Changes in Extracellular Dopamine During Cocaine Self-Administration in Squirrel Monkeys

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ABSTRACT Environmental cues are thought to play a role in drug craving, leading to the high relapse rate observed in cocaine abusers. Cocaine-paired cues can reinstate cocaine-maintained behavior in rodents and nonhuman primates and can induce changes in dopamine levels in the rodent striatum. In the present study, squirrel monkeys were trained to self-administer cocaine under a second-order schedule, and then were implanted with guide cannulae targeted at the caudate nucleus. Caudate dopamine levels were measured while the animals performed the behavioral task, self-administering cocaine or saline under extinction conditions. In addition, animals received noncontingent (passive) cocaine infusions yoked to the drug self-administration sessions. Cocaine administration increased dopamine levels 2-fold, but a leftward shift was seen in the peak effect in animals self-administering cocaine as compared to animals passively receiving cocaine. Moreover, dopamine levels began to decline during the experimental session when animals were self-administering cocaine, even though they continued to receive cocaine injections. In contrast, dopamine levels declined below baseline during the session when animals were given access to saline. The results suggest that the environmental context associated with drug self-administration can modulate cocaine-induced elevations in extracellular dopamine. Synapse 56:129–134, 2005. © 2005 Wiley-Liss, Inc.

INTRODUCTION

Addiction to cocaine and other drugs of abuse is difficult to treat, due to the high rate of relapse in cocaine abusers (Carroll et al., 1994; Mendelson and Mello, 1996). Environmental cues associated with the subjective effects of cocaine can induce drug craving and contribute to relapse in humans (Carter and Tiffany, 1999; Childress et al., 1988; Rohsenow et al., 1990). Similarly, exposure to cocaine-paired environmental stimuli can reinstate cocaine-maintained behaviors in rats (de Wit and Stewart, 1981; Meil and See, 1996; Spealman et al., 2000; Weiss et al., 2000, 2001) and nonhuman primates (Spealman et al., 1999).

Cocaine binds to the dopamine transporter (DAT), thus inhibiting the reuptake of dopamine (DA) and increasing extrasynaptic DA levels (Ritz et al., 1987). For example, systemic administration of cocaine produces dose-dependent increases in extracellular DA in the rat striatum (Bradberry and Roth, 1989; Church et al., 1987) and in the squirrel monkey caudate (Czoty et al., 2000). Similarly, self-administered cocaine also increases extracellular DA in the rat nucleus accumbens (Pettit and Justice, 1989) and in the rhesus monkey striatum (Bradberry et al., 2000). This increase in DA is thought to mediate the reinforcing effects and abuse liability of cocaine (Richardson and Roberts, 1991; Ritz et al., 1987; Roberts et al., 1977; Volkow et al., 1997).

It appears that DA may also play a role in cocaine-seeking behavior in response to drug-associated stimuli. For example, microdialysis studies in rats have shown conditioned increases in mesolimbic DA induced by stimuli associated with cocaine (Fontana et al., 1993; Weiss et al., 2000). Also, DA receptor antagonists can suppress cue-induced reinstatement of cocaine-seeking behavior (de Wit and Stewart, 1981). This increase in DA is thought to mediate the reinforcing effects and abuse liability of cocaine (Richardson and Roberts, 1991; Ritz et al., 1987; Roberts et al., 1977; Volkow et al., 1997).

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self-administration, suggesting a role for DA in cue-induced drug-seeking behavior (Weiss et al., 2001). More recent studies utilizing fast-scan cyclic voltammetry have revealed that rapid DA transmission occurs during components of cocaine-seeking behavior and during presentation of cocaine-associated stimuli (Phillips et al., 2003). However, similar findings have not been established in nonhuman primate species. In rhesus monkeys trained to self-administer cocaine, cocaine-associated visual and auditory cues did not produce changes in striatal DA levels, although cocaine-seeking behaviors were reinstated (Bradberry et al., 2000). In the present studies, the effect of self-administered cocaine on extracellular DA in the caudate of squirrel monkeys was compared to noncontingent (passive) administration of cocaine yoked to the same schedule. In addition, a saline extinction paradigm was used to characterize further the effects of drug-associated cues on extracellular DA.

**MATERIALS AND METHODS**

Three adult male squirrel monkeys (*Saimiri sciureus*) weighing 900-1,200 g served as subjects. Animals lived in individual home cages and had daily access to food (monkey chow, Harlan Teklad, Madison, WI; fresh fruit and vegetables) and unlimited access to water. Animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Emory University.

During daily test sessions, each animal was seated in a Plexiglas chair within a ventilated, sound-attenuating chamber. The chair was equipped with stimulus lights, a response lever, and a motor-driven syringe pump used to deliver drug injections. During drug self-administration experiments, Teflon and polyvinyl chloride tubing passed through a small hole in the chamber and connected the catheter to a syringe situated within a computer-controlled pump located outside the chamber. Behavioral test sessions lasted ~70 min each day, 5 days per week. Subjects were seated in the chair and fitted with an adjustable Lexan neckplate that was positioned perpendicular to the medial plane of the body just above the shoulder. All subjects had been acclimated to the chair and neckplate over several months, during which they were maintained on the second-order schedule of i.v. cocaine (0.1 mg/infusion) self-administration. At least 1 week elapsed between microdialysis experiments.

Animals were prepared with chronically indwelling venous catheters under sterile surgical conditions. Animals were initially anesthetized with a cocktail consisting of Telazol (tiletamine hydrochloride and zolazepam hydrochloride, 5.0 mg), ketamine HCl (20 mg), and atropine (0.1 mg). Anesthesia was maintained with supplements of ketamine HCl. A catheter made of polyvinyl chloride tubing (0.38 mm i.d.; 0.76 mm o.d.) was inserted via the femoral or external jugular vein and passed to a point near the right atrium. The proximal end of the catheter was passed subcutaneously to the interscapular region of the monkey’s back where it exited the skin. A nylon mesh vest was worn by the monkey at all times to protect the externalized end of the catheter. Veterinary staff prescribed preoperative antibiotics (Monocid (cefonicid), Rocephin (ceftiraxone)) and postoperative analgesics (Banamine (flunixin meglumine)). Catheters were flushed with saline solution several times each week and were filled with heparinized saline and sealed with a stainless steel obturator when not in use.

A stereotaxic apparatus was used to implant CMA/11 guide cannulae (CMA/Microdialysis, Acton, MA) bilaterally to target the caudate nuclei of three monkeys as described previously (Czoty et al., 2000). Anesthesia was initiated with Telazol (tiletamine hydrochloride and zolazepam hydrochloride, 5.0 mg) and atropine (0.1 mg). Inhaled isoflurane (1.0–2.0%) was administered to maintain depth of anesthesia during the procedure. A stainless steel stylet was placed in the guide cannula when not in use. Analgesics (flunixin meglumine) and antibiotics (ceftriaxone) were prescribed as necessary by veterinary staff. Animals were closely monitored during recovery from anesthesia, and a minimum of 1 month was allowed before microdialysis experiments were performed. Following guide cannulae implantation, accurate placement was verified in each monkey through use of MRI as described previously (Czoty et al., 2000).

Self-administration behavior was maintained under a second-order schedule of i.v. drug delivery. At the beginning of a testing session the behavioral chamber was illuminated with a red light for 600 sec (fixed-interval (FI) 600 sec). Completion of 20 responses (FR20) during the FI 600 sec produced a 2-sec flash of white light. When the 600-sec interval had elapsed, the monkey had 10 sec to complete an FR20 to terminate the red light and received an infusion of 0.1 mg of cocaine in 0.2 ml. Upon termination of the red light, a white light was illuminated for 15 sec, followed by a 60-sec timeout. If an FR20 was not completed in the 10-sec period, the animal did not receive the cocaine infusion, followed by a 60-sec timeout. Responding during timeout periods had no scheduled consequences. Each daily session consisted of five FI 600-sec (FR20:S) components. When rates and patterns of responding had stabilized, animals were acclimated to the neckplate and guide cannulae were implanted as described above. After a 2-week recovery period, animals were allowed to self-administer the training dose for more than 10 sessions to reestablish steady baseline response rates. During the active cocaine self-administration schedule, animals were given access to cocaine under the same second-order schedule described above. Saline was substituted for cocaine in the infusion syringe during the
extinction paradigm, but the stimulus lights remained active throughout the session. Extinction sessions were infrequent and unpredictable so that lever-pressing and stimulus presentations were maintained in the absence of cocaine. During the noncontingent cocaine infusion paradigm, the experimenter activated the infusion pump at the same intervals as in the behavioral schedule described above, but no stimulus lights were presented during the session and the chamber remained darkened. During the current studies, animals received infusions of only saline or cocaine.

CMA/102 dialysis probes with a shaft length of 14 mm and active dialysis membrane measuring 4 x 0.24 mm were flushed with artificial cerebrospinal fluid (1.0 mM Na₂HPO₄, 150 mM NaCl, 3 mM KCl, 1.3 mM CaCl₂, 1.0 mM MgCl₂, and 0.15 mM ascorbic acid) for 20 min. Probes were inserted into the guide cannulae and connected to a CMA/102 microinfusion pump via FEP Teflon tubing. Probes were perfused with artificial cerebrospinal fluid at 2.0 μl/min for the duration of the experiment. Samples were collected every 10 min in microcentrifuge tubes. Following a 60-min equilibration in which no samples were collected, three consecutive 10-min samples were collected for determination of baseline DA concentration. Following collection of baseline samples, samples were collected at the end of each of the five 10-min components. Three more samples were collected at 10-min intervals following the behavioral session. After the final experimental sample was collected, the KCl concentration of the perfusate aCSF was increased to 100 mM for 10 min to induce voltage-dependent DA release and a final 10-min sample was collected. A robust increase in extracellular DA in response to high KCl verified tissue integrity.

High-performance liquid chromatography (HPLC) and electrochemical detection were used to quantify levels of DA. The HPLC system consisted of a small-bore (3 mm i.d. x 100 mm) column (5 μm C18 stationary phase; Thermo Hypersil, Keystone Scientific Operations, Bellefonte, PA) with a commercially available mobile phase (ESA, Chelmsford, MA) delivered by an ESA 582 solvent delivery pump at a flow rate of 0.6 ml/min. Samples (20 μl) were mixed with 3 μl of ascorbate oxidase, and 11 μl of the mixture was injected into the HPLC system by a CMA/200 autosampler. Electrochemical analyses were performed using an ESA Coulochem II detector. Before each in vivo experiment, probes were tested in vitro to determine suitability of the probes. Percent recovery was similar for all probes (10–20%). DA values reported were not adjusted for recovery because in vitro recovery of microdialysis probes has been demonstrated to be an inaccurate measure of in vivo recovery (Parsons and Justice, 1992).

Mean baseline DA concentrations for an individual were defined as the mean of the three samples preceding behavioral sessions or noncontingent drug administration. Samples were collected from both the left and right caudate in separate experiments. Microdialysis was conducted a minimum of three times per side corresponding to self-administration, passive administration, and extinction conditions. In limited cases, an experiment had to be repeated due to technical problems. However, only one session was included in the data analyses for each condition. The treatment effect and time-courses were similar between the two sides in individual subjects. Therefore, DA levels from each side were averaged, resulting in a single value for individual subjects on each experimental condition. Microdialysis data were analyzed using a two-way repeated-measures ANOVA to determine overall experimental condition effects, followed by a Tukey's post-hoc multiple comparisons test to determine whether the experimental conditions differed from each other over time. Each experimental condition was analyzed using separate one-way repeated-measures ANOVAs to identify significant experimental condition effects, each followed by a Tukey's post-hoc multiple comparisons test to determine whether DA concentrations in single samples were significantly different from the baseline value at time-point 0. Statistical significance was accepted at the 95% level of confidence (P < 0.05).

Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) was dissolved in 0.9% saline. Drug dose was determined as the salt.

RESULTS

Both noncontingent and self-administered cocaine increased DA levels in the squirrel monkey caudate to a peak of ~200% of baseline DA levels (Fig. 1). The first sample obtained after baseline determinations (shown at 10 min) corresponds to the first FI component of a session and was collected prior to the first saline or drug infusion. Noncontingent cocaine infusions increased DA levels starting at the end of the second component and steadily increased DA levels until 10 min after the final infusion. Animals that were actively self-administering cocaine showed a slight (25%) increase in DA levels in the first component, before cocaine administration. DA levels continued to increase until the end of the third component, then DA levels declined to about 150% of baseline during the behavioral session. Substitution of saline for cocaine under extinction conditions decreased DA levels in the caudate following the fifth behavioral component. A two-way repeated-measures ANOVA determined that there was a time effect [F(2,28) = 5.285, P = 0.004] but not an effect of treatment [F(2,28) = 2.771, P = 0.176] on DA levels. A significant interaction between treatment and time was detected [F(14,28) = 3.719, P = 0.002]. Individual one-way repeated-measures ANOVA determined that each treatment had an overall effect: noncontingent cocaine administration [F(2,16) = 3.613,
P \approx 0.014], active cocaine self-administration [F(2,16) = 4.681, P \approx 0.004], and saline extinction [F(2,16) = 4.788, P \approx 0.004]. At time-point 60, post-hoc tests showed animals that received noncontingent cocaine infusions exhibited DA levels that were significantly higher than those at time-point 0 (P < 0.05). However, when animals were actively self-administering cocaine, post-hoc tests showed that DA levels were significantly higher at time-points 30 and 40 than at time-point 0 (P < 0.05). During saline extinction, post-hoc tests showed that DA levels at time-points 50, 60, and 70 min were significantly lower than DA levels at time-point 0 (P < 0.05).

Animals reliably self-administered 0.1 mg/infusion cocaine throughout the behavioral session, with a response rate that was slightly elevated above baseline levels in the first two components and that declined to a level slightly below baseline in the fourth and fifth components (Fig. 2). In the saline extinction paradigm, the rate of responding was 60% of baseline in the first two components and declined to 20% of baseline in the final three components.

**DISCUSSION**

Repeated injections of cocaine produced graded increases in extracellular DA that peaked at ~200% of basal levels. Importantly, there was a leftward shift in the peak effect during self-administration of cocaine compared to noncontingent cocaine administration.

Moreover, DA levels began to decline during the self-administration sessions even though the animals continued to receive cocaine injections. Consistent with the latter observation, basal levels of extracellular DA also showed a modest, gradual decline during extinction conditions. In contrast, DA levels did not decline until 20 min after the last noncontingent cocaine injection. The results indicate that active self-administration behavior in the context of drug-associated environmental stimuli can modulate cocaine-induced changes in extracellular DA in nonhuman primates.

Only modest increases in extracellular DA were observed during the first component of the self-administration sessions prior to drug delivery. Also, no changes in extracellular DA were observed in the first component when saline was substituted for cocaine under extinction conditions. The latter results are consistent with those reported in rhesus monkeys (Bradberry et al., 2000) and indicate that drug-associated stimuli alone did not increase DA levels. Apparently, there is a complex interaction among self-administration behavior, environmental context, and the pharmacological effects of cocaine in nonhuman primates. It is unclear why cue-induced increases in DA observed in rodent studies have not been replicated in squirrel monkeys or rhesus monkeys. Rodent studies have focused primarily on the nucleus accumbens as the region of interest. Although the present study in squirrel monkeys measured extracellular DA in the caudate nucleus, studies conducted in rhesus monkeys targeted sensorimotor and mesolimbic striatum (Bradberry et al., 2000). Certainly, species differ-
ences may be responsible for the different experimental outcomes in rodent and nonhuman primate studies. It is well established that primes differ from rodents in the organization and anatomical distribution of dopaminergic projections (Berger et al., 1991). In the present study, we focused our neurochemical measurements to the caudate, as the dopaminergic input from the midbrain is more broadly distributed across the ventromedial and medial striatum (Haber and McFarland, 1999). While the neuronal inputs and outputs from the caudate and accumbens may differ, the high density of DAT in the caudate makes it an ideal target for neurochemical studies involving compounds that bind to DAT, such as cocaine. In addition, the caudate is a larger brain region than the nucleus accumbens, facilitating the current microdialysis studies. Future studies should also examine the differences between self-administered and noncontingent cocaine in the nucleus accumbens. Another important region for study is the prefrontal cortex, which has been shown to play a role in cue- and stress-induced cocaine reinstatement in rodents (Capriles et al., 2003; McFarland and Kalivas, 2001) and in cocaine craving and relapse humans (Childress et al., 1999; Grant et al., 1996).

In addition, behavioral training and drug history may play an important role in determining the impact of drug cues on neurochemistry. The squirrel monkeys in the present study underwent extensive behavioral training and testing under a complex second-order schedule of reinforcement. Similarly, the rhesus monkeys involved in the study reported by Bradberry et al. (2000) had prior drug histories sufficient to induce sensitization to cocaine-induced elevations in extracellular DA (Bradberry, 2000). The observed differences in motor behavior during the different test conditions may also play a role in the changes in DA levels. However, the present results support the influence of the schedule itself on extracellular DA levels. In the present studies, during saline extinction, the animals still responded at a rate of 60% of baseline during the first two components, and DA levels during these components were comparable to those during the baseline. Lever-pressing dropped to 20% of control during the final three components, but a significant decrease in DA was not observed until the fifth and final component. In addition, squirrel monkeys are typically very active and exhibit a great deal of motor behavior when in the testing chamber, regardless of whether this behavior is directed at the response lever.

In summary, the present study demonstrated that self-administration behavior in the context of drug-associated stimuli can modulate cocaine-induced elevations in extracellular DA in nonhuman primates. However, there was no evidence for robust increases in extracellular DA in response to drug-associated stimuli during extinction conditions. The latter results are consistent with previous studies in nonhuman primates reporting no change in mesolimbic DA in response to cocaine cues (Bradberry et al., 2000). The finding indicates a complex interaction among self-administration behavior, environmental context, and the pharmacological effects of cocaine.

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REFERENCES


