine (SAL + COC) displayed greater cumulative locomotor activity than drug-naïve rats (SAL + SAL) ($p < 0.001$). Pretreatment with 5 mg/kg AMD3100 [AMD (5) + COC] ($p < 0.01$) or 10 mg/kg AMD3100 [AMD(10) + COC] ($p < 0.001$) reduced cocaine-induced locomotor activity compared to pretreatment with saline (SAL + COC). In cocaine-naïve rats, locomotor activity in saline-injected rats (SAL + SAL) was not significantly different from rats treated with 5 mg/kg AMD3100 [AMD(5) + SAL] ($p > 0.05$) or 10 mg/kg AMD3100 [AMD(10) + SAL] ($p > 0.05$).

3.3. Repeated cocaine exposure increased CXCL12 gene expression in the VTA (Fig. 4)

The effect of a 4-day cocaine exposure on CXCL12 mRNA expression in the VTA and nucleus accumbens was investigated. Repeated cocaine exposure significantly increased CXCL12 mRNA levels in the VTA relative to saline-injected controls (1.49-fold increase, $p < 0.05$, Student's t-test). CXCL12 mRNA expression in the nucleus accumbens was 1.18-fold greater in cocaine- versus saline-injected rats but the effect did not reach statistical significance ($p > 0.05$, data not shown).

4. Discussion

We demonstrated that CXCL12/CXCR4 systems in the brain reward circuit are impacted by cocaine exposure and influence behavioral effects related to cocaine's abuse liability. AMD 3100 inhibited development and expression of cocaine CPP and reduced locomotor activation produced by cocaine. Repeated cocaine exposure increased CXCL12 gene expression in the VTA but not in the nucleus accumbens. Our data are the first to demonstrate that cocaine increases CXCL12 levels in the brain. Our results also identify the VTA as a potential anatomical locus in which CXCL12 interacts with dopamine to influence behavioral effects of cocaine. Since dopamine mediates cocaine's rewarding and stimulant effects and CXCL12 enhances dopamine transmission through CXCR4 receptor activation (Cervo and Samanin, 1995), AMD 3100 would be expected to inhibit both responses.

The mechanism responsible for the efficacy of AMD 3100 is unclear. However, on the basis of our gene expression data, AMD 3100 may have acted in the VTA to dampen CXCL12/dopamine signaling. In this model, CXCL12 mRNA levels in the VTA are increased during repeated cocaine exposure, leading to CXCR4 receptor activation and enhanced dopamine transmission in the nucleus accumbens that mediates cocaine reward and locomotor activation. In the case in which cocaine is administered with AMD 3100, the ensuing receptor block prevents CXCR4 activation, thereby dampening dopamine transmission and reducing cocaine's behavioral effects. Our explanation is supported by data showing that CXCL12 injected icv or into the VTA enhances locomotor activation produced by cocaine (Trecki and Unterwald, 2009). Furthermore, CXCL12 injected into the substantia nigra enhances extracellular dopamine in the dorsal striatum in a CXCR4 receptor-dependent manner (Skrzydelski et al. 2007; Guyon et al., 2014). Although a cellular location for CXCR4 in the VTA is not yet clear, expression by dopaminergic neurons is possible since CXCR4 receptors are expressed by dopamine neurons in the substantia nigra (Banisadr et al., 2002).

While our data are the first to show that cocaine increases CXCL12 levels in the brain, it is not yet known how cocaine produces this increase. One possibility is through interactions with TNF- α (tumor necrosis factor). Dopamine, but not cocaine directly, increases TNF- α mRNA expression in microglia through activation of dopamine D₂ receptors (Lewitus et al., 2016). The concentration of dopamine (1 μ M) that enhances TNF- α expression is consistent with the concentration of dopamine found in the nucleus accumbens following systemic cocaine administration (Hooks et al., 1992). Recent evidence also indicates that TNF- α increases CXCL12 mRNA expression in astrocytes (Blaževski et al., 2015). Thus, a conceivable explanation for our gene expression results is that cocaine, through elevation of extracellular dopamine in the VTA (Groves et al., 1975; Beart et al., 1979), increases TNF- α levels. The enhancement in TNF- α then increases CXCL12 levels, which enhances mesolimbic dopamine activity through CXCR4 receptor activation.

Despite our finding that cocaine exposure did not affect CXCL12 gene expression in the nucleus accumbens, the possibility that AMD 3100 acted in substrates outside of the VTA cannot yet be discounted. CXCR4 receptors are located in the caudate putamen and nucleus accumbens shell, where individual neurons express both CXCR4 and D1 receptors (Trecki et

al., 2010), and in the substantia nigra, where CXCR4 is expressed by dopamine neurons (Banisadr et al., 2002). Additionally, current technology, with the exception of mass spectrometry, is unable to effectively measure CXCL12 release into the extracellular compartment. The increase in CXCL12 mRNA levels that we detected in the VTA following cocaine exposure may be predictive of an increase in CXCL12 release/turnover that we cannot yet detect. Moreover, the inability of cocaine to increase CXCL12 gene expression in the nucleus accumbens does not exclude the possibility that cocaine produced release of CXCL12 that contributed to its behavioral effects following both acute and repeated exposure.

Consistent with a role for dopamine, AMD3100 reduced locomotor activation caused by a dose of cocaine that produced place preference. CXCL12-dopamine interactions have been identified in the context of locomotor activity, as CXCL12 injected into the midbrain increases striatal dopamine levels, induces rotational behavior, and enhances cocaine-induced hyperlocomotion (Skrzydelski et al., 2007; Trecki et al., 2009). In the present experiments, the efficacy of AMD3100 suggests that hyperlocomotion produced by cocaine exposure requires tonically active CXCR4 receptors. Basal locomotor activity was not significantly affected by AMD3100, although a downward trend was detected at the highest dose of 10 mg/kg. Since lower doses of AMD3100 effectively reduced CPP, it is unlikely that inhibition of cocaine place preference by AMD3100 was due to suppression of locomotor activity.

In summary, we showed that the CXCL12/CXCR4 system in the brain reward circuit is impacted by cocaine exposure and influences cocaine's rewarding and locomotor-stimulant effects. When considered in the context of prior work showing that plasma levels of CXCL12 are increased in mice exposed to acute cocaine, and decreased in human cocaine abusers during abstinence (Araos et al., 2015), our results suggest that interactions between neuroimmune and brain reward systems exist and contribute to the rewarding effects of cocaine.

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Research Highlights

- Repeated cocaine exposure increases CXCL12 gene expression in the ventral tegmental area.
- Chemokine CXCR4 receptor antagonist AMD3100 reduces development of cocaine place preference.
- CXCR4 antagonist AMD3100 inhibits expression of cocaine place preference.
- CXCR4 antagonist AMD3100 reduces locomotor activation produced by cocaine.
- Chemokine and neuroimmune systems may influence the abuse liability of cocaine.

Fig. 1. AMD3100 inhibited development of cocaine CPP

Rats were conditioned with cocaine (10 mg/kg) or saline for 4 days. On each day of conditioning, rats were pretreated with saline or AMD 3100 (5 mg/kg) 15 min prior to cocaine or saline administration. Data are presented as a difference score (difference in time spent on cocaine-paired side between post-test and pre-test). $N=7-8$ rats/group. $**p < 0.01$ compared to SAL + SAL and $*p < 0.05$ compared to SAL + COC. **Box)** Dose-effects of AMD3100 (1, 2.5, 5 mg/kg) on cocaine CPP. Data are presented as the percentage cocaine CPP in the absence of AMD3100. $N=8-11$ rats/group. $*p < 0.05$ compared to the group treated only with cocaine (*i.e.*, AMD3100 naïve-group).

Fig. 2. AMD3100 inhibited expression of cocaine CPP

All rats were conditioned with cocaine (10 mg/kg) and then challenged with AMD 3100 (5 mg/kg) or saline on the day of CPP testing. Data are presented as a difference score (difference in time spent on cocaine-paired side between post-test and pre-test). N= 7–8 rats/group. ** $p < 0.01$, Student's t-test.

Fig. 3. AMD3100 reduced cocaine-induced locomotor activity

Rats pretreated with AMD 3100 (5, 10 mg/kg) or saline were injected with cocaine (15 mg/kg) or saline, and locomotor activity was measured for 90 min. Time-course data are presented as activity counts/5 min. $N = 5=6$ rats/group. **Box)** Data are presented as cumulative locomotor counts from 0–60 min. *** $p < 0.001$ compared to SAL + SAL or ### $p < 0.001$, ## $p < 0.01$ compared to SAL + COC.

Fig. 4. Repeated cocaine exposure increases CXCR4 expression in the VTA

Rats were injected with cocaine (10 mg/kg) or saline for 4 days, and CXCR4 mRNA in the VTA was quantified by rt-PCR. Data are presented as the relative fold change in mRNA expression compared with saline-injected controls. N = 5-6 rats/group. * $p < 0.05$, Student's t-test.