

# Serotonergic Attenuation of the Reinforcing and Neurochemical Effects of Cocaine in Squirrel Monkeys

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## ABSTRACT

Preclinical studies have documented that serotonin (5-HT) can modulate the behavioral effects of cocaine. The present study examined the ability of 5-HT to attenuate the reinforcing and neurochemical effects of cocaine in nonhuman primates. In squirrel monkeys trained to self-administer cocaine (0.1 and 0.3 mg/injection) under a second-order schedule of i.v. drug delivery, the 5-HT uptake inhibitor alaproclate (3.0 and 10.0 mg/kg) and the 5-HT direct agonist quipazine (0.3–1.0 mg/kg) decreased response rates at doses that had no significant effect on behavior maintained by an identical schedule of stimulus termination. The neurochemical bases of the observed drug interactions on behavior were investigated further using *in vivo* microdialysis techniques in a separate group of awake mon-

keys to monitor drug-induced changes in extracellular dopamine (DA). Cocaine (1.0 mg/kg) elevated the concentration of DA in the caudate nucleus to approximately 300% of basal levels. Pretreatment with alaproclate or quipazine attenuated cocaine-induced increases in extracellular DA at the same pretreatment doses that decreased cocaine self-administration. The results obtained suggest that increasing brain 5-HT activity can attenuate the reinforcing effects of cocaine, ostensibly by decreasing the ability of cocaine to elevate extracellular DA in brain areas that mediate the behavioral effects. These findings extend those reported previously for the behavioral-stimulant effects of cocaine and identify a potential neurochemical mechanism underlying drug interactions on behavior.

Abuse of stimulant drugs such as cocaine persists as a major health problem in the United States (Chilcoat and Johanson, 1998). Clearly, there is a great need for pharmacological treatments to combat abuse of these drugs; however, no pharmacotherapy has demonstrated sufficient efficacy for widespread clinical use (Carroll et al., 1999). A better understanding of the effects of cocaine on central nervous system neurochemistry will help identify effective approaches in developing medications for treating cocaine dependence.

The behavioral-stimulant and reinforcing effects of cocaine have been linked to its ability to enhance dopaminergic neurotransmission by inhibiting DA uptake via transporter blockade (Ritz et al., 1987). The affinities of several cocaine-like drugs for DA transporter correlate well with their potencies for supporting self-administration behavior (Ritz et al.,

1987; Bergman et al., 1989). In humans, a significant correlation has been observed between DA transporter binding and the intensity of subjective effects produced by intravenous cocaine (Volkow et al., 1997). Cocaine affects neurotransmission in various brain DA systems, leading to a variety of behavioral effects. For example, the DA neurons of the substantia nigra pars compacta project to the caudate nucleus and putamen, the primate homologues of the rodent dorsal striatum (Parent et al., 1995), to modulate motor function. Facilitation of DA transmission in the nigrostriatal system likely contributes to the prominent behavioral-stimulant effects of cocaine. The mesolimbic/mesocortical DA system consists of dopaminergic cell bodies in the ventral tegmental area (VTA), which project to subcortical areas such as the nucleus accumbens (NAcc) and ventral pallidum and to cortical areas including the medial prefrontal cortex (Oades and Halliday, 1987; Domesick, 1988). This system is understood to be an important mediator of the reinforcing effects of cocaine (for reviews, see Kuhar et al., 1991; Wise, 1998).

While DA plays a primary role in mediating the behavioral pharmacology of cocaine, recent evidence suggests that brain serotonin (5-HT) systems can modulate these effects. For example, the reinforcing efficacy of cocaine can be enhanced

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**ABBREVIATIONS:** DA, dopamine; VTA, ventral tegmental area; NAcc, nucleus accumbens; 5-HT, 5-hydroxytryptamine (serotonin); GBR 12909, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine; FI, fixed interval; FR, fixed ratio; ANOVA, analysis of variance; HPLC, high-performance liquid chromatography; GABA,  $\gamma$ -aminobutyric acid.

by chemical lesions of brain 5-HT systems (Roberts et al., 1994), whereas dietary supplement with 5-HT precursors can attenuate cocaine self-administration in rodents (Carroll et al., 1990). Furthermore, self-administration of cocaine is decreased by 5-HT uptake inhibitors in rats (Richardson and Roberts, 1991) and monkeys (Kleven and Woolverton, 1993), although similar effects are obtained on food-maintained behavior. More recent work in squirrel monkeys has demonstrated that 5-HT can attenuate the behavioral-stimulant effects of cocaine (Howell and Byrd, 1995) and the selective DA uptake inhibitor GBR 12909 (Howell et al., 1997) in a qualitatively similar fashion.

Brain 5-HT systems are ideally situated to modulate the activity of DA neurons and the behavioral effects of DA indirect agonists such as cocaine. 5-HT neurons from the dorsal and median raphe nuclei innervate the dopaminergic cell bodies and terminal regions of the nigrostriatal and mesolimbic DA systems, and the convergence of 5-HT terminals and DA neurons has been visualized in the VTA and NAcc at the light and electron microscopic levels (Herve et al., 1987; Phelix and Broderick, 1995). It is possible that 5-HT acts on cell bodies to decrease the firing rate of DA neurons or at terminals to decrease DA release. In either case, the ability of 5-HT to attenuate the behavioral effects of cocaine could result from an attenuation of cocaine-induced elevation of extracellular DA. Alternatively, 5-HT may act postsynaptically to DA neurons, attenuating the effects of cocaine-induced increases of extracellular DA on a downstream component of the pathway.

The present studies were conducted to evaluate further the interactions between 5-HT and DA in nonhuman primates regarding the behavioral pharmacology of cocaine. The effects of a 5-HT uptake inhibitor, alaproclate, and a 5-HT direct agonist, quipazine, on cocaine self-administration were characterized to determine whether serotonergic modulation of the behavioral-stimulant effects of cocaine reported previously extends to its reinforcing effects. Alaproclate has high nanomolar affinity for the 5-HT transporter and greater than 50-fold selectivity for the 5-HT transporter compared with DA and norepinephrine transporters (Lindberg et al., 1978). Quipazine is a nonselective 5-HT agonist with low nanomolar affinity for 5-HT<sub>2</sub> binding sites (Glennon, 1986). In addition, microdialysis experiments in awake squirrel monkeys characterized the effects of serotonergic drugs on cocaine-induced increases in extracellular DA measured in the caudate nucleus to identify potential neurochemical mechanisms that mediate drug interactions on behavior. The results obtained have important implications toward an understanding of cocaine neuropharmacology in nonhuman primate models of drug addiction.

## Materials and Methods

**Subjects.** Nineteen adult male squirrel monkeys (*Saimiri sciureus*) weighing 900 to 1200 g served as subjects. Animals lived in individual home cages and had daily access to food (Harlan Teklad monkey chow; Harlan Teklad, Madison, WI; fresh fruit and vegetables) and unlimited access to water. All monkeys had prior exposure to cocaine and other drugs with selective dopaminergic or serotonergic activity in various behavioral studies (Howell and Byrd, 1995; Howell et al., 1997). 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.

**Apparatus.** 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 either equipment for delivering a mild electrical stimulus to the tail or a motor driven syringe pump used to deliver self-administered 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 approximately 70 min each day, 5 days per week. During microdialysis experiments in a separate group of animals, 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 over several months. At least 2 weeks elapsed between microdialysis experiments.

**Catheter Implantation.** Seven monkeys 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 (15 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 (ceftriaxone)] 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.

**Guide Cannula Implantation.** 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 effect 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 cannula implantation, accurate placement was verified in each monkey through use of magnetic resonance imaging as described previously (Czoty et al., 2000).

**Self-Administration Procedures.** Seven animals were initially trained under a second-order FI 900-s (FR20:S) schedule of stimulus termination. At the beginning of a testing session, the behavioral chamber was illuminated with a red light for 900 s. Completion of 20 responses (FR20) produced a 2-s flash of white light. When the 900-s interval had elapsed, the monkey had 10 s to complete an FR20 to terminate the red light, which was associated with an impending electric stimulus. Upon termination of the red light, a white light was illuminated for 15 s, followed by a 60-s timeout. If an FR20 was not completed in the 10-s period, the animal received one 3-mA stimulus for approximately 200 ms to the tail, followed by a 60-s timeout. Responding during timeout periods had no scheduled consequences. Each daily session consisted of four FI 900-s (FR20:S) components. When rates and patterns of responding had stabilized, venous catheters were implanted as described above. Subsequently, the method of reinforcement was changed such that termination of the red light resulted in the injection of 0.1 mg of cocaine. All other events and schedule parameters were identical to those of the sec-

ond-order schedule of stimulus termination. Drug experiments began when rates and patterns of responding had stabilized. Animals were allowed to self-administer the training dose for more than ten sessions. Subsequently, a range of doses of cocaine (0.03–1.0 mg/injection) was substituted for the training dose to establish a full dose-effect curve for cocaine self-administration. Mean response rates at each dose were calculated by averaging daily mean response rates over more than ten sessions. Extinction sessions with saline substitution were conducted on consecutive days until responding decreased to less than 20% of response rate obtained with the maintenance dose of cocaine (0.1 mg/injection). Saline substitution conditions were routinely imposed to ensure that the subjects would rapidly extinguish in the absence of cocaine.

During drug interaction studies, saline was administered 5-min pre-session for 3 consecutive days (typically Tuesday, Wednesday, and Thursday). Subsequently, 1 dose of a pretreatment drug was administered 5-min pre-session on the Tuesday, Wednesday, and Thursday of the following week. Mean rate of responding during self-administration sessions was determined for individual monkeys by averaging response rate in the presence of the red light during all components of a session. Mean response rate for a given pretreatment dose was determined across the three drug sessions and expressed as a percentage of the mean rate during the 3 days of the previous week on which saline was administered. Each dose of each pretreatment drug was administered at least twice. Initially, self-administration of cocaine (0.03–1.0 mg/injection) was characterized in seven monkeys. Subsequently, alaproclate (3.0 and 10.0 mg/kg,  $n = 4$ ) and quipazine (0.3–3.0 mg/kg,  $n = 3$ ) were administered as pretreatments 5 min prior to the start of cocaine (0.1 and 0.3 mg/injection) self-administration sessions. A repeated-measures ANOVA and Tukey's post hoc multiple comparisons tests were used to determine statistical significance, defined at the 95% level of confidence ( $p < 0.05$ ).

**Stimulus Termination Procedures.** Nine additional monkeys were trained under a second order FI 900-s (FR20:S) schedule of stimulus termination as described above. Subjects were divided into two groups with one subject assigned to both groups. Alaproclate (3.0 and 10.0 mg/kg,  $n = 5$ ) and quipazine (0.3 and 1.0 mg/kg,  $n = 5$ ) were administered 5 min prior to the start of the session. No systematic trend toward progressively increasing or decreasing response rates over the 3 pretreatment days was observed for saline or either of the pretreatment drugs. For each drug dose, response rate data were averaged over the 3 pretreatment days to provide control data for the pretreatment drugs administered alone. Group data were derived and expressed as mean  $\pm$  S.E.M. for the group based on the percentage change in the rate of responding during drug sessions compared with all sessions in which saline was administered. Each dose of pretreatment drug was administered at least twice in a quasi-random order. A repeated-measures ANOVA with Tukey's post hoc multiple comparisons was used to determine statistical significance, defined at the 95% level of confidence ( $p < 0.05$ ).

**Microdialysis Procedures.** CMA/11 dialysis probes with a shaft length of 14 mm and active dialysis membrane measuring  $4 \times 0.24$  mm were flushed with artificial cerebrospinal fluid (1.0 mM  $\text{Na}_2\text{HPO}_4$ , 150 mM NaCl, 3 mM KCl, 1.3 mM  $\text{CaCl}_2$ , 1.0 mM  $\text{MgCl}_2$  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  $\mu\text{l}/\text{min}$  for the duration of the experiment. Samples were collected every 10 min in microcentrifuge tubes and immediately refrigerated. Following a 60-min equilibration, three consecutive 10-min samples were collected for determination of baseline DA concentration. Following collection of baseline samples, saline or a dose of a pretreatment drug was administered, and 30 min later, cocaine (1.0 mg/kg) was administered. Alaproclate (3.0 and 10.0 mg/kg;  $n = 3$ ) and quipazine (0.3 and 1.0 mg/kg;  $n = 3$ ) were administered in combination with cocaine while microdialysis samples were collected from the caudate nucleus. 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.  $\times$  100 mm) column (5  $\mu\text{m}$   $\text{C}_{18}$  stationary phase; Thermo Hypersil, Keystone Scientific Operations, Bellefonte, PA) with a commercially available mobile phase (ESA, Inc., Chelmsford, MA) delivered by an ESA 582 solvent delivery pump at a flow rate of 0.3 ml/min. Samples (6  $\mu\text{l}$ ) were mixed with 6  $\mu\text{l}$  of ascorbate oxidase, and 11  $\mu\text{l}$  of the mixture was injected into the HPLC system by a CMA/200 autosampler. Electrochemical analyses were performed using an ESA Coulochem II detector (ESA, Inc.). The neurochemical effects of the combinations were compared with neurochemical effects of cocaine following saline administration.

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%), but DA values reported herein 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). Data were analyzed with a two-way ANOVA designed to determine whether pretreatment with serotonergic drugs differed from pretreatment with saline in regard to the effects of cocaine on extracellular DA. Tukey's post hoc multiple comparisons determined whether pretreatment with a particular dose of serotonergic drug differed from pretreatment with saline at individual time points (i.e., sampling intervals).

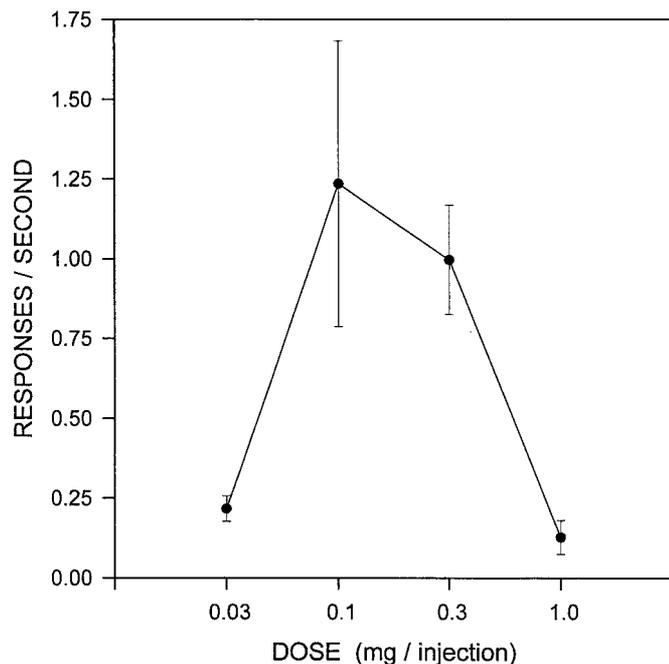
**Drugs.** Alaproclate HCl, quipazine dimaleate (Sigma/RBI, Natick, MA) and cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) were dissolved in 0.9% saline. Drug doses were determined as salts. All drug injections were administered into the thigh muscle at a volume of 0.4 to 0.8 ml.

## Results

**Cocaine Self-Administration.** All animals readily acquired self-administration of the 0.1 mg/injection training dose of cocaine (Fig. 1). Rates and patterns of responding were characteristic of performance under second-order FI schedules with FR components. Characteristic FR rates and patterns were maintained during each FR component by the 2-s presentation of white light after the completion of each FR20. A brief pause in responding after each 2-s flash of white light was followed by a steady, high rate of responding. Mean response rates during individual FR components typically were lower during the start of each 900-s interval and increased during subsequent FR components as the interval elapsed. Mean  $\pm$  S.E.M. response rate for seven animals during maintenance sessions was  $1.23 \pm 0.48$  responses/s. Other doses were subsequently substituted for the training dose for a minimum of ten sessions. Mean  $\pm$  S.E.M. response rates during substitution with 0.03, 0.3, and 1.0 mg/injection cocaine were  $0.21 \pm 0.04$ ,  $0.99 \pm 0.19$ , and  $0.13 \pm 0.06$  responses/s, respectively, resulting in an inverted U-shaped dose-effect curve. Two doses of cocaine (0.1 and 0.3 mg/injection) were selected for drug interaction studies based on their effectiveness in maintaining peak rates of responding. Response rates for individual subjects are shown in Table 1.

**Effects of Serotonergic Drugs on Cocaine Self-Administration.** Response rates maintained by cocaine (0.1 and 0.3 mg/injection) were decreased following pretreatment with the 5-HT uptake inhibitor alaproclate (3.0 and 10.0 mg/kg; Fig. 2, left). Statistical analyses revealed a significant main effect of alaproclate ( $p < 0.05$ ) in combination with both doses of cocaine. Post hoc multiple comparisons confirmed a significant effect ( $p < 0.05$ ) of 10.0 mg/kg alaproclate with both doses of cocaine. The 5-HT direct agonist quipazine (0.3–3.0 mg/kg) also decreased response rates maintained by

## COCAINE SELF-ADMINISTRATION



**Fig. 1.** Mean ( $\pm$ S.E.M.) rate of responding maintained by i.v. injection of cocaine (0.03–1.0 mg/injection) under a second-order FI 900-s (FR20:S) schedule in a group of seven squirrel monkeys. Data for each dose were derived from at least ten consecutive sessions. Abscissa, dose log scale; ordinate, mean response rate expressed as responses per second.

TABLE 1

Mean response rates for cocaine self-administration in individual subjects

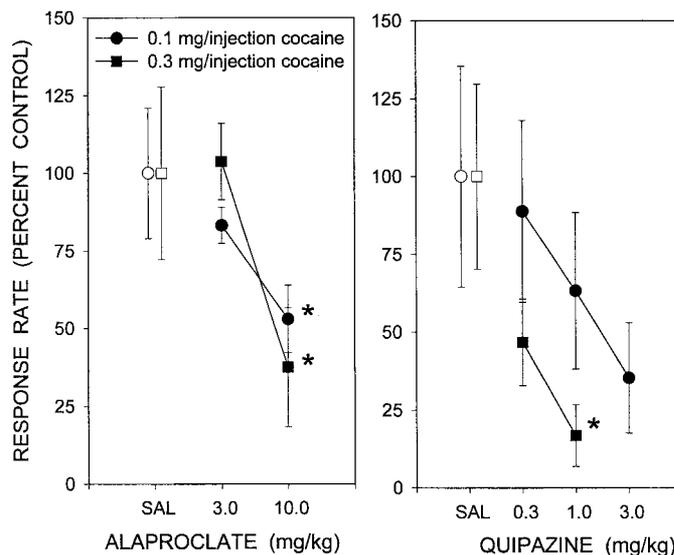
Mean response rates (responses/second) are reported for individual subjects during the last ten sessions of each condition. Data are shown for the two unit doses of cocaine used in drug interaction studies. The doses were selected based on their effectiveness in maintaining peak rates of responding.

Subject	Cocaine Dose (mg/injection)	
	0.1	0.3
S-124	3.60	2.00
S-125	0.75	1.14
S-126	2.01	0.65
S-127	0.94	0.92
S-129	0.72	0.71
S-136	0.32	0.89
S-140	0.32	0.72
Mean ( $\pm$ S.E.M.)	1.23 $\pm$ 0.48	1.00 $\pm$ 0.19

cocaine self-administration (Fig. 2, right). Although a clear dose-dependent trend toward decreasing response rates was observed in animals self-administering 0.1 mg/injection cocaine, the effect did not reach statistical significance. However, a significant main effect of quipazine pretreatment was observed when animals self-administered 0.3 mg/kg cocaine ( $p < 0.05$ ). Post hoc multiple comparisons confirmed a significant effect of 1.0 mg/kg quipazine in combination with 0.3 mg/injection cocaine.

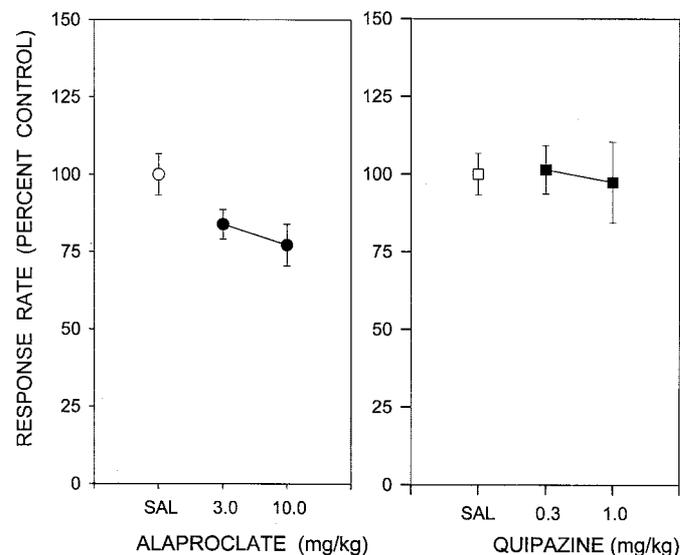
**Effects of Serotonergic Drugs on Stimulus Termination.** Alaproclate (3.0 and 10.0 mg/kg) and quipazine (0.3 and 1.0 mg/kg) were administered to two groups of five animals whose behavior was maintained by a second-order FI 900-s (FR20:S) schedule of stimulus termination (Fig. 3). The mean  $\pm$  S.E.M. response rate for the two groups when saline was given 5-min pre-session was  $0.93 \pm 0.17$  and  $0.85 \pm 0.27$

## COCAINE SELF-ADMINISTRATION



**Fig. 2.** Mean ( $\pm$ S.E.M.) rate of responding maintained by a second-order FI 900-s (FR20:S) schedule of i.v. self-administration of cocaine (0.1 and 0.3 mg/injection) after pretreatment with saline (SAL), alaproclate (left, 3.0 and 10.0 mg/kg,  $n = 4$ ), or quipazine (right, 0.3–3.0 mg/kg,  $n = 3$ ). Abscissae, dose log scale; ordinates, mean response rate expressed as a percent of control response rate obtained following saline pretreatment. Open symbol indicates mean ( $\pm$ 95% confidence limits) for cocaine self-administration following saline pretreatment (control). Asterisks indicate a significant difference ( $p < 0.05$ ) when compared with control response rate.

## STIMULUS TERMINATION



**Fig. 3.** Mean ( $\pm$ S.E.M.) rate of responding maintained by a second-order FI 900-s (FR20:S) schedule of stimulus termination after pretreatment with saline (SAL), alaproclate (left, 3.0 and 10.0 mg/kg), or quipazine (right, 0.3 and 1.0 mg/kg) in separate groups of five monkeys. Open symbol indicates mean ( $\pm$ 95% confidence limits) for response rate following saline pretreatment (control). Otherwise, as in Fig. 2.

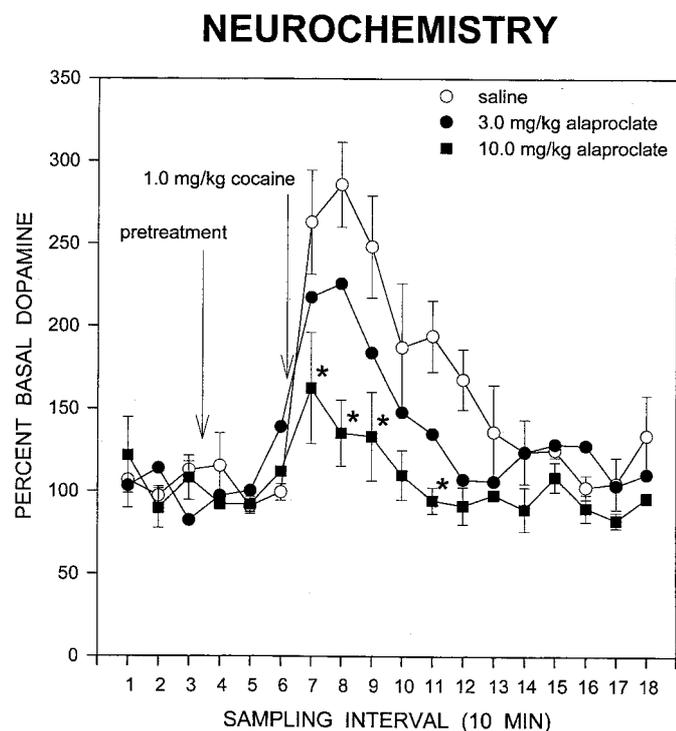
responses/s. No statistically significant effects of the pretreatment drugs on response rates were observed.

**Effects of Serotonergic Drugs on the Neurochemical Effects of Cocaine.** In all experiments, DA levels stabilized

within 1 h of probe insertion, at which point three 10-min samples were collected, the mean of which served as a baseline value. Mean basal DA levels, unadjusted for probe recovery, were  $6.21 \pm 1.85$  nM. Following determination of basal levels, saline was administered. No systematic changes in DA levels were observed in the 30 min following saline administration (Fig. 4). Cocaine (1.0 mg/kg) increased extracellular DA in the caudate nucleus to approximately 300% of basal levels in the first 10-min sample following drug administration, and DA levels returned to baseline approximately 60 min postinjection (Fig. 4). When alaproclate (3.0 and 10.0 mg/kg) was administered 30 min prior to cocaine, an attenuation of the effects of cocaine on extracellular DA was observed. Statistical analyses revealed a significant main effect of alaproclate ( $p < 0.05$ ), and post hoc multiple comparisons revealed significant effects ( $p < 0.05$ ) on DA levels following 10.0 mg/kg alaproclate at several sampling intervals compared with saline pretreatment. Quipazine (0.3 and 1.0 mg/kg) also attenuated cocaine-induced elevations in extracellular DA (Fig. 5). A significant main effect of quipazine was observed ( $p < 0.05$ ), and post hoc multiple comparisons revealed significant effects ( $p < 0.05$ ) on DA levels following 0.3 and 1.0 mg/kg quipazine at several sampling intervals compared with saline pretreatment.

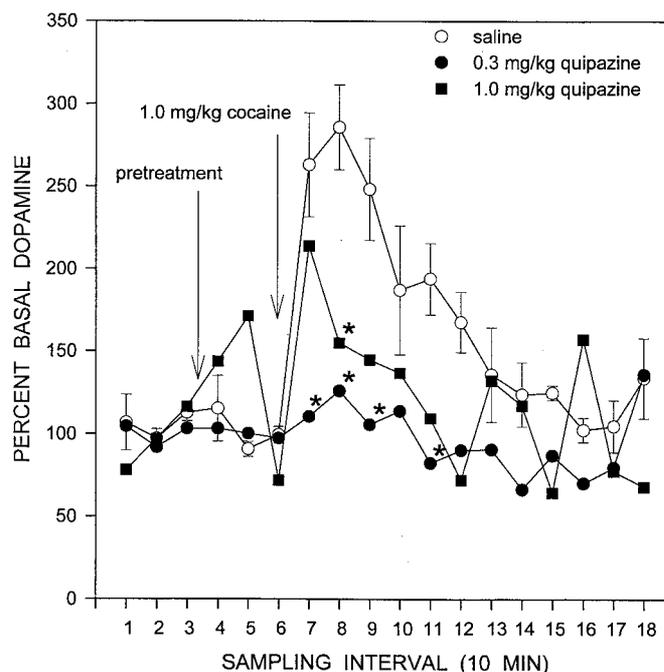
### Discussion

The 5-HT uptake inhibitor alaproclate and the 5-HT direct agonist quipazine decreased response rates for cocaine self-administration, suggesting that enhancing 5-HT activity de-



**Fig. 4.** Effects of cocaine (1.0 mg/kg) on extracellular levels of dopamine after pretreatment with saline (open symbols) or alaproclate (3.0 and 10.0 mg/kg; filled symbols) in three monkeys. Asterisks indicate significant ( $p < 0.05$ ) difference from the effect of cocaine following saline pretreatment. Abscissa, 10-min sampling intervals beginning 1 h after probe implantation; ordinate, dopamine levels expressed as mean ( $\pm$ S.E.M.) percentage of baseline levels.

### NEUROCHEMISTRY



**Fig. 5.** Effects of cocaine (1.0 mg/kg) on extracellular levels of dopamine after pretreatment with saline (open symbols) or quipazine (0.3 and 1.0 mg/kg; filled symbols) in three monkeys. Otherwise, as in Fig. 4.

creases the reinforcing effects of cocaine. These results are consistent with previous results in squirrel monkeys in which the 5-HT antagonist ritanserin increased response rates for injections for cocaine (Howell and Byrd, 1995) and GBR 12909 (Howell et al., 1997). To examine the possibility that decreases in response rate for cocaine injections were due to direct (i.e., rate-decreasing) effects of the pretreatment drugs, alaproclate and quipazine were administered to animals whose behavior was maintained by a second-order schedule of stimulus termination. This schedule was identical to that used in self-administration experiments, except that behavior was reinforced by termination of a light previously paired with an electrical stimulus rather than by an injection of cocaine. Response rates under the stimulus termination schedule were not significantly affected by alaproclate or quipazine, suggesting that decreases in cocaine self-administration behavior following administration of the pretreatment drugs resulted from an attenuation of the behavioral effects of cocaine rather than nonspecific (i.e., direct rate-decreasing) effects of the pretreatment drugs. Although some studies reporting 5-HT-induced decreases in cocaine self-administration have found similar effects on behavior maintained by nondrug reinforcers (Kleven and Woolverton, 1993; Roberts et al., 1994), the present results are consistent with the interpretation that alaproclate and quipazine selectively decreased the reinforcing effects of cocaine. Moreover, this modulation of cocaine self-administration is similar to that observed with the behavioral-stimulant effects of cocaine (Howell and Byrd, 1995) and GBR 12909 (Howell et al., 1997) in squirrel monkeys.

Microdialysis studies were used to investigate the neurochemical mechanism of the observed serotonergic attenuation of the behavioral effects of cocaine. One plausible hy-

pothesis is that 5-HT acts presynaptically with respect to DA terminals in brain areas that mediate these behavioral effects to attenuate cocaine-induced elevations of extracellular DA. This inhibition could take place in the terminal regions through interference with the DA release mechanism or at the cell bodies through a decrease in firing rate of DA neurons. Conversely, 5-HT could act downstream of DA neurons, attenuating the effects of cocaine-induced DA increases on a distal component of the pathway. Alaproclate and quipazine attenuated cocaine-induced increases in extracellular DA in the caudate nucleus, the location of the axon terminals of midbrain DA neurons. The decreased effectiveness of cocaine to elevate caudate DA supports the hypothesis that 5-HT acts presynaptically with respect to terminals of these DA neurons to modulate the behavioral effects of cocaine. However, the results do not rule out the potential contribution of postsynaptic effects of 5-HT.

One limitation of the present study is that the neurochemical effects were characterized after noncontingent administration of cocaine, whereas the drug interactions on behavior were characterized in monkeys self-administering cocaine. Therefore, future studies should examine neurochemical changes during active drug self-administration and determine whether serotonergic manipulations would have a similar effect on neurochemistry under conditions of response-contingent cocaine delivery. However, it is important to note that the subjects assigned to microdialysis experiments were not drug-naïve. Similar to the self-administration monkeys, the microdialysis subjects had been used previously in a variety of behavioral and pharmacological studies that involved extensive histories of exposure to cocaine and related stimulants.

The mesolimbic DA system, particularly the dopaminergic projections from the VTA to the NAcc, has been strongly implicated in mediating the reinforcing effects of cocaine and other DA indirect agonists (for reviews, see Kuhar et al., 1991; Wise, 1998). Observation of neurochemical interactions between 5-HT uptake inhibitors and cocaine in the NAcc that are similar to those observed in the present studies in the caudate nucleus would provide more direct evidence that the ability of these 5-HT uptake inhibitors to decrease cocaine self-administration is based on a decrease in the effectiveness of cocaine in elevating NAcc DA. It is reasonable to expect that the effects observed in the caudate will extend to the NAcc based on the anatomical organization of the primate striatum. In rodents, the NAcc is delineated from surrounding striatal tissue on the basis of afferent cortical and subcortical fibers. In primates, however, limbic afferent input from the cortex and thalamus and dopaminergic input from the midbrain are more broadly distributed across the ventromedial and medial striatum, whereas neurons from motor areas of the cortex and thalamus innervate the dorso-lateral striatum (for review, see Haber and McFarland, 1999). Thus, a broad distinction has been made between striatal areas subserving a limbic function (i.e., ventromedial striatum, including the NAcc, and central/medial striatum, including the caudate nucleus) and a sensorimotor function (i.e., dorso-lateral striatum) (Haber and McFarland, 1999; Bradberry et al., 2000). In the present study, probes were placed in the medial caudate nucleus within the mesolimbic division of the striatum. Although definitive studies remain to be conducted, the diffuse nature of afferent innervation

throughout the mesolimbic striatum suggests that the interactions of 5-HT uptake inhibitors and cocaine observed in the present study also occur in the NAcc.

There is a growing consensus that stimulation of 5-HT<sub>2C</sub> receptors inhibits the function of the mesolimbic DA system (Di Matteo et al., 2001). Firing rates of DA neurons in the VTA are decreased by 5-HT uptake inhibitors (Di Mascio et al., 1998) and selective 5-HT<sub>2C</sub> agonists (Prisco et al., 1994), resulting in a decrease in NAcc DA levels (Di Matteo et al., 2000). Conversely, selective 5-HT<sub>2C</sub> antagonists increase the activity of these neurons (Ashby and Minabe, 1996), leading to increased DA release in the NAcc (Di Matteo et al., 1999). Since 5-HT<sub>2C</sub> receptors are located exclusively on GABA neurons (Eberle-Wang et al., 1997), the effects of 5-HT<sub>2C</sub> receptor stimulation are likely to be indirectly mediated by an enhancement of GABA-mediated inhibition of VTA DA neurons. Localization of 5-HT<sub>2C</sub> receptors on GABAergic terminals may also explain the conflicting results that have been obtained in previous electrophysiological, neurochemical, and behavioral studies. Some studies investigating interactions between 5-HT and DA have concluded that 5-HT can exert an excitatory influence on DA activity (Cameron and Williams, 1994) and release (Blandina et al., 1989; De Deurwaerdere et al., 1997). Stimulation of 5-HT<sub>1B</sub> receptors can enhance cocaine reinforcement (Parsons et al., 1998), likely by decreasing GABA-mediated inhibition in the VTA (Cameron and Williams, 1994). 5-HT<sub>3</sub> receptors also appear to play a facilitatory role in the behavioral effects of DA agonists (Layer et al., 1992; Kankaanpää et al., 1996). Additional studies have failed to find alterations in cocaine-maintained responding resulting from administration of serotonergic drugs (Porrino et al., 1989; Lacosta and Roberts, 1993; Peltier and Schenk, 1993). These seemingly disparate results likely reflect the complexity of interactions between 5-HT and DA systems and the 5-HT receptor subtypes influenced by drug administration.

In summary, drugs that increase brain 5-HT activity decreased self-administration of the DA indirect agonist cocaine. This modulation apparently was not due to nonspecific behavioral effects of the 5-HT pretreatments. Microdialysis studies suggest that the serotonergic modulation involves presynaptic mechanisms with respect to DA neurons, decreasing the ability of cocaine to elevate extracellular DA. These findings extend those reported previously for the behavioral-stimulant effects of cocaine and identify a potential neurochemical mechanism underlying drug interactions on behavior. The information provided may have important implications for the development of medications to treat cocaine abuse.

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