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The glutamatergic projection from the prefrontal cortex to the nucleus accumbens core is required for cocaine-induced decreases in ventral pallidal GABA

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Abstract

Cocaine-primed reinstatement of drug-seeking is associated with a decrease in extracellular GABA in the ventral pallidum (VP). The present study investigated the neural mechanism of this cocaine-induced decrease in VP GABA by determining if activity of the glutamatergic projection from the medial prefrontal cortex (PFC) to the nucleus accumbens is required for the effect. Microdialysis was performed to measure extracellular GABA in the VP while simultaneously, either a combination of the GABA agonists baclofen and muscimol was microinjected into the PFC, or the AMPA/kainate glutamate receptor antagonist CNQX was microinjected into the accumbens core. Inhibition of the PFC with GABA agonists and blockade of AMPA glutamate receptors in the accumbens core were both sufficient to prevent the cocaine-induced decrease in VP GABA, further implicating increased activity of the cortico-striato-pallidal circuit in relapse to drug-seeking.

Keywords

cocaine; prefrontal cortex; nucleus accumbens; ventral pallidum; GABA; dialysis; glutamate

Introduction

As originally outlined by Mogenson (1980) [12], it has long been proposed that motor activity elicited by amphetamine-like psychostimulants arises from inhibition of the GABAergic projection from the nucleus accumbens to the ventral pallidum (VP). This hypothesis is based primarily upon the fact that acute administration of psychostimulants inhibits accumbens medium spiny neurons, and that microinjecting GABA agonists into the VP prevents psychostimulant-induced motor activity [13,19]. However, apparent contradictions to this hypothesis have emerged over the last 20 years, notably that the microinjection of compounds into the accumbens that increase spiny cell firing frequency, such as ionotropic glutamate receptor agonists or the GABA_A receptor antagonist picrotoxin, promote motor activity [2,4, 18]. One possible explanation for this apparent paradox lies in the fact that the GABAergic projection from the accumbens to the VP is co-localized with various neuropeptides, including enkephalin [22]. Accordingly, mu opioid receptor stimulation in the VP is thought to promote motor activity, in part, by reducing presynaptic release of GABA, and repeated cocaine

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administration results in reduced extracellular GABA in the VP due to increasing stimulation of presynaptic mu opioid receptors [1,20]. Moreover, stimulation of mu opioid receptors in the VP is necessary for cocaine-seeking to be induced in the reinstatement animal model of relapse [20].

The reinstatement of cocaine-seeking is measured by training animals to self-administer cocaine, and then extinguishing this responding by no longer administering cocaine when a response is made. After extinction, re-exposing the animal to cocaine by non-contingent injection reinstates responding [17]. Using this model, it has been shown that neuronal activity not only in the VP, but also in the dorsomedial prefrontal cortex (PFC) and core of the accumbens (NAcore) is necessary for cocaine-seeking to occur [7]. Moreover, it was shown that the PFC, NAcore and VP form a series circuit in regulating reinstatement [8]. In addition, increased glutamate release from the PFC to NAcore [9], and reduced extracellular GABA in the VP are associated with cocaine-seeking [20]. This association between glutamate in the accumbens and GABA in the VP is paradoxical given that glutamate in the accumbens activates spiny cells [5], and would therefore be predicted to increase GABA transmission in the VP.

While the aforementioned presynaptic regulation of GABA release by mu receptors in the VP offers an explanation for how stimulating spiny cells can reduce GABA transmission, it is not clear from the studies to date whether the cocaine-induced release of glutamate into the NAcore from PFC afferents is capable of reducing extracellular GABA in the VP. Accordingly, the goal of the present study was to determine if the glutamatergic projection from the PFC to the NAcore is necessary for the cocaine-induced decrease in GABA in the VP of animals pretreated with chronic cocaine.

Materials and Methods

Male Sprague-Dawley rats (Charles River, Indianapolis, IN) weighing 250–275 grams were housed individually in humidity and temperature controlled rooms, and maintained on a 12/12 hr light/dark cycle. Rats were allowed *ad libitum* access to food and water. All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Medical University of South Carolina Animal Care and Use Committee. Cocaine hydrochloride was generously provided by the National Institute on Drug Abuse and was dissolved in 0.9% sterile saline. CNQX, muscimol, and (RS)-baclofen were purchased from Tocris Cookson Inc. (Ellsville, MO), and were dissolved in artificial cerebral spinal fluid (aCSF) for microinjection.

Rats were anesthetized with a combination of 87.5 mg/kg ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) and 5 mg/kg xylazine (Rompun; Bayer, Shawnee Mission, KS) administered intramuscularly (i.m.). Animals were also given the analgesic Ketoralac (3 mg/kg, i.p., Hospira Inc., Lake Forest, IL) before surgery. Animals were implanted bilaterally with 20 gauge microdialysis guide cannula (Plastics One, Roanoke, VA) aimed 2 mm dorsal to the VP (from bregma: 0 mm AP, ± 4.0 mm ML, -6.5 mm DV at an angle of 10°), and 26 gauge microinjection cannula (Small Parts Inc., Miami Lakes, FL) aimed 1 mm above the PFC or NAcore (all coordinates are relative to bregma; PFC: $+3.0$ mm AP, ± 0.7 mm ML, -2.0 mm DV; NAcore: $+2.5$ mm AP, ± 1.8 mm ML, -5.8 mm DV). All coordinates were based on the rat stereotaxic atlas by Paxinos and Watson [16], and after securing the cannula with 3 screws and dental acrylic resin, dummy cannula (C313DC, Plastics One, Roanoke, VA) or obturators were inserted the length of the microdialysis and microinjection guide cannula, respectively. Animals were allowed 7 days to recover from surgery before experimentation.

Dialysis probes were constructed with 1.5–2.0 mm active membrane as described elsewhere [20]. The night before microdialysis experiments animals were implanted with dialysis probes

extending into the VP. The next morning the probes were perfused for 2 hours with aCSF (5 mM D-glucose, 2.5 mM KCl, 140 mM NaCl, 1.4 mM CaCl₂, 1.2 mM MgCl₂, and 0.15% PBS, pH 7.4) at a rate of 2 μ L/min, and 6 baseline samples were collected over a period of 2 hours (20 min/sample). Samples were stored at -80°C until analysis, and the concentration of GABA in samples was determined by electrochemical detection as described elsewhere [20].

All rats were administered a saline injection on day 0, and then divided into a chronic cocaine or saline group. The cocaine regimen was 15 mg/kg i.p. on days 1 and 7 and 30 mg/kg on days 2–6. Rats were then not injected with cocaine or saline for 3 weeks, during which time the animals had surgery to implant both microinjection and dialysis cannula (see above). At 3 wks after the last daily injection, a unilateral microinjection of aCSF or a baclofen (0.3 nmol)/ muscimol 0.03 nmol combination was made in a volume of 0.3 μ L over 1 min into the hemisphere ipsilateral to the microdialysis probe, or rats received a unilateral microinjection of aCSF or CNQX (1 nmol/0.3 μ L) into the NAc core ipsilateral to the microdialysis probe. Immediately following either microinjection, rats were administered a cocaine injection (15 mg/kg ip), and dialysis samples were collected for an additional 2 hours (6 samples).

At the end of each experiment animals were anesthetized with pentobarbital, and the brains were transcardially perfused with saline and placed in 4% paraformaldehyde. Coronal brain sections (80 μ m thick) were placed on gelatin subbed slides, stained with cresyl violet, coverslipped, and correct cannula placements were determined using a light microscope. Placement of cannula into the PFC, NAc core and VP was considered correct according to previously established guidelines [8,20]. Animals with incorrect cannula placements were not included in the data analysis. Statistical analyses of the data were performed using Graph Pad Prism software. All dialysis data were normalized to the average of the last three baseline samples. Group differences were estimated using a two-way ANOVA with repeated measures over time, followed by a post hoc least significant difference test when a significant interaction effect was measured [10].

Results

Using a two-way repeated measures ANOVA, it was determined that the control aCSF injections into the PFC or NAc core resulted in identical effects on extracellular GABA in the VP, so these data were pooled into chronic saline or chronic cocaine groups termed aCSF in figure 1. Figure 1A shows that inhibiting the PFC with the baclofen/muscimol (B/M) cocktail, or inhibiting AMPA/kainate receptors in the NAc core with CNQX prevented the cocaine-induced decrease in extracellular GABA in the VP of animals withdrawn for 3 wks from a week of repeated cocaine administration. A two-way ANOVA with repeated measures over time revealed a significant main effect of treatment ($F_{(2,176)}=9.79$, $p=0.006$), a significant interaction between treatment and time ($F_{(16,176)}=11.32$, $p=0.005$), and a trend towards a main effect of time ($F_{(8,176)}=4.72$, $p=0.064$). A post hoc least significant differences test further showed that the B/M group was significantly different from the aCSF group at 20, 40 and 60 min after injection, and that the CNQX group differed at 60 and 80 min. Only in the aCSF group did acute cocaine administration significantly reduce extracellular GABA compared to the last baseline sample (-20 min).

Figure 1B shows parallel data to figure 1A except that all animals were pretreated with chronic saline. In this experiment, the B/M group and aCSF groups were identical, while CNQX was found to significantly elevate extracellular GABA compared to aCSF at 20–80 min after injection. Similarly, only CNQX significantly altered extracellular GABA relative to the last baseline sample. A two-way ANOVA revealed a significant main effect of treatment ($F_{(2,184)}=13.08$, $p=0.015$) and a significant interaction between treatment and time ($F_{(16,184)}=11.13$, $p<0.001$), but no main effect of time ($F_{(8,184)}=2.64$, $p=0.215$).

Discussion

The present data confirm that the reduction produced in extracellular GABA in the VP by acute cocaine administration in animals withdrawn from chronic cocaine is prevented by inhibiting neuronal activity in the PFC or by blocking AMPA/kainate receptors in the NAc. This cocaine-induced reduction in extracellular GABA in the VP has recently been linked to the reinstatement of cocaine-seeking [20], and the present data are consistent with the established role of the glutamatergic projection from the PFC to the NAc in both cocaine-induced reinstatement [7], and the expression of locomotor sensitization [21]. However, the present study did not compare the effect of the mPFC and NAc manipulations on VP GABA after an acute saline injection, so it is possible that these treatments produce an opposing increase in VP GABA by a separate process than that which mediates the cocaine-induced decrease in GABA. The finding that blockade of AMPA receptors in the NAc produces an increase in VP GABA in response to cocaine in chronic saline treated animals, supports the hypothesis that CNQX may produce an increase in VP GABA on its own, which opposes the cocaine induced decrease.

Having a reduction in extracellular GABA in the VP associated with increased glutamate transmission in the projection from the PFC to NAc is problematic given that increasing NAc spiny cell activity would be predicted to elevate GABA release in the NAc to VP GABAergic projection. Moreover, it has long been postulated that psychostimulant-induced locomotor activity results from direct inhibition of this GABAergic projection [11,12]. There are a number of potential explanations for this paradox. The previous hypotheses have been based upon acute psychostimulant administration, and as shown herein and elsewhere [3,20], acute cocaine or amphetamine administration produces only a slight reduction in extracellular GABA in the VP, and the multiple neuroadaptations produced in the NAc by chronic psychostimulant administration may alter the effect of psychostimulants on the NAc to VP projection [6,7]. One of these adaptations is reduced GABA in the VP, and this cocaine-induced reduction is blocked by inhibiting mu opioid receptors in the VP [20]. It has long been known that GABA and enkephalin co-localize in the NAc to VP projection, and the effect of mu opioid receptor blockade indicates that co-released enkephalin may be presynaptically inhibiting GABA release [14,15,20]. A final consideration is that the source of extracellular GABA in the VP is not well defined, and may arise from the NAc afferents, recurrent collaterals from the GABAergic VP projection neurons, or from non-neuronal sources. However, the fact that the cocaine-induced reduction in extracellular GABA was blocked by pharmacological manipulation of the PFC and NAc supports involvement of the PFC to NAc to VP series projection in the measured effects on extracellular GABA. Indeed, previous studies employing contralateral, unilateral microinjections of B/M into the PFC and VP demonstrated that a series projection between the PFC and VP was critical for cocaine induced reinstatement of drug-seeking, and the likely intervening synapse between the PFC and VP is in the NAc since B/M injections in the NAc also inhibited reinstatement [8].

However, as stated above, the results could also be interpreted as two synergistic, independent processes, one produced by cocaine to decrease VP GABA, which sensitizes during withdrawal from chronic cocaine, and one produced by tonic glutamatergic activity in the NAc to suppress GABA release in the VP. If the effect of CNQX in the NAc to increase GABA in saline treated animals were independent of cocaine's effects, one would still hypothesize that activation of glutamatergic AMPA receptors in the NAc suppresses VP GABA release, most likely via pre-synaptic inhibition of release by co-localized peptides. The large increase in accumbens glutamate produced by cocaine after withdrawal from chronic cocaine [9], likely enhances this AMPA receptor activity to produce a significant decrease in VP GABA that is necessary for the expression of addiction-like behaviors [20]. This hypothesis is supported by the slight, non-significant decrease in VP GABA produced by cocaine in the saline treated

animals, because acute cocaine at the dose used in the present study does not produce a large increase in NAc core glutamate [23] compared to that produced in animals withdrawn from chronic cocaine.

In addition, inhibition of the PFC to reduce glutamatergic input to the NAc did not increase VP GABA in saline treated rats, indicating that glutamatergic inputs from other sources, such as the amygdala or hippocampus, were sufficient to maintain the suppression of VP GABA release, but that when glutamatergic activation from all inputs is inhibited by CNQX a rise in VP GABA is revealed. However, it is clear that without activation of the mPFC and AMPA receptors in the NAc core, cocaine does not reduce VP GABA.

In conclusion, these data demonstrate that cocaine-induced changes in extracellular GABA in the VP are regulated by the PFC and glutamatergic transmission in the NAc core. Combined with the previously demonstrated association between increased PFC to NAc core glutamate release and reduced extracellular GABA in the VP, and cocaine reinstatement and behavioral sensitization, these findings support the important role played by the PFC-NAc core-VP series projection for cocaine-induced neuroplasticity and vulnerability to relapse.

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Figure 1.

The glutamatergic projection from the PFC to the NAc core is required for the cocaine-induced decrease in VP GABA. The control group in both panels consists of pooled values for animals administered aCSF into either the NAc core or PFC ($N \geq 5$ for each group). **A**) Effect of acute cocaine administration on extracellular GABA in the VP in animals withdrawn from chronic cocaine administration. Rats were pretreated with either intracranial aCSF, intra-NAc core CNQX or intra-PFC Bac/mus just prior to injecting acute cocaine. **B**) Chronic saline treated subjects after intracranial aCSF, CNQX or B/M followed by acute cocaine. Arrow indicates time of intracranial injection and acute cocaine administration (e.g. between 60 and 80 min).

* $p < 0.05$, compared to aCSF using a least significant difference test [10]

+ $p < 0.05$, compared to the last baseline sample within each group