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Interactions between the mGluR2/3 agonist, LY379268, and cocaine on *in vivo* neurochemistry and behavior in squirrel monkeys

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ABSTRACT

Recent evidence indicates that group II metabotropic glutamate receptors (mGluR2 and mGluR3) may play a role in the pathology of cocaine addiction. The purpose of the current study was to determine the effects of the mGluR2/3 agonist, LY379268, on cocaine-induced changes in DA neurochemistry in nonhuman primates. Furthermore, the current study aimed to determine if changes in DA neurochemistry would correlate with LY379268-induced changes in the behavioral effects of cocaine. *In vivo* microdialysis was conducted in conscious squirrel monkeys ($n=4$) in order to monitor cocaine-induced changes in extracellular DA in the caudate nucleus. Separate groups of subjects were trained on a fixed-interval schedule of stimulus termination ($n=4$) or a second-order schedule of cocaine self-administration ($n=5$) to characterize the behavioral-stimulant and reinforcing effects, respectively. LY379268 significantly attenuated cocaine-induced increases in DA. LY379268 also significantly attenuated cocaine-induced behavioral-stimulant effects following a short pretreatment time, but not following a longer pretreatment time. Cocaine self-administration was significantly attenuated but only at an intermediate pretreatment dose of LY379268. Moreover, reinstatement of previously extinguished cocaine self-administration was not significantly attenuated by LY379268. Hence, drug interactions on neurochemistry did not correlate well with behavioral measures.

1. Introduction

Glutamate is the major excitatory neurotransmitter and essential for many functions in the mammalian central nervous system (Watkins, 2000). Glutamate receptors can be divided into two classes: ionotropic glutamate receptors and metabotropic glutamate receptors (mGluRs) (Conn and Pin, 1997; Nakanishi, 1994; Nakanishi et al., 1998). The G-protein coupled mGluRs have the capacity to modulate the actions of the ionotropic glutamate receptors as well as other neurotransmitter receptors, which make them good candidates for pharmacotherapeutic targets (Pin and Duvoisin, 1995). Eight mGluR subtypes have been identified and classified into three distinct groups based on pharmacology and sequence homology. Group I mGluRs (mGluR1 and 5) are positively coupled to phospholipase C. Group II mGluRs (mGluR2 and 3) and III (mGluR4, 6, 7 and 8) are negatively coupled to adenyl cyclase. Group II mGluRs are primarily localized perisynaptically on presynaptic neurons and function as autoreceptors to regulate neurotransmitter release. Group II mGluRs have been associated with several neurological and psychiatric disorders including anxiety (Grillon et al., 2003; Muly et al., 2007), schizophrenia and PCP-induced psychosis (Gupta et al., 2005; Moghaddam, 2004), and psychostimulant abuse (Peters and Kalivas, 2006; Xi et al., 2002a).

Chronic administration of cocaine can alter glutamate neurotransmission. In rats chronically treated with cocaine, there is a reported decrease in extracellular glutamate concentrations in the nucleus accumbens (Baker et al., 2003; Pierce et al., 1996). The reduction in basal levels of glutamate is associated with enhanced release of glutamate by an acute infusion of cocaine and may play an important role in cocaine-induced reinstatement of previously extinguished self-administration behavior (Baker et al., 2003). A possible mechanism for the observed augmentation of glutamate release may be the decreased stimulation of mGluR2/3 autoreceptors by extracellular glutamate. Therefore, it may be therapeutically beneficial to reduce glutamatergic signaling by stimulating these receptors.

In rats, mGluR2/3s are highly expressed in many regions of the brain including (but not restricted to) the prefrontal cortex, ventral tegmental

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area, nucleus accumbens and striatum, areas which have been shown to play a pivotal role in addiction (Ohishi et al., 1994; Ohishi et al., 1993; Testa et al., 1998; Xi et al., 2002b). Studies have demonstrated a significant glutamatergic tone on mGluR2/3 receptors to suppress dopaminergic release in the nucleus accumbens (Hu et al., 1999). Furthermore, activation of mGluR2/3 receptors decreased basal DA and the magnitude of DA response to systemic phencyclidine administration (Greenslade and Mitchell, 2004). Activation of mGluR2/3 receptors also attenuated behavioral responses believed to be mediated via dopaminergic pathways. Amphetamine-induced increases in locomotor activity were attenuated by mGluR2/3 agonist treatment (David and Abraini, 2003), while locomotor activity was enhanced by selective mGluR2/3 antagonists (O'Neill et al., 2003). Furthermore, mGluR2/3 agonists attenuated amphetamine self-administration in rats (Kim et al., 2005) and cocaine self-administration in nonhuman primates (Adewale et al., 2006), and blocked cue-induced reinstatement of extinguished heroin self-administration in rats (Bossert et al., 2005) and drug-primed reinstatement of extinguished cocaine self-administration in rats (Peters and Kalivas, 2006) and nonhuman primates (Adewale et al., 2006). The current studies characterized the effects of the mGluR2/3 agonist, LY379268, on cocaine-induced increases in extracelluar DA in conscious nonhuman primates, as well as on the behavioral-stimulant and reinforcing effects of cocaine. It was hypothesized that pharmacological activation of mGluR2/3 would result in an attenuation of cocaine-induced increases in DA and this attenuation would be associated with decreased behavioral-stimulant and reinforcing properties of cocaine.

2. Methods

2.1. Subjects

Fourteen male adult squirrel monkeys (*Saimiri sciureus*) (5–15 years) weighing between 800 and 1200 g served as subjects. Between experimental sessions, subjects were individually housed and allowed access to food twice daily (Harlan Teklad monkey chow; Harlan Teklad, Madison, WI; fresh fruit and vegetables) and had access to water ad libitum. All subjects had served in previous experiments involving acute administration of cocaine and monoaminergic drugs. These studies were conducted in strict accordance with the NIH "Guidelines for the Care and Use of Laboratory Animals" and were approved by the Institutional Animal Care and Use Committee of Emory University.

2.2. Apparatus

Experimental sessions were conducted daily in a ventilated, sound-attenuating chamber in which each monkey was seated comfortably in a commercially available primate chair (Modular Primate Chair, Med Associates Inc, St. Albans, VT). The front wall of the chair facing the monkey was equipped with a response lever and red and white lights. The response lever registered responses monitored by computers and integrated circuitry interfaced with the chambers. Typically, operant behavioral sessions lasted 1 h and were conducted five days a week. Drug time-course determinations lasted one and a half hours. *In vivo* microdialysis sessions were limited to 5 h and were conducted no more frequently than once per week in individual animals.

2.3. Surgical procedures

For *in vivo* microdialysis experiments, subjects (s159, s168, s175, and s181) were implanted with bilateral guide cannulae (CMA/11, CMA/Microdialysis, North Chelmsford, MA) using stereotaxic procedures to target the caudate nuclei utilizing sterile surgical procedures (Czoty et al., 2000). Coordinates for the stereotaxic procedures were obtained from a standard squirrel monkey atlas (Gergen et al., 1962) (from the earbar: A/P + 15.0, L/M ± 3.0). Subjects were initially sedated using a cocktail of 0.1 mg/kg atropine, 20 mg/kg ketamine, and 2 mg/kg Telazol to prepare the animal for surgery and position the subject's head in the stereotaxic frame. Anesthesia was maintained with isoflurane (1–2%) during the surgery. Nylon screws (2.4 mm, Plastics One, Inc., Roanoke, VA) were implanted surrounding the guide cannulae and the preparation was secured in place with dental cement (Plastics One, Inc., Roanoke, VA). After the surgery, a stainless steel stylet was placed in each guide cannulae to protect the site when not in use. Subjects were allowed two to three weeks to recover before the experiments were initiated.

Subjects involved in behavioral protocols in which drugs were administered i.v. were surgically implanted with a chronic indwelling PVC catheter (0.38 mm ID; 0.76 mm OD) as described previously (Howell and Wilcox, 2001). Subjects were anesthetized with 0.1 mg/kg atropine, 20 mg/kg ketamine, and 2 mg/kg Telazol, and anesthesia was maintained by ketamine supplements during the surgery. The catheter was implanted in either a right or left femoral or external jugular vein to the level of the right atrium using sterile surgical techniques. The catheter was routed subcutaneously to the interscapular region and exited the skin under a protective nylon-mesh jacket. The catheter was filled with heparinized saline and sealed with a stainless-steel obturator when not in use. Subjects were allowed to recover for one week before beginning any experiments. If the catheter became occluded or damaged, the catheter was removed and another catheter was implanted in the same vein if possible, or another vein.

2.4. Microdialysis procedures

Teflon tubing passed through the ceiling of the chamber to connect the probe implanted in the monkey to the perfusion pump. In order to prevent the subject from disturbing the probe or tubing, a Lexan plate was positioned perpendicularly to the medial plane of the body just above the shoulders. Commercially available probes (CMA/11) with a shaft length of 14 mm and active dialysis membrane measuring 4 × 0.24 mm were inserted into the guide cannulae. A Harvard PicoPlus microinfusion pump continuously flushed artificial cerebrospinal fluid (1.0 mM Na_2HPO_4, 150 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 0.15 mM ascorbic acid) through the probes via FEP Teflon tubing to the probe at a flow rate of 2.0 µL/min for the duration of the experiment. Following a 60-min equilibration, four consecutive 10-min samples were collected for determination of baseline DA concentration. Following collection of baseline samples, saline or LY379268 was administered i.m. Three 10-min samples were collected after the pretreatment, allowing for a 30-min pretreatment before administering saline or cocaine. After cocaine or saline was administered i.m., samples were collected every 10 min for 2 h. All samples were collected in microcentrifuge tubes and immediately refrigerated. Probes were tested *in vitro* to determine the suitability of probe efficiency and performance before and after each experiment. Subjects were tested no more frequently than once per week and each site was accessed no more frequently than once every 2 weeks. Under the conditions described, consistent responses to drug treatments have been observed following repeated access to the caudate without significant gliosis (Czoty et al., 2000).

Small-bore, high-performance liquid chromatography (HPLC) and electrochemical detection quantified levels of DA according to previously established protocols (Kimmel et al., 2005; Kimmel et al., 2007). The HPLC system consisted of a small-bore (3 mm i.d. × 100 mm) column (5 µm 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 0.6 mL/min. After loading onto the refrigerated sample tray, samples (20 µl) were automatically mixed with 3 µl of ascorbate oxidase, and 5 µl of the mixture was injected into the HPLC system by an ESA model 542 autosampler. Samples were

analyzed within 12 h of collection, remaining either in a refrigerator or in the refrigerated autosampler tray during this time. Electrochemical analyses were performed using an ESA dual-channel analytical cell (model 5040) and guard cell (model 5020, potential = 350 mV) and an ESA Coulochem II detector. The potential of channel 1 was set to −150 mV for oxidation, while the potential of channel 2 was set to 275 mV for reduction. A full range of dopamine standards (0.5–25 nM) was analyzed before and after each set of samples to evaluate possible degradation of dopamine. Levels of dopamine below 0.5 nM were considered below the limit of detection. A desktop computer collected data and chromatograms were generated by EZChrome Elite software (version 3.1, Scientific Software, Pleasanton, CA). The chromatograms were analyzed using the EZChrome software, comparing the experimental samples with the standards.

2.5. Fixed-interval stimulus termination

Each subject (s178, s185, s187, and s190) was trained on a 300s fixed-interval (FI) schedule of stimulus termination with a 3s limited hold (LH). During the 300s FI, the chamber was illuminated with a red light. After the interval elapsed, the animal had 3s to press a response lever and extinguish the red light associated with an impending electric stimulus. When the animal pressed the lever during the 3s LH and extinguished the red light, a white light was illuminated for 15s followed by a 60s timeout. In the absence of a response in the 3s LH, the animal received a single, brief (200 ms) 4-mA electrical stimulus to a shaved area near the tip of the tail. Each session consisted of 13 consecutive FI 300s components, each followed by a 60s timeout. This schedule has been used to reliably characterize the behavioral-stimulant effects of drugs (Kimmel et al., 2007). Pretreatment doses of the mGluR2/3 agonist, LY3799268, or saline were given via i.m. injection (volume of 0.4–0.8 mL in the thigh) in the test chamber 5 min prior to the initiation of the session. Cocaine (0.03–1.0 mg/kg) was administered via i.v. infusion (volume of 0.25 mL and flushed with 0.25 mL saline) in increasing cumulative doses after components 1, 4, 7 and 10. During control sessions, saline was administered via i.v. infusion (volume of 0.5 mL) after the same components.

2.6. Cocaine self-administration

In order to extend the life of the catheter, subjects (s183, s184, s186, s207, and s209) were initially trained under a second-order FI-600-s schedule of stimulus termination with fixed-ratio (FR) 20 components and a 20-s limited hold (LH). A red light illuminated the chamber during the fixed interval, and every 20th response (FR 20) during the FI changed the red light to a white light for 2-s. After the FI 600-s elapsed, the subject had 20-s to complete an FR 20 to terminate the red light, which was associated with an impending electrical stimulus. Termination of the red light resulted in a 15-s illumination of a white light followed by a 60-s timeout. If the red light was not extinguished during the 20-s period, the subject received a single, brief (200 ms) 4 mA electrical stimulus to the tail followed by a 60-s timeout. Responding during timeout periods had no scheduled consequences. Animals were tested daily, 5 days each week, and each daily session consisted of 5 consecutive FI 600-s (FR 20: S) components. When rates and patterns of responding were stable, venous catheters were implanted as previously described. Subsequently, the method of reinforcement was changed such that termination of the red light resulted in injection of 0.1 mg/kg of cocaine and the LH was increased to 200s. All other events and schedule parameters remained identical to those of the second-order schedule of stimulus termination. This technique has been effective at maintaining high rates of responding during drug self-administration (Kimmel et al., 2007). Drug experiments began once rates and patterns of responding were stable. Initially, 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 and to determine the dose that maintained peak rates of responding. Each dose was tested for 5 consecutive sessions, and the order of drug dose was randomized. Subsequently, subjects were maintained on the dose that maintained peak rates of responding (0.1 mg/kg/infusion: s183, s184, s207, s209; 0.3 mg/kg/infusion: s186). During drug interaction studies, saline was administered i.m. as a control 5 min pre-session for 3 consecutive days (typically Tuesday, Wednesday, and Thursday). Subsequently, a single dose of LY379268 was administered i.m. 5 min pre-session for 3 consecutive days 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 which saline was administered on the previous week.

2.7. Reinstatement

Subjects (s183, s186, s201, and s209) were initially trained on a second-order schedule of stimulus termination as described above [FI 10 min (FR 20: S)]. Once rates of responding were stable, subjects were implanted with an indwelling catheter as described above. The schedule parameters for the reinstatement procedures were the same as those for the self-administration procedures, and each daily session consisted of 5 components. A full dose–effect curve for cocaine self-administration was established to determine the dose that maintained peak rates of responding before reinstatement studies began (0.1 mg/kg/infusion: s183, s201, s209; 0.3 mg/kg/infusion: s186). Once rates of responding were stable at the maintenance dose for 4–5 days, subjects were then extinguished for at least two days. During extinction, the schedule parameters remained the same, except that the white stimulus light was not illuminated after completion of each FR and the red light remained illuminated, and cocaine was replaced with vehicle (saline) for self-administration. Reinstatement experiments were initiated once the extinction rates were less than 30% of the cocaine-maintained response rates. During the reinstatement experiments, vehicle was available for self-administration and the cocaine-paired stimulus was restored. Previous studies demonstrated that reinstatement of previously extinguished cocaine self-administration was greatest when cocaine priming was accompanied by presentations of the cocaine-paired stimulus (Spealman et al., 2004). Reinstatement sessions were conducted on three consecutive days utilizing the same pretreatment dose. LY379268 was administered i.m. 5 min prior to administering either an i.v. saline prime or cocaine prime (0.3 or 1.0 mg/kg) in a pseudo-randomized order. The prime was administered 5 min prior to the start of the session. Before testing a new pretreatment dose, subjects were allowed to self-administer cocaine until baseline cocaine self-administration rates were re-established, and behavior was extinguished again. Baseline rates of responding between reinstatement sessions did not vary by more than 20% and were reached within 4–5 days of cocaine self-administration. All subjects readily extinguished within 2 days of implementing extinction parameters.

2.8. Drugs

Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD), was dissolved in 0.9% saline. LY379268 (generously supplied by Dr. Jeffrey Witkin, Dept. of Psychiatry, Eli Lilly, Indianapolis, Indiana) was dissolved in sterile water. LY379268 was selected based on high potency (ED50 values of 2.7 and 4.5 nM at human mGluR2 and mGluR3 receptors, respectively) and selectivity (Monn et al., 1999; Cartmell et al., 1999), documentation of behavioral effects in nonhuman primates followed systemic administration (Adewale et al., 2006) and availability. Drug doses were determined as salts. All i.m. drug injections were administered into the thigh muscle at a volume of 0.4–0.8 mL.

2.9. Statistical analyses

The time-course data from the microdialysis experiments were analyzed using repeated-measures ANOVA. In addition, a one-way ANOVA was used to determine the statistical significance of LY379268 pretreatments on the peak DA response to cocaine. Tukey's post hoc multiple comparison tests were used to determine the statistical significance of individual pretreatment doses. The peak effects were determined by an obvious maximal effect or the first data point of a plateau. The effects of cocaine alone on response rate in stimulus-termination experiments were analyzed using repeated-measures ANOVA. In addition, a two-way ANOVA was used to determine the statistical significance of LY379268 pretreatments. Effects of LY379268 pretreatments in self-administration experiments were analyzed using a Mann–Whitney nonparametric test because these data did not meet the assumptions of equal variance and normality for an ANOVA. Lastly, a two-way ANOVA was used to determine the statistical significance of LY379268 pretreatments on cocaine-induced reinstatement. Statistical significance was defined at the 95% level of confidence ($p<0.05$).

3. Results

3.1. Microdialysis

Basal levels of DA were between 3 and 5 nM, unadjusted for probe recovery, as reported previously (Czoty et al., 2000). As shown in previous studies of squirrel monkeys, systemic administration of cocaine (1.0 mg/kg; i.m.) produced a significant increase in extracellular DA as measured by *in vivo* microdialysis ($F[1,14]=14.284$, $p=0.032$, Fig. 1). In drug interaction studies, subjects were given a 30 min pretreatment of LY379268 (1.0 or 3.0 mg/kg). Administration of LY379268 as a pretreatment before a saline injection had no obvious effect on basal extracellular DA. However, there was a significant main effect of pretreatment on the peak cocaine-induced increases in DA ($F[2,8]=7.767$, $p=0.013$). Post hoc analysis demonstrated that both doses of LY379268 significantly attenuated the peak increase in DA induced by cocaine. However, cocaine still induced a significant increase in DA compared to saline even following pretreatment with LY379268, and the peak increases in DA were not significantly different when comparing

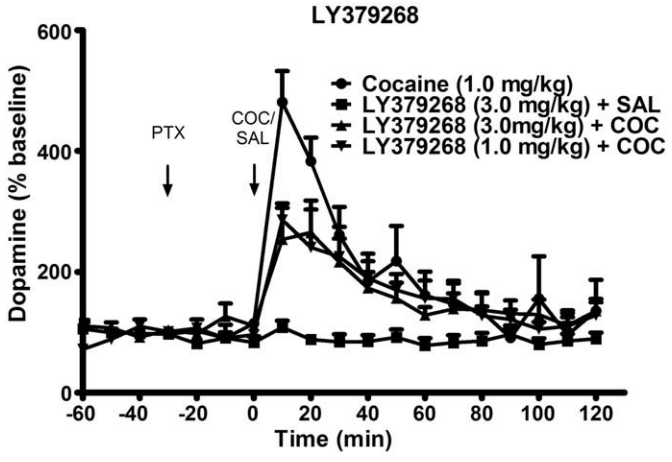

Fig. 1. Cocaine (1.0 mg/kg) significantly elevated extracellular levels of DA in the caudate nucleus in four squirrel monkeys. In contrast, LY379268 (1.0–3.0 mg/kg) administration did not significantly alter extracellular DA when administered as a pretreatment to saline. However, LY379268 pretreatments significantly attenuated cocaine-induced increases in extracellular DA. The 10 min sampling intervals indicated began 60 min post probe insertion. Arrows indicate the points at which the pretreatment, cocaine or saline was administered. Data points represent mean ± SEM DA levels as a percent of values obtained prior to drug administration. Abscissa: time. Ordinate: extracellular concentrations of DA expressed as a percent of baseline control levels.

the two pretreatment doses. At the highest dose (3.0 mg/kg), adverse side-effects were evident including weight loss, emesis and scratching behavior, so higher doses were not examined.

3.2. Fixed-interval stimulus termination

A cumulative-dosing paradigm was utilized to determine the effects of LY379268 on the behavioral-stimulant effects of cocaine. Response rates for individual subjects ranged from 0.19 to 0.47 responses/s with a mean (±S.E.M.) of 0.34±0.13 responses/s. Cumulative dosing of saline produced no significant change in rates of responding throughout the session (Fig. 2). However, increasing doses of cocaine produced a typical inverted U-shaped dose–response curve with a significant main effect of dose ($F[4,30]=7.217$, $p<0.001$). A 5 min pretreatment of LY379268 (1.0–3.0 mg/kg; i.m.) had no significant effect when administered prior to repeated injections of saline ($p=0.566$; Fig. 2A), although rate-decreasing effects were evident at 1.7 and 3.0 mg/kg. However, a 5 min pretreatment of LY372968 produced a significant downward shift in the cocaine dose response curve ($F[3,60]=1.204$, $p=0.002$; Fig. 2B). A 30-min pretreatment of LY379268 did not significantly alter rates of responding during cumulative saline dosing ($p=0.499$; Fig. 2C). Interestingly, the downward shift of the cocaine dose response curve was no longer apparent when LY379268 was given at the longer pretreatment time ($F[3,60]=2.013$, $p=0.122$, Fig. 2D). In fact, a 30-min pretreatment tended to increase response rates relative to cocaine alone.

3.3. Self-administration

Once stable cocaine self-administration was established under the second-order schedule, cocaine dose–response curves were determined for individual subjects. Subsequently, each subject was maintained on the unit dose that maintained peak rates of responding. The response rates for individual subjects ranged from 1.71 to 3.87 responses/s with a mean (±S.E.M.) of 2.34±0.87 responses/s. Pretreatment with LY379268 (1.0–3.0 mg/kg; i.m.) for three consecutive days at each dose produced a significant decrease in rates of responding only at the 1.7 mg/kg dose [Mann–Whitney Rank Sum Test ($p=0.029$)] (Fig. 3), though there was a trend towards an attenuation with the higher dose. It should be noted that frequent emesis was observed when the subjects were given the 3.0 mg/kg dose.

3.4. Reinstatement

Response rates for individual subjects at the maintenance dose of cocaine ranged from 1.75 to 3.88 responses/s with a mean (±S.E.M.) of 2.37±0.22 responses/s. Saline was then substituted for cocaine until rates of responding were extinguished to less than 30% of the rates maintained by cocaine ($F[1,3]=6583.213$, $p=<0.001$; Fig. 4). All subjects extinguished within two days of saline substitution. Initially, subjects were administered a 5 min vehicle pretreatment prior to the reinstatement prime (vehicle or cocaine, 0.3, or 1.0 mg/kg; i.v.) which was administered 5 min prior to the start of the session. Once baseline reinstatement behavior was established, subjects were administered LY379268 (1.0 or 1.7 mg/kg) 5 min prior to the reinstatement prime. Cocaine significantly increased rates of responding at the doses tested ($F[2,6]=23.980$, $p=0.001$, Fig. 4). Neither dose of LY379268 significantly altered cocaine-induced reinstatement, although the effect of LY379268 in combination with 1.0 mg/kg cocaine approached significance ($F[2,5]=5.272$, $p=0.059$). Because of the emesis observed in other experiments in this study with the 3.0 mg/kg LY379268, this dose was not administered as a pretreatment to reinstatement.

4. Discussion

The purpose of the current studies was to determine the effects of the mGluR2/3 agonist, LY379268, on cocaine-induced changes in DA

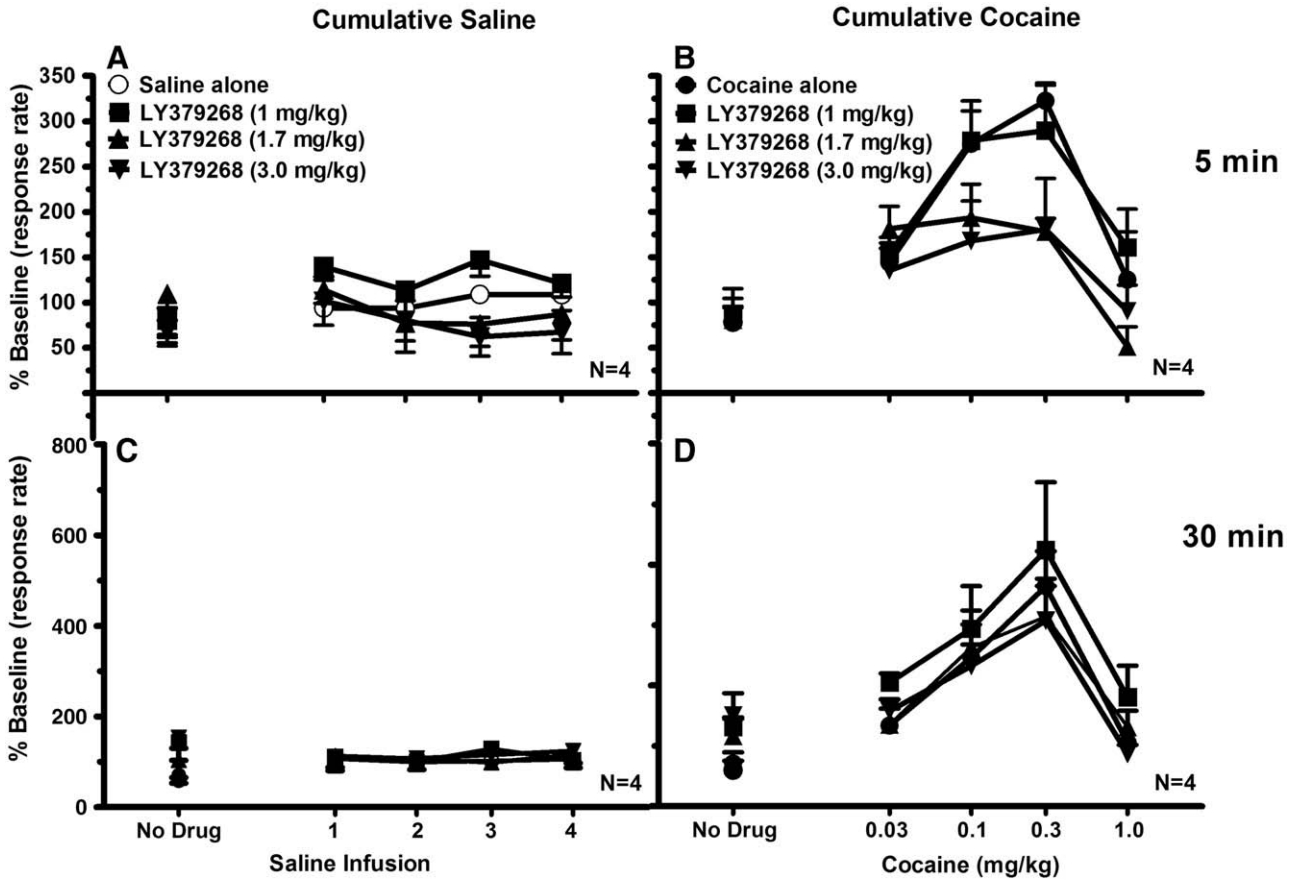

Fig. 2. Cumulative infusions of saline did not significantly alter baseline rates of responding maintained by a fixed-interval schedule of stimulus termination in four squirrel monkeys (A). Cumulative doses of cocaine (0.03–1.0 mg/kg) produced a typical inverted U-shaped dose–effect curve (B). Both 0.1 and 0.3 mg/kg cocaine produced significant increases in rates of responding. LY379268 (1.0–3.0 mg/kg) pretreatments did not significantly alter rates of responding maintained by a fixed-interval stimulus-termination schedule when repeated injections of saline were administered (A,C). However, a 5-min LY379268 pretreatment produced a downward shift of the cocaine dose–effect curve (B). In contrast, a 30-min pretreatment time was ineffective in attenuating the behavioral-stimulant effects of cocaine (D). Abscissae: drug dose or saline infusion. Ordinates: rates of responding expressed as a percent of baseline control rates.

neurochemistry in conscious nonhuman primates. Furthermore, the current studies aimed to determine if changes in DA neurochemistry would correlate with the behavioral effects of cocaine. LY379268 attenuated cocaine-induced increases in extracellular DA without directly altering basal extracellular DA. There are conflicting reports regarding the effects of activation of mGluR2/3 receptors on basal extracellular dopamine in rodents. Early studies demonstrated increases in basal DA by selective mGluR2/3 agonists in the mPFC (Pintor et al., 1998), NAcc (Taber and Fibiger, 1995), and striatum (Cartmell et al., 2000b). Conversely, Greenslade and Mitchell (2004) demonstrated that LY379268 could block phencyclidine induced increases in DA in the rat nucleus accumbens, but only at doses in which LY379268 also decreased basal DA. Further, group II activation produced a biphasic effect on dopamine, with low and high doses having little effect and the intermediate dose decreasing DA in the NAcc (Moghaddam and Adams, 1998). The effects of LY379268 on basal extracellular DA have not been reported previously in nonhuman primates.

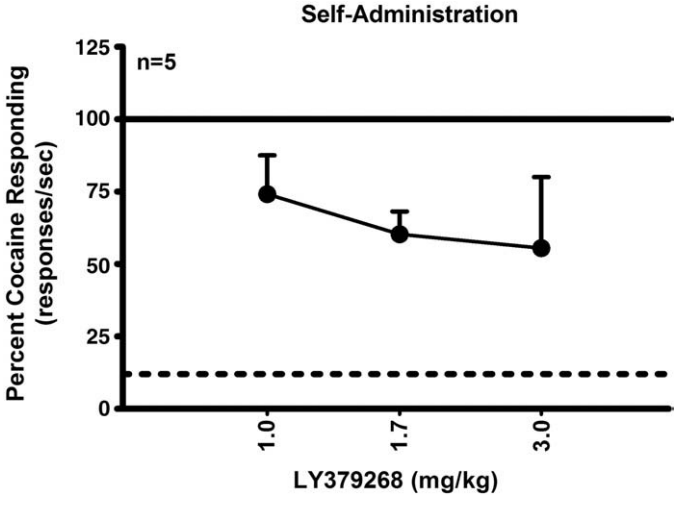

Fig. 3. Administration of LY379268 significantly attenuated rates of responding maintained by a second-order schedule of cocaine self-administration only at the intermediate dose, though there was a trend towards attenuation with the higher dose. Each dose of LY379268 was administered for three consecutive days on separate occasions in five squirrel monkeys. Abscissa: drug dose. Ordinate: rates of responding expressed as a percent of baseline control rates.

LY379268 significantly attenuated the behavioral-stimulant effects of cocaine when administered as a 5-min pretreatment, consistent with previous studies in rodents which demonstrated that LY379268 attenuated amphetamine-induced ambulations and rearing in rats (Cartmell et al., 2000a). Because the most pronounced attenuation was seen at the peak dose of cocaine and this dose was administered approximately 30 min into the session, a longer pretreatment time was utilized to evaluate the potential influence of drug time course of LY372968. When LY379268 was administered as a 30-min pretreatment, there was no significant interaction with cocaine. Hence, the effectiveness of LY379268 to attenuate the behavioral-stimulant effects of cocaine dissipated over the course of the session following a longer pretreatment time. Note that the effects of LY379268 on cocaine-induced increases in DA also were most evident at early time points during the session.

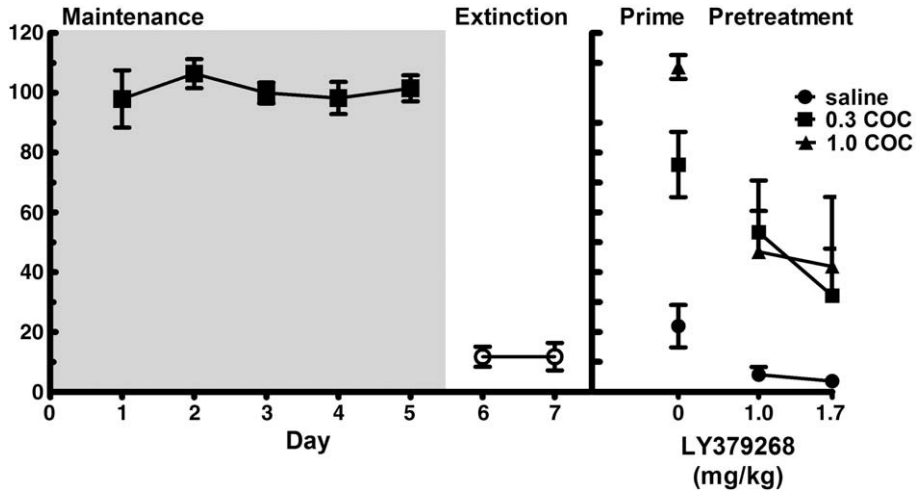

Fig. 4. Cocaine self-administration was reliable during the maintenance phase, and extinguished readily in 2 sessions in four squirrel monkeys. Subsequently, non-contingent injections of cocaine (0.3 and 1.0 mg/kg) reinstated responding. LY379268 pretreatment demonstrated a strong trend toward significantly attenuating reinstatement of responding induced by the high dose (1.0 mg/kg) of cocaine. Abscissa: day of treatment and pretreatment dose of LY379268 prior to reinstatement tests. Ordinate: rates of responding expressed as a percent of baseline control rates.

In contrast to previous studies (Adewale et al., 2006; Peters and Kalivas, 2006), the effects of LY379268 on the reinforcing properties of cocaine were not robust or consistent across doses. LY379268 significantly attenuated cocaine self-administration, but only at the intermediate dose. Moreover, the observed effects of LY379268 only occurred at doses that partially suppressed behavior maintained by the fixed-interval schedule of stimulus termination, and induced scratching and emesis. Importantly, administration of LY379268 had no significant effect on cocaine-induced reinstatement of previously extinguished self-administration, although the effects of LY379268 in combination with the high dose of cocaine approached significance. The apparent short time course of LY379268 in dialysis and stimulus-termination experiments raised the possibility that LY379268 may have produced large but transient decreases in self-administration and reinstatement that were minimized by averaging across multiple components. However, analysis of response rates for individual components (data not shown) provided no evidence that drug effects were pronounced early in the session and dissipated in later components. Overall, these data provide little evidence for a correlation between neurochemical and behavioral results. However, it should be noted that LY379268 pretreatment did not completely abolish cocaine-induced increases in dopamine. Interestingly, the effects of 1.0 mg/kg cocaine in combination with LY379268 in the present study were similar to the effects of 0.3 mg/kg cocaine reported previously in squirrel monkeys (Ginsburg et al., 2005). It is well documented that 0.3 mg/kg cocaine is a dose that is highly reinforcing and induces robust reinstatement effects in nonhuman primates. Thus, a more significant attenuation of cocaine-induced increases in extracellular dopamine may be necessary for robust changes in behavior to be observed.

Recent studies have demonstrated that LY379268 may also have partial agonist activity at DA D2 receptors (Seeman et al., 2008). Accordingly, it is unclear whether the current observations are due, in part, to the effects of LY379268 at DA D2 receptors. Further studies, utilizing selective antagonists for DA receptors and mGluR2/3, should elucidate which receptors mediate the effects of LY379268 on the pharmacology of cocaine. Compounds with higher potency and increased selectivity for the mGluR2/3 will be especially useful in isolating the effects of mGluR2/3 on cocaine pharmacology. Additionally, LY379268 does not distinguish between mGluR2 or mGluR3. Increasing evidence suggests that selective mGluR2 (Corti et al., 2007; Fell et al., 2008) activation may be a more effective target for pharmacotherapeutics of psychiatric disorders such as anxiety and schizophrenia. Selective potentiators of mGluR2 may be more effective in attenuating the behavioral-stimulant and reinforcing effects of cocaine.

The current studies provide the first evidence that an mGluR2/3 agonist can attenuate the neurochemical effects of cocaine in nonhuman primates. LY379268 significantly attenuated cocaine-induced increases in extracelluar DA. However, this attenuation was only partial as cocaine still induced significant increases in DA following pretreatment with LY379268. Moreover, the effects of LY379268 on the reinforcing properties of cocaine and cocaine-induced reinstatement were not robust or consistent across doses, and adverse effects precluded the administration of higher doses of LY379268. Overall, the results obtained with LY379268 did not reveal a prominent attenuation of the abuse-related effects of cocaine in nonhuman primates.

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