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Cocaine facilitates glutamatergic transmission and activates lateral habenular neurons

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ABSTRACT

Cocaine administration can be both rewarding and aversive. While much effort has gone to investigating the rewarding effect, the mechanisms underlying cocaine-induced aversion remain murky. There is increasing evidence that the lateral habenula (LHb), a small epithalamic structure, plays a critical role in the aversive responses of many addictive drugs including cocaine. However, the effects of cocaine on LHb neurons are not well explored. Here we show that, in acute brain slices from rats, cocaine depolarized LHb neurons and accelerated their spontaneous firing. The AMPA and NMDA glutamate receptor antagonists, 6, 7-dinitroquinoxaline-2, 3-dione, DL-2-amino-5-phosphono-valeric acid, attenuated cocaine-induced acceleration. In addition, cocaine concentration-dependently enhanced glutamatergic excitation: enhanced the amplitude but reduced the paired pulse ratio of EPSCs elicited by electrical stimulations, and increased the frequency of spontaneous EPSCs in the absence and presence of tetrodotoxin. Dopamine and the agonists of dopamine D1 (SKF 38393) and D2 (quinpirole) receptors, as well as the dopamine transporter blocker (GBR12935), mimicked the effects of cocaine. Conversely, both D1 (SKF83566) and D2 (raclopride) antagonists substantially attenuated cocaine's effects on EPSCs and firing. Together, our results provide evidence that cocaine may act primarily via an increase in dopamine levels in the LHb that activates both D1 and D2 receptors. This leads to an increase in presynaptic glutamate release probability and LHb neuron activity. This may contribute to the aversive effect of cocaine observed *in vivo*.

1. Introduction

Cocaine abuse is prevalent throughout the world. Like many other addictive drugs, cocaine has both rewarding and aversive properties (Ettenberg, 2004; Riley, 2011; Serafine et al., 2012a), and a balance between these two effects influences drug abuse potential (Hunt and Amit, 1987). The dopamine transporter (DAT) is a key player in dopamine neurotransmission. Inhibition of the DAT is a major factor that contributes to cocaine reward (Sora et al., 2001). Although recent evidence suggests that dopamine may also contribute to cocaine's aversive property (Freeman et al., 2005; Serafine et al., 2012b), the underlying mechanisms remain murky.

There is increasing evidence that the lateral habenula (LHb), an epithalamic nucleus, plays a critical role in fear, anxiety, stress, drug abuse, and aversion (Bromberg-Martin and Hikosaka, 2011; Matsumoto and Hikosaka, 2007; Pobbe and Zangrossi, 2010; Shabel et al., 2012; Winter et al., 2011). Converging anatomical, physiological, and behavioral studies show that the LHb is one of the few regions of the brain to be inhibited by hedonic stimuli and reward, but activated by various aversive stimuli and reward prediction error (Bromberg-Martin and Hikosaka, 2011; Matsumoto and Hikosaka, 2007). Recent evidence links the LHb to cocaine addiction and aversion. Acute cocaine exposure selectively increases Fos protein expression (an index of neural activity) in the LHb (Zahm et al., 2010; Zhang et al., 2007). Chronic cocaine administration specifically induces degeneration in axons from the LHb to fasciculus retroflexus (Ellison, 2002). Lesions of the LHb increased

Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4- propionic acid; AP5, DL-2-amino-5-phosphono-valeric acid; DA, dopamine; DNQX, 6, 7-dinitroquinoxaline-2, 3-dione; Hb, Habenula complex; LHb, Lateral habenula; sEPSCs, spontaneous excitatory postsynaptic currents; mEPSCs, miniature excitatory postsynaptic current; TTX, tetrodotoxin.

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a delayed cocaine seeking behavior, whereas, deep-brain-stimulation, that depicts glutamate transmission to the LHb, reduced cocaine seeking behavior (Friedman et al., 2010). Cocaine selectively potentiates AMPA receptor-mediated EPSCs in LHb neurons that send axons to GABAergic neurons in the rostromedial tegmental nucleus (RMTg), which send inhibitory projection to the dopamine neurons in the ventral tegmental area (Maroteaux and Mameli, 2012).

The LHb is subject to dopamine regulation. There is mutual innervation between the LHb and the midbrain dopaminergic structures. The LHb not only send their projections (directly and indirectly) to the midbrain dopaminergic neurons but also receive dopaminergic projections (Goncalves et al., 2012; Gruber et al., 2007; Skagerberg et al., 1984). Dopamine activates D1-like (consisting of D1 and D5) and D2-like (D2, D3, and D4) receptor families; both of which play critical roles in cocaine addiction (Friedman et al., 2010; McCulloch et al., 1980; Ramm et al., 1984), and are richly expressed on the LHb (GENSAT, 2008; Savasta et al., 1986; Weiner et al., 1991). Systemic administration of dopamine agonists induces aversive responses such as nausea, vomiting (Perez-Lloret et al., 2010), and conditioned taste aversions (Asin and Montana, 1989), as well as increases firing of LHb neurons and c-fos expression in the LHb. These effects were prevented by the dopamine antagonist haloperidol (Kowski et al., 2009; Wirtshafter et al., 1994). In the current study, we investigated the effects of acute cocaine on the activity and glutamate transmission of the LHb neurons in brain slices of rats.

2. Materials and methods

2.1. Animals and brain slice preparation

All procedures were in accordance with the National Institutes of Health guidelines and with the approval of the Animal Care and Utilization Committee of the University of Medicine and Dentistry of New Jersey. We minimized the number of animals used and their suffering. We studied 70 Sprague–Dawley rats at postnatal days 20–30. These rats were housed two per cage with food and water available *ad libitum*, and kept on a standard 12-h light/dark cycle with lights on at 7:00 A.M and off at 7:00 P.M. On the experimental days, rats were deeply anesthetized with ketamine/xylazine (IP) and then decapitated. Brains were rapidly dissected and placed in a cold glycerol-based artificial cerebrospinal fluid (aCSF) containing (in mM): 251 glycerol, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_26H_2O$, 2 $CaCl_2$, 25 $NaHCO_3$, 1 L-ascorbate, and 11 glucose (Ye et al., 2006), and oxygenated with 95% O_2/5% CO_2 (carbogen). Coronal slices (200–250 μm thick) containing the LHb were cut with a Compresstome VF-200 slicer (Precisionary Instruments Inc., Greenville, NC, USA), then immediately transferred to a holding chamber and incubated for at least 1 h at room temperature (24°-25 °C) in cabogenated (95% O_2/5% CO_2) regular aCSF which has almost the same composition as glycerol-based aCSF, the exception being that the 252 mM glycerol was replaced with 126 mM NaCl. In a low Ca^{2+} solution, $CaCl_2$ was replaced by an equivalent amount of $MgCl_2$. For electrophysiological recording, a single slice was transferred to a submersion-type recording chamber and was stabilized with a platinum ring. Only one neuron was recorded from each slice.

2.2. Electrophysiology

Electrophysiological recordings were obtained with an Axon 200B amplifier, a Digidata 1440A A/D converter, and Clampfit 10.3 software (Molecular Devices Co., Union City, CA, USA). Data was filtered at 2 kHz and sampled at 5 kHz with the Digidata 1440A interface. The experiments were done at 32.6 °C and maintained by an automatic temperature controller (Warner Instruments, Hamden, CT, USA). ACSF was perfused at a rate of 1.5–2 ml/min. Patch pipettes had a resistance of 6–8 MΩ when filled with an internal solution containing (in mM) 140 cesium methanesulfonate, 5 KCl, 2 $MgCl_2$, 10 Hepes, 2 MgATP, 0.2 GTP for voltage clamp recordings or 140 potassium gluconate, 5 KCl, 2 $MgCl_2$, 10 Hepes, 2 MgATP, and 0.2 GTP for current clamp recordings. The pH of these pipette solutions were adjusted to 7.2 with Tris-base and the osmolality to 310 mOsmol/L with sucrose. Excitatory postsynaptic currents (EPSCs), mediated by α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, including both evoked and spontaneous, were recorded at a holding potential (V_H) of −70 mV in the presence of gabazine (10 μM) and strychnine (1 μM) to block GABA and glycine receptors. To elicit EPSCs (eEPSCs), a nichrome wire bipolar stimulating electrode was positioned within 200 μm of the soma, electrical stimuli (100–200 μs in duration) were given at the rate of 0.05 Hz. Near the start of the recording, an input/output curve was obtained and the stimulation was then set to 20–30% of the maximum, an intensity that resulted in stable responses with no failures. Paired eEPSCs were elicited with a pair of identical stimuli separated by an interval of 50 ms. Inhibitory postsynaptic currents (IPSCs), mediated by GABAA receptors were recorded at a V_H of +40 mV in the presence of AP5(50 μM) and DNQX (20 μM) to block glutamate receptors. Spontaneous discharges of LHb neurons were recorded by the loose-patch cell-attached technique, which permits long-lasting recordings without perturbing the cytoplasmic contents. In some experiments, firing was recorded in whole-cell mode, which allowed for the measurement of the resting membrane potential, input resistance, and synaptic currents.

2.3. Chemicals

We purchased cocaine, cadmium chloride ($CdCl_2$), 6, 7-dinitroquinoxaline-2, 3-dione (DNQX), DL-2-amino-5-phosphono-valeric acid (DL-AP5), strychnine, SKF38393, SKF83566, raclopride, quinpirole, dopamine, GBR12953, gabazine, and tetrodotoxin (TTX) from Sigma (St Louis, MO). Drugs at final concentration were added to the superfusate.

2.4. Data analysis and statistics

The baseline mean values of electrophysiological data were obtained during the initial control period (3–5 min) and after washout. To assess the effects of a drug, data over a 3 min period at the peak of a drug response from each cell were obtained and then averaged. All data were expressed as mean ± S.E.M. Statistical significance was assessed using a two-tailed Student's *t* test, a one-way ANOVA with a Tukey's *post hoc* test for multiple group comparisons, or a Kolmogorov–Smirnov (K–S) test. Dose-response data were fitted to the logistic equation: $y = 100x^a/(x^a + x_0^a)$, where y is the percentage change, x is the concentration of cocaine, a is the slope parameter, and x_0 the cocaine concentration which induces a half-maximal change (e.g., Fig. 2F). Significance for all analyses was determined at $p < 0.05$.

3. Results

Electrophysiological data were obtained from 200 neurons, located in both the lateral and medial sub-regions of the LHb, but mostly from the former. No significant difference was observed between data from these two sub-regions and between data from juvenile male and female rats, data were pooled.

3.1. Cocaine activates LHb neurons involving glutamate transmission

We first examined the effects of cocaine on the spontaneous firings of LHb neurons. Under current clamp, 10 μM cocaine reversibly depolarized the cells (Fig. 1A and B), by an average of 4.6 ± 1.0 mV ($n = 6$, $t = 4.6$, $p < 0.01$, paired t test; Fig. 1B). Meanwhile, cocaine robustly accelerated the ongoing discharges. A similar acceleration was observed in the cell-attached mode (Fig. 1C–E). Data obtained from current clamp and cell-attached configurations were not significantly different and therefore were pooled. The initial spontaneous firing rate was stable in aCSF. Bath application of 10 μM cocaine increased the firing rate by 72.3 ± 14.9%, from 6.0 ± 0.8 Hz in control to 10.1 ± 1.1 Hz ($n = 17$, $t = -4.8$, $p < 0.001$; Fig. 1F). The effects of cocaine were reversed after wash. Repeated cocaine application to the same neuron produced a similar increase (Fig. 1C).

Glutamate is a major excitatory neurotransmitter, playing a crucial role in the neuronal function of many brain areas and in cocaine addiction (Cornish and Kalivas, 2000; Mameli et al., 2011; Uys and LaLumiere, 2008). The LHb receives a lot of glutamatergic inputs, including those from the lateral hypothalamus, lateral preoptic area and basal ganglia (Kowski et al., 2008; Matsumoto and Hikosaka, 2007), and the LHb neurons are largely glutamatergic (Omelchenko et al., 2009). To determine whether glutamate transmission plays a role in cocaine-induced acceleration of LHb firing, we compared the effect of cocaine in the absence and presence of the NMDA and AMPA/KA-type glutamate antagonists. The application of AP5 (50 μM) and DNQX (20 μM) substantially lower the spontaneous firing rate (by 43.6 ± 13.4%, $n = 6$, $t = 3.3$,