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Beta-funaltrexamine affects cocaine self-administration in rats responding on a progressive ratio schedule of reinforcement

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Abstract

Many studies have shown interactions between μ-opiates and the mesolimbic dopamine (DA) system. Mu-opiate receptor antagonists have been reported to either increase or decrease the rate of cocaine self-administration, and the interpretation of these data has been difficult. In an attempt to further characterize and localize the effect of opiate receptor blockade on the reinforcing effects of cocaine, the μ-opiate irreversible antagonist beta-funaltrexamine (βFNA) was administered locally to different regions of the mesocorticolimbic system. Microinjection of βFNA into the ventral tegmental area (VTA) or the nucleus accumbens (NAcc) had no effect on cocaine self-administration under a fixed ratio (FR) schedule of reinforcement. However, blockade of opiate receptors in both brain regions did attenuate responding for cocaine maintained by a progressive ratio (PR) schedule. Administration of βFNA in the dorsal striatum had no effect under either schedule condition. The present findings suggest that endogenous opiate systems within the mesolimbic DA system modulate the reinforcing effects of cocaine; however, this modulation seems to be schedule dependent.

Keywords: Cocaine; μ Receptor; Beta-funaltrexamine; Progressive ratio; Self-administration

1. Introduction

A wealth of pharmacological, neurochemical and lesion experiments support the hypothesis that the principle site of action for the reinforcing effects of cocaine is at the dopamine (DA) transporter within the mesolimbic system. By blocking DA reuptake into the presynaptic terminal, cocaine acts as an indirect agonist at dopaminergic receptors (see Koob et al., 1998; Koob, 1992; Fibiger et al., 1992; Wise and Bozarth, 1987 for reviews). However, several other neurotransmitter systems, such as gamma-aminobutyric acid (GABA) (Brebner et al., 2000, 2002; Negus et al., 2000; Campbell et al., 1999), serotonin (Howell et al., 1997; Richardson and Roberts, 1991; Porrino et al., 1989), and acetylcholine (Levin et al., 2000) may be involved indirectly in the reinforcing effects of cocaine through interactions with either DAergic terminals or DAergic cells bodies in the ventral tegmental area (VTA). Among these interactions, the relationship between DA and the endogenous opiate system has received much attention and may be particularly important.

Electrophysiological literature has shown an intimate association between opiate receptors and the DA mesolimbic system (You et al., 1999; Yoshida et al., 1999; Tanda et al., 1997). Mu-opiate receptors are located on both GABAergic and DAergic neurons within the VTA (Dilts and Kalivas, 1989; Mansour et al., 1995; Svingos et al., 2001; Garzon and Pickel, 2001) and on medium spiny neurons of the nucleus accumbens (NAcc) (Svingos et al., 1996; Moriwaki et al., 1996). Furthermore, stimulation of μ receptors in the VTA has been shown to increase DA release in the NAcc (Leone et al., 1991; Spanagel et al., 1990; Devine et al., 1993), and administration of μ agonists stimulates the release of DA and its metabolites in the NAcc (Di Chiara and Imperato, 1988).

Given the close interaction between μ receptors and the mesolimbic DA system, it might be predicted that opiate receptor antagonists would influence the reinforcing effects of cocaine. Indeed, decreases in cocaine self-administration were reported following naltrexone administration on an FR5 schedule in the rat (Corrigall and Coen, 1991) and on an FR4 schedule in the monkey (Mello et al., 1990). Carroll

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et al. (1986), however, reported increases in cocaine-maintained responding on an FR1 schedule in the rat after naltrexone pretreatment. This was interpreted by the authors as either an increase or decrease in the reinforcing effects of cocaine. Also, there are reports that indicate no effect of opiate antagonism on cocaine self-administration (Ettenberg et al., 1982; Corrigall and Vaccarino, 1988; Hemby et al., 1996). Rather than assessing rates of self-administration in cocaine-experienced animals, acquisition studies have also been used to investigate the effects of opiate antagonists. Naltrexone was reported to affect acquisition of cocaine self-administration at threshold doses of cocaine (De Vry et al., 1989; Ramsey and VanRee, 1991; Ramsey et al., 1999). It appears that the effects of naloxone and naltrexone on cocaine self-administration can be dependent upon many parameters of a self-administration study, especially the type of operant schedule used and the unit dose tested (see Ramsey and VanRee, 1991; Ramsey et al., 1999 for reviews). Despite difficulties in interpreting the discrepant data, the majority of these findings support the hypothesis that μ-opiate receptors play a role in the self-administration of cocaine, and the need for more thorough investigation is evident.

The development of an irreversible μ-opiate receptor antagonist, beta-funaltrexamine (βFNA), has provided an alternate method to study μ-opiate receptor function and interactions. As is the case with other μ-opiate antagonists, both intracerebroventricular (Negus et al., 1993; Martin et al., 1995, 1998) and intra-accumbens (Martin et al., 2002) microinjection of βFNA significantly affected self-administration of heroin on a fixed ratio (FR) schedule. Also, like naloxone, βFNA attenuates the effects of morphine on increasing extracellular DA levels in the NAcc (Di Chiara and Imperato, 1988; Piepponen and Ahtee, 1995).

βFNA has been used to study interactions between opiate receptors and DA levels in the NAcc. βFNA significantly attenuated the amphetamine-induced increase in extracellular DA content in the NAcc (Schad et al., 1996). Also, while βFNA was reported to antagonize the increases in extracellular DA induced by μ-opiate agonists, Devine et al. (1993) reported increases in NAcc DA concentrations when βFNA was administered by itself. Given both the anatomical location of μ-opiate receptors and their influence on extracellular DA in the NAcc, one may expect that blockade of these receptors by βFNA administration would also alter the reinforcing properties of cocaine.

In light of the previous discussion and conclusion that opiate antagonists are sensitive to many parameters of a self-administration study, we investigated the effect of intracerebral injections of βFNA on cocaine self-administration using both the FR and the progressive ratio (PR) schedule. In the present study, rats trained to self-administer cocaine received a microinjection of βFNA or vehicle into either the VTA or the NAcc, two brain regions that are located within the mesolimbic DA system. βFNA was also administered into the dorsal striatum, an area known to contain μ receptors, but located dorsal to the mesolimbic pathway.

2. Method

2.1. Subjects

Subjects were male Fisher-344 rats (Charles River, North Carolina) weighing 275–300 g at the start of the experiment. All animals were placed under quarantine for 1 week following arrival at the facility and were maintained on a 12 L:12 D cycle (lights on at 1500 h). Food and water were available ad libitum throughout all phases of the experiment. The care and treatment of all animals conformed to the standards of the Wake Forest University Animal Care and Use Committee and the National Institutes of Health.

2.2. Drug self-administration

Following quarantine, rats were anesthetized with a combination of ketamine (75 mg/kg) and xylazine (8 mg/kg) and implanted with a chronically indwelling Silastic jugular cannula that exited through the skin on the dorsal surface in the region of the scapulae (Roberts and Goeders, 1989). Rats were individually housed and trained in 25-cm^3 operant testing apparatus containing a retractable lever and stimulus light mounted directly above the lever. A motor-driven syringe pump was located in front of the chamber. The cannula was connected through a stainless steel protective spring to a counterbalanced swivel apparatus that allowed free movement within the operant chamber. Ticarcillin disodium (0.1 ml of 67 mg/ml in saline) was administered through the catheter immediately after surgery to forestall infection, and Butorphanol was administered (0.03 mg/kg sc) as an analgesic agent. For the next 4 days, rats were allowed to recover from surgery, and ticarcillin disodium was administered as before.

Following antibiotic treatment, animals were given access to a single-response lever that controlled the delivery of cocaine (1.5 mg/kg per injection over 3–5 s based on body weight, in 0.08 ml saline) on an FR1 schedule. Concurrent with the start of each drug injection, a stimulus light located above the lever was activated to signal a 20-s postinfusion time-out period, during which the lever was retracted and no response could be made. Rats received daily 6-h test sessions (0900–1500 h) that began with one priming injection. After establishing a stable daily pattern of intake of cocaine (3 consecutive days of >30 injections/6 h and regular postinfusion pauses) on an FR1 schedule, rats were either administered a microinjection of βFNA or were switched to a PR schedule of reinforcement. Under this schedule, the animal had to make a progressively greater number of responses to obtain each subsequent injection in a particular session. Drug infusions were contingent upon an increasing number of responses incremented through the following

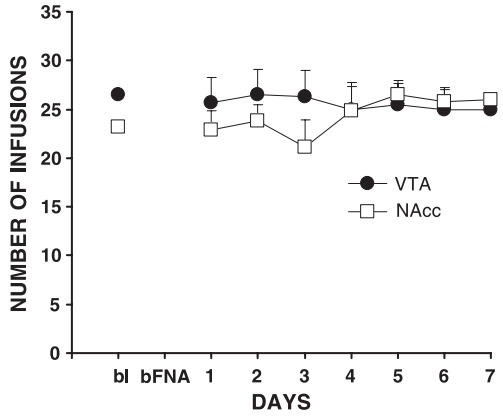

Fig. 1. Effect of intracerebral injections of βFNA on cocaine self-administration under an FR1 schedule. Points represent the mean (±S.E.M.) number of infusions throughout a 3 h test session in groups receiving βFNA into either the VTA (●, $n=6$) or NAcc (□, $n=6$). No significant effect of βFNA was observed.

progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603. The number of steps completed in the series each day (i.e., number of infusions) was defined as the breakpoint (BP). After achieving three consecutive days of stable BPs (±2BP), a microinjection of βFNA was administered to the animal. For statistical purposes, the BP was defined as the number of steps in the series rather than "final ratios" so as not to violate assumptions of homogeneity of variance (see Richardson and Roberts, 1996). For clarity, the final ratios corresponding to the BPs are shown in the figures.

The first group of rats ($n=12$) were trained under the FR schedule of reinforcement. Six animals received a microinjection of βFNA into the VTA, and six animals received a microinjection of βFNA into the NAcc.

A second group of rats ($n=32$) were trained under the PR schedule of reinforcement. Eight animals received an injection of βFNA into the VTA, and another eight received an injection of βFNA into the NAcc. A third group of animals ($n=8$) received an injection of βFNA into the dorsal striatum. For the remaining group of rats ($n=8$), saline was microinjected into the VTA of four subjects and into the NAcc for the remaining four subjects.

2.3. βFNA administration

All microinjections were performed under identical anesthesia as described above for jugular catheterization. Animals were placed into a stereotaxic apparatus with the nose piece set at 2.5 mm below the horizontal, and 2.5 nmol βFNA was administered in 0.9% saline through a microinjector attached to a Hamilton gastight microsyringe into either site.

For the VTA, the microinjector was stereotaxically lowered into the brain +3.2 mm anterior to lambda, ±1.0 mm lateral to the midline, and −8.2 mm ventral to the skull surface. Coordinates for the NAcc were +7.5 mm anterior to lambda, ±1.5 mm lateral to the midline, and −6.5 mm ventral to skull surface. Coordinates for the dorsal striatum were +7.5 mm anterior to lambda, ±1.5 mm lateral to the midline, and −5.0 ventral to skull surface. The total volume of injection was 4 μl (2 μl into each hemisphere) and was administered at a rate of 2 μl/min using a syringe pump

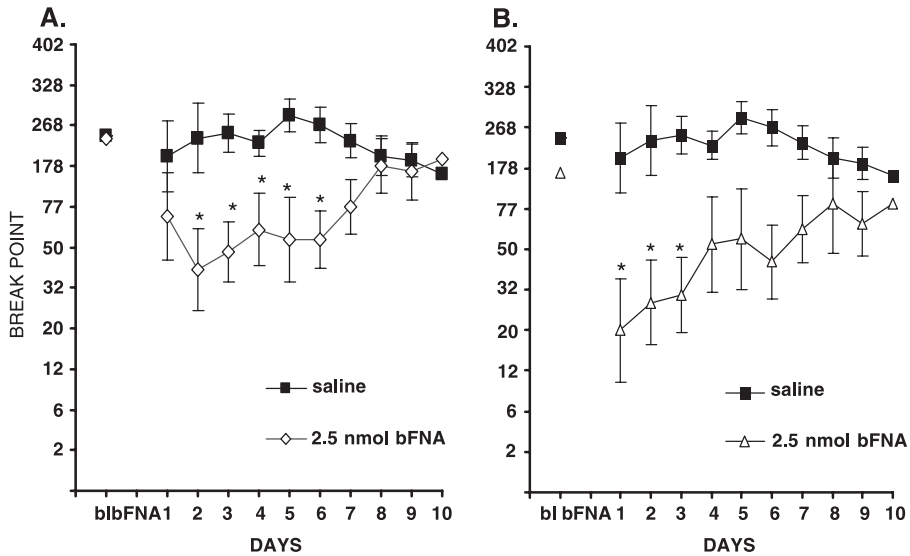

Fig. 2. Effect of intracerebral injections of βFNA on cocaine self-administration under a PR schedule. In panel A, points represent the mean (±S.E.M.) BP established across days after microinjection of either βFNA (◇, $n=8$) into the VTA. In panel B, points represent the mean (±S.E.M.) BP across days after microinjection of either βFNA (△, $n=8$) into the NAcc. The control group in both panels (■, $n=8$) was composed of animals that received saline into either the VTA ($n=4$) or the NAcc ($n=4$). The number of steps completed in the PR series was defined as the BP. For clarity, the final ratios corresponding to the BPs are shown in. Asterisk (*) indicates a statistically significant difference between groups ($P \leq .05$).

(Razel Scientific Instruments, Stamford, CT). The microinjector was left in place for 5 min to allow for pressure equilibration. Animals were allowed 1 day to recover before being given access to the cocaine lever.

3. Results

3.1. FR schedule of reinforcement

Fig. 1 shows that as seen in previous studies, βFNA had no effect on cocaine self-administration under an FR schedule of reinforcement when administered into either the VTA [$F(7,35)=0.444$, *ns*)] or the NAcc [$F(7,35)=1.402$, *ns*] over a 3-h session.

3.2. PR schedule of reinforcement

Fig. 2A presents the decreased BP following βFNA administration into the VTA [$F(10,87=3.9$, $P<.001$]. Post hoc analysis (Dunnett's method) shows BPs to be significantly lower than baseline on Days 1 through 5 post injection. Microinjection of saline alone had no effect on cocaine self-administration ($F<1$).

Microinjection of βFNA into the NAcc (Fig. 2B) also significantly decreased responding on the PR schedule [$F(10,87)=3.369$, $P<.001$]. Post hoc analysis (Dunnett's method) shows BPs to be significantly lower than baseline on Days 1 through 3 post injection. Fig. 3 shows no significant effect of βFNA on BP when microinjected into the dorsal striatum [$F(10,65)=1.885$, *ns*].

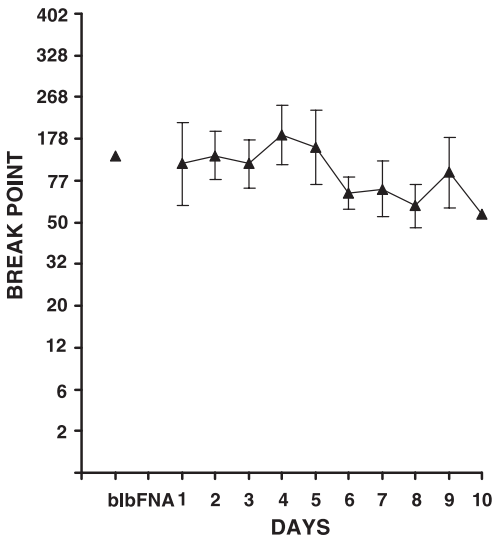

Fig. 3. Effect of βFNA into the dorsal striatum (▲, $n=8$) on cocaine self-administration under a PR schedule. Points represent mean (±S.E.M.) BP established during daily sessions. The number of steps completed in the PR series was defined as the BP. For clarity, the final ratios corresponding to the BPs are shown in. No significant effect of βFNA was observed.

4. Discussion

Irreversible blockade of μ-opiate receptors within the mesolimbic DA system attenuated cocaine-reinforced responding on a PR but not on an FR schedule of reinforcement. Significant attenuation of cocaine self-administration was seen for up to 5 days, after which responding returned to baseline levels by Day 10. The time course of this recovery of cocaine self-administration is similar to the time course of self-administration behavior reported by Martin et al. (1995) regarding the effects of βFNA on heroin-reinforced responding. Also, the effect of βFNA on cocaine self-administration was regionally specific, in that βFNA administration produced significant effects in the VTA and NAcc, both within the mesolimbic DA system. Conversely, no effect was seen after blockade of μ receptors in the dorsal striatum. The dorsal striatum is primarily innervated from DA cell bodies in the substantia nigra, and the literature suggests that this pathway is not as involved in the reinforcing effects of cocaine (Wise and Bozarth, 1987; Koob, 1992; Di Chiara and Imperato, 1988; Carboni et al., 1989). These results are consistent with the hypothesis that μ receptors within the NAcc and VTA play a modulatory role in the reinforcing effects of cocaine in the rat.

The attenuation of cocaine self-administration under a PR schedule and not an FR schedule is consistent with the conclusion that the type of schedule used can greatly impact detection of opiate–DA interactions. As was previously discussed, detection of modulatory effects of opiate receptors within the mesolimbic DA system are dependent upon experimental design, including experience of the animal, the type of schedule used, as well as doses used. We have reported, in accordance with Martin et al. (1998), that opiate antagonism did not affect the rate of cocaine self-administration on an FR schedule. Detecting modulations of the reinforcing effects of cocaine has been shown to be dependent on the schedule of reinforcement, e.g., FR versus PR (see Richardson and Roberts, 1996 for a review). Cocaine-reinforced BPs on a PR schedule have been shown to be sensitive to unit injection dose and various DA agonists and antagonists, and are sometimes more sensitive than FR to hormonal (Roberts et al., 1989) and neurotoxic manipulations (Loh and Roberts, 1990). The PR and FR schedules appear to be measuring different aspects of drug reinforcement. Whereas FR data provide an exquisitely sensitive measure of rate of drug intake, the PR schedule was designed to assess motivational aspects related to drug reinforcement. βFNA is yet another example wherein responding on an FR and PR schedule are differential affected by a drug treatment. Although elimination of μ receptors in either the VTA or the NAcc had no effect on the rate of cocaine self-administration, the PR data suggest an attenuation of the motivation to respond for cocaine. As previously mentioned, data collected with μ-opiate agonists, such as morphine, have shown that activation of these

receptors in the VTA increases extracellular DA in the NAcc. The apparent decrease in the motivation to self-administer cocaine following blockade of μ receptors in this study supports the impact of opiates on the mesolimbic DA system. Mu receptors have been implicated in the motivational aspects of opiate reinforcement (Narita et al., 2001; Becker et al., 2000; Rowlett et al., 1998). The present results extend the involvement of μ receptors in motivational processes to cocaine reinforcement as well.

The potential importance of this interaction between opiate and DAergic systems is evident in studies of the coadministration of cocaine and heroin. The coadministration of heroin and cocaine in the rat has synergistic effects both neurochemically and behaviorally. The self-administration of speedball resulted in increases in extracellular levels of DA within the NAcc well above that seen during cocaine self-administration alone (Hemby et al., 1999), even though self-administered heroin had no effect (Hemby et al., 1995). Significant potentiation of cocaine action by μ-opiate receptor activation could be partially responsible for the potent reinforcing efficacy of the coadministration of cocaine and heroin. Also, combinations of heroin and cocaine have been reported to increase the BP for self-administration above that of cocaine alone on a PR schedule, indicating an enhancement of reinforcing efficacy (Ranaldi and Munn, 1998; Duvauchelle et al., 1998).

The present results support a critical and modulatory role of endogenous opiate systems within the mesolimbic DA system in the motivational aspects of cocaine reinforcement, but do not provide information as to the neurobiology behind this effect. As previously mentioned, cocaine acts as an indirect DA agonist, and its ability to enhance DA neurotransmission in the mesolimbic pathway has been widely implicated as underlying the reinforcing properties of cocaine (Koob, 1992; Fibiger et al., 1992; Wise and Bozarth, 1987). One explanation of the present findings is that blockade of μ opiate receptors may be preventing increases in extracellular DA in the NAcc that has been regarded as the mechanism underlying the reinforcing effects of cocaine.

Administration of βFNA into either the VTA or the NAcc produced similar decreases in responding for cocaine under a PR schedule, and blockade of μ receptors in either of these brain regions could result in a decrease in NAcc DA levels. Mu receptors within the VTA are located presynaptically on GABAergic interneurons. Because μ receptors mainly inhibit synaptic input (Yuan et al., 1992), antagonism of the μ receptors on these GABAergic interneurons may disinhibit the firing of these neurons onto their DAergic targets. The net result of this would be a decrease in firing of DAergic neurons projecting to the NAcc. In the NAcc, μ receptors preferentially colocalize with the GABA/substance P/D1 population of medium spiny neurons that projection back to the VTA (Guttenberg et al., 1996). Again, these μ receptors are thought to be inhibitory; therefore, blockade of these μ receptors would again disinhibit the GABAergic medium spiny neurons. This would lead to an increased inhibitory feedback by these cells via their projection into the VTA. In summary, presynaptic μ receptors in the VTA and postsynaptic μ receptors on medium spiny neurons in the NAcc are thought to be inhibitory, and antagonism of either of these receptor populations may enhance the inhibitory output of these brain regions, thereby decreasing DAergic cell firing. This is a possible mechanism by which administration of βFNA into either the VTA or the NAcc attenuates the motivational aspects of cocaine reinforcement.

This explanation, however, runs the risk of being overly simplistic when other findings are considered. It is unclear, for instance, why rats continue to respond for cocaine on an FR schedule after βFNA treatment, as seen in this study and in Martin et al. (1998), if the result of βFNA was to decrease DA levels in the NAcc. The mechanism of the attenuating effects of naloxone on cocaine self-administration also remains inconclusive. Although some reports indicate an attenuation of cocaine self-administration, Schad et al. (1995) demonstrated that naloxone did not attenuate cocaine-induced increases in DA in NAcc. Additionally, there are conflicting reports regarding the effects of βFNA on release of DA in the NAcc in the absence of cocaine. Results from Devine et al. (1993) showed that βFNA administered into the VTA elevated DA and metabolite concentrations within the NAcc. This effect suggests that a complex local circuitry mediates opiate–DA interactions within the mesolimbic DA system. In another intriguing report, βFNA had no modulatory effect on the release of DA in the NAcc in a slice preparation, but significantly enhanced the stimulation-evoked release of acetylcholine after DAergic blockade (Sandor et al., 1992).

In conclusion, in an effort to find potential sites of action of modulation of the reinforcing effects of cocaine by opiate blockade, a μ-opiate receptor antagonist in either the VTA or NAcc attenuated cocaine intake under a PR schedule, but not an FR schedule. This study shows that consideration of various operant schedules is necessary to improve sensitivity when assessing the impact of various manipulations on the reinforcing effects of cocaine. These findings do suggest that opiate mechanisms at the level of the VTA and NAcc are involved in the reinforcing efficacy of cocaine, probably by interacting with the mesolimbic DA system. Although a common interpretation of decreases in the reinforcing effects of cocaine following pharmacological manipulations involves attenuation of increased extracellular DA in the NAcc, other evidence suggests a more complex circuitry behind the present results. More behavioral studies in concert with a further characterization of the neurochemical effects of βFNA in animals self-administering cocaine are necessary to further understand the interactions between opiate receptors and DA activity within the mesolimbic DA system.

Acknowledgements

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