Acute Cocaine Reduces Excitatory Synaptic Transmission in Pyramidal Neurons of the Mouse Medial Prefrontal Cortex

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The medial prefrontal cortex (mPFC) plays critical roles in the development of cocaine addiction. Numerous studies have reported about the effects of cocaine on neuronal and synaptic activities in the nucleus accumbens and ventral tegmental area, which are brain regions associated with cocaine addiction; however, a limited number of studies have reported the effect of cocaine on mPFC neuronal activity. In this study, using whole-cell patch-clamp recordings in brain slices, we present that under the condition where synaptic transmission is enhanced by increasing extracellular K⁺ concentration, cocaine significantly reduced the frequency but not amplitude of spontaneous excitatory postsynaptic currents. These findings suggest that cocaine exposure could be a trigger to induce hypofrontality, which is related to the compulsive craving for cocaine use.

Key words cocaine; medial prefrontal cortex; excitatory postsynaptic current; addiction; glutamate; mouse

INTRODUCTION

The medial prefrontal cortex (mPFC) plays critical roles in the development of cocaine addiction.5–7) The mPFC receives dopamine (DA), noradrenaline (NA) and serotonin (5-HT) projections from the ventral tegmental area (VTA), locus coeruleus and dorsal raphe, respectively. Systemic administration of cocaine increases extracellular levels of these neurotransmitters through the blockade of transporters for DA (DAT), NA (NET) and 5-HT (SERT), which are expressed in the mPFC.6,7) Contrary to the numerous studies reporting the effect of cocaine administration on the neuronal activity in the nucleus accumbens (NAc) and the VTA,8–10) few studies have addressed the effect of cocaine injection on mPFC neuronal activity. Cocaine administration reduces mPFC blood flow in humans, suggesting a decreased neuronal activity.11) In support of this finding, acute cocaine administration decreases neuronal activity in the rat mPFC.12) These cocaine-induced changes, so-called hypofrontality, may contribute to the development of cocaine addiction. However, the mechanisms underlying these inhibitory effects of cocaine remains unclear. Considering that synaptic transmission plays a crucial role in regulating neuronal activity, it is hypothesized that cocaine modulates the excitatory synaptic transmission in the mPFC. To address this hypothesis, we performed whole-cell patch-clamp recordings in mPFC layer V (L5) pyramidal neurons using mouse brain slices.

MATERIALS AND METHODS

Animals C57BL/6J mice (female, 4–6 weeks of age) were used in the present study. Mice were maintained in a constant ambient temperature (22 ± 1°C) under a 12-h light/dark cycle with food and water available ad libitum. All experiments were conducted in accordance with the National Institutes of Health guidelines and performed with the approval of the Institutional Animal Care and Use Committee at Kanazawa University. All efforts were made to minimize the suffering and number of animals used in this study.

Slice Preparation and Electrophysiology Mice were anesthetized with isoflurane and decapitated. The brains were submerged in ice-cold modified Ringer’s solution containing the following (in mM): choline chloride, 125; KCl, 2.5; NaH₂PO₄, 1.25; MgCl₂, 2.5; CaCl₂, 2.0; NaHCO₃, 26; and glucose, 25; and bubbled with 95% O₂/5% CO₂ (pH 7.4). Coronal slices (250 μm thick) of the mPFC were cut with a microslicer (VT1200S, Leica, Wetzlar, Germany) and incubated at 32–34°C for 15–30 min in standard Ringer’s solution containing the following (in mM): NaCl, 125; KCl, 2.5; NaH₂PO₄, 1.25; MgCl₂, 1.5; CaCl₂, 2.0; NaHCO₃, 26; and glucose, 25; and bubbled with 95% O₂/5% CO₂ (pH 7.4). The slices were transferred to standard Ringer’s solution at room temperature, and then mounted in a recording chamber on an upright microscope (BX-51WI, Olympus, Tokyo, Japan). The chamber was continuously superfused with standard Ringer’s solution at a flow rate of 2–2.5 mL/min. Whole-cell voltage-clamp recordings were obtained from mPFC L5 pyramidal neurons by visually controlling patch pipettes, which were prepared from borosilicate glass capillaries and filled with an internal solution containing the following (in mM): CsOH, 150; CsCl, 5.0; MgCl₂, 2.0; Na₂ATP, 4.0; Na₂GTP, 0.3; ethylene glycol bis(2-aminoethyl ether)-N,N',N'N'-tetraacetic acid (EGTA), 10; N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid (HEPES), 10; QX-314, 3.0 (pH 7.3 with gluconic acid). The resistance of the electrodes was 3–9 MΩ in the Ringer’s solution. All recordings were performed at 32–34°C.

To record spontaneous excitatory postsynaptic currents (sEPSCs), the membrane potentials were held at −70 mV. In some experiments, recordings were performed in high-K⁺ Ringer’s solution, which was prepared by equimolar substitution of NaCl with 3.5–4 mM KCl. Frequencies and amplitudes
of sEPSCs and the holding current were measured in the periods of 0–1 min before and 4–5 min after cocaine hydrochloride (Takeda Pharmaceutical, Osaka, Japan, 5 µM) application and 0–1 min before high-K⁺ Ringer’s solution substitution with the use of Clampfit 10.5 (Molecular Devices) and Mini Analysis 6.0 (Synaptosoft). To record miniature EPSCs (mEPSCs), tetrodotoxin (TTX, Nacalai Tesque, Kyoto, Japan, 500 nM) was bath applied. The effects of the drugs were evaluated by comparing the average values of these periods.

**Statistical Analyses** Data are expressed as means ± standard error of the mean (S.E.M.) and were compared using Student’s t-test or paired t-test when comparing two groups or one-way repeated measures ANOVA with post hoc Tukey’s multiple comparison test when comparing more than two groups using Prism 6 (GraphPad Software, La Jolla, CA, U.S.A.). Results were considered significant if the p value was <0.05.

**RESULTS** We first investigated whether cocaine affects sEPSCs in mPFC L5 pyramidal neurons in normal Ringer’s solution. Bath application of cocaine (5 µM) did not affect the frequency and amplitude of sEPSCs (frequency, control, 5.3 ± 1.2 Hz, vs. cocaine, 5.4 ± 1.6 Hz, n = 5 from 4 mice, p = 0.7970; amplitude, control, 19.1 ± 1.8 pA, vs. cocaine, 16.7 ± 1.2 pA, p = 0.0507; Figs. 1A–C). Additionally, cocaine failed to change the holding currents (control, −36.3 ± 3.6 pA, vs. cocaine, −30.8 ± 6.0 pA, p = 0.1578; Fig. 1D). Thus, under normal conditions, cocaine does not affect excitatory synaptic transmission in mPFC L5 pyramidal neurons.

Because cocaine exerts its effect by blocking DAT, NET and/or SERT, we hypothesized that extracellular levels of these neurotransmitters are not sufficient to observe the effect of cocaine in vitro. Thus, we next increased the extracellular K⁺ concentration, which has been reported to enhance synaptic activity.¹¹ When the extracellular fluid was replaced by high-K⁺ Ringer’s solution, the frequency of sEPSCs and the holding current were significantly increased (frequency, control, 5.8 ± 1.3 Hz, vs. high-K⁺, 8.1 ± 1.8 Hz, n = 7 from 6 mice, p = 0.0351; Figs. 2A, B; holding current, control, −23.8 ± 7.5 pA, vs. high-K⁺, −40.0 ± 8.0 pA, p = 0.0013; Fig. 2D), without affecting the amplitude of sEPSCs (control, 20.1 ± 2.5 pA, vs. high-K⁺, 18.8 ± 2.2 pA, p = 0.5796; Figs. 2A, C). Under this high-K⁺ condition, a bath application of cocaine significantly reduced the frequency but not the amplitude of sEPSCs (frequency, high-K⁺, 8.1 ± 1.8 Hz, vs. high-K⁺ + cocaine, 6.0 ± 1.7 Hz, p = 0.0014; Figs. 2A, B; amplitude, high-K⁺, 18.8 ± 2.2 pA, vs. high-K⁺ + cocaine, 18.9 ± 2.2 pA, p = 0.9985; Figs. 2A, C). On the other hand, cocaine did
not affect the holding current (holding current, high-K\(^+\), 
\(-40.0 \pm 8.0\) Hz, vs. high-K\(^+\) + cocaine, 
\(-37.7 \pm 8.1\) Hz, \(p = 0.1859\); Fig. 2D).

We also examined the effect of cocaine at a higher concentration (50 \(\mu M\)). The inhibitory effect of the higher concentration on sEPSC frequency was larger than that of the lower concentration; the reductions of sEPSC frequency by 5 and 50 \(\mu M\) were 30.4 \(\pm 5.6\)% and 53.7 \(\pm 10.3\)% of high-K\(^+\), respectively (\(p = 0.0627\)), indicating the concentration-dependent effect of cocaine.

Together, these results indicate that cocaine attenuates the sEPSC frequency in mPFC L5 pyramidal neurons under the condition where synaptic transmission is relatively high.

We next tested whether inhibitory effect of cocaine is still observed in the presence of TTX, which blocks action potential-dependent synaptic transmission. Under the high-K\(^+\) condition, a bath-application of TTX (500 nM) significantly reduced the mEPSC frequency but not amplitude and holding current (frequency, high-K\(^+\), 7.5 \(\pm 0.8\) Hz, vs. high-K\(^+\) + TTX, 3.0 \(\pm 0.6\) Hz, \(n = 5\) from 3 mice, \(p = 0.0097\); Figs. 3A, B; amplitude, high-K\(^+\), 20.1 \(\pm 3.5\) pA, vs. high-K\(^+\) + TTX, 12.9 \(\pm 1.1\) pA, \(p = 0.1258\); Figs. 3A, C; holding current, high-K\(^+\), \(-47.1 \pm 10.4\) pA, vs. high-K\(^+\) + TTX, \(-41.0 \pm 10.8\) pA, \(p = 0.1899\); Fig. 3D). Additional application of cocaine failed to affect the frequency and amplitude of mEPSCs (frequency, high-K\(^+\) + TTX, 3.0 \(\pm 0.6\) Hz, vs. high-K\(^+\) + TTX + cocaine, 2.9 \(\pm 0.6\) Hz, \(p = 0.8559\); Figs. 3A, B; amplitude, high-K\(^+\) + TTX, 12.9 \(\pm 1.1\) pA, vs. high-K\(^+\) + TTX + cocaine, 12.3 \(\pm 0.9\) pA, \(p = 0.3005\); Figs. 3A, C) and holding current (holding current, high-K\(^+\) + TTX, \(-41.0 \pm 10.8\) pA, vs. high-K\(^+\) + TTX + cocaine, \(-42.4 \pm 11.9\) pA, \(p = 0.8031\); Fig. 3D).

**DISCUSSION**

We found that although cocaine did not affect the frequency of sEPSC in the standard Ringer’s solution, it significantly decreased the sEPSC frequency under high-K\(^+\) conditions in mPFC L5 pyramidal neurons. This finding is consistent with that of a previous report showing that cocaine application reduced the amplitude of evoked excitatory postsynaptic potentials (EPSPs) in mPFC neurons. Extracellular K\(^+\) levels affect axonal excitability, and high-K\(^+\) has been reported to increase synaptic activity. Under high-K\(^+\) conditions, DA, NA, and 5-HT release may be enhanced, and cocaine could increase extracellular levels of these neurotransmitters via a blockade of DAT, NET, and SERT in the mPFC, leading to a decrease in the sEPSC frequency. Although we have not examined whether the reduced effect is mediated by a presynaptic or postsynaptic mechanism, the non-significant effect of cocaine on the amplitude of sEPSCs suggests a presynaptic mechanism. In support of this hypothesis, previous studies have demonstrated that DA activates presynaptically located D1 or D2 receptors, which leads to a decrease in evoked EPSC/EPSP amplitudes. Likewise, NA stimulates presynaptic α2 adrenoceptors, which decreases glutamate release. Additionally, 5-HT decreases the frequency of miniature EPSCs via activation of 5-HT1B receptors, presumably located in presynaptic terminals. Therefore, the inhibitory effects of cocaine observed in the present study are likely to be exerted by stimulation of any or all of these receptors. However, considering the findings that in the presence of TTX cocaine failed to affect mEPSC frequency, the inhibitory effect of cocaine on sEPSCs might be mediated by a postsynaptic mechanism. Nevertheless, the possibility that the effect of cocaine is mediated by a presynaptic mechanism cannot be discarded, because TTX may dramatically reduce extracellular monoamines, without which cocaine might not be able to exert its effect. Further studies would be necessary to clarify this issue and to determine the receptors involved in the effects of cocaine.

The cocaine-induced reduction of sEPSCs observed in this study may be at least partly account for the reduced activity of mPFC neurons recorded after cocaine administration in rats in vivo. The reduction of mPFC neuronal activity may be related to cocaine-induced hypofrontality, in which mPFC neuronal responses to cocaine and cocaine-associated cues are amplified. Thus, our finding might be one of the mechanisms for generating hypofrontality, which is critically involved in the development of cocaine addiction.

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**Conflict of Interest** The authors declare no conflict of interest.

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