Published in final edited form as:

Psychopharmacology (Berl). 2020 February ; 237(2): 409–417. doi:10.1007/s00213-019-05377-y.

Effects of the mGluR2/3 receptor agonist LY379268 on the reinforcing strength of cocaine in rhesus monkeys

Paul W. Czoty¹, Bruce E. Blough², Antonio Landavazo², Michael A. Nader¹

¹Department of Physiology & Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA

²Center for Drug Discovery, Research Triangle Institute, Research Triangle Park, NC 27709, USA

Abstract

Rationale: Because chronic cocaine exposure produces profound effects on brain glutamate function, this system has been investigated as a target for novel medications for cocaine use disorder. Studies in animal models have provided encouraging results for drugs that target metabotropic glutamate receptors (mGluR), particularly Group II mGluRs which includes mGluR2 and mGluR3 receptors.

Objective: The present study examined the effects of the mGluR2/3 receptor-selective agonist, (–)-2-oxa-4-aminobicyclo hexane-4,6-dicarboxylic acid (LY379268), in male rhesus monkeys self-administering cocaine under two procedures that assess the strength of cocaine as a reinforcer.

Methods and Results: In four monkeys, acute effects of LY379268 on food and cocaine self-administration were characterized using a multiple 10-response fixed-ratio food, progressive-ratio cocaine schedule of reinforcement. When monkeys self-administered 0.03 mg/kg per injection cocaine, a dose that resulted in maximal or near-maximal injections, LY379268 (0.001–0.56 mg/kg, i.v.) increased cocaine injections and disrupted food-maintained responding. Another group of monkeys (n=3) responded under a food-cocaine choice procedure in which a dose-effect curve for self-administered cocaine (0.0, 0.003–0.1 mg/kg per injection) was generated daily. Acute LY379268 (0.01–0.1 mg/kg, i.v.) produced a shift in allocation of responding towards cocaine without affecting the total reinforcers delivered. When treatment was extended to 5 consecutive days, tolerance developed to LY379268-induced increases in cocaine choice.

Conclusions: These data from two complimentary nonhuman primate models of cocaine use disorder are consistently negative with respect to the potential of LY379268 as a pharmacotherapy for reducing ongoing cocaine use.

Corresponding author: Paul W. Czoty, Ph.D., Department of Physiology and Pharmacology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1083, Phone: 336-713-7112, pczoty@wakehealth.edu.

Publisher's Disclaimer: This Author Accepted Manuscript is a PDF file of a an unedited peer-reviewed manuscript that has been accepted for publication but has not been copyedited or corrected. The official version of record that is published in the journal is kept up to date and so may therefore differ from this version.

CONFLICT OF INTEREST STATEMENT.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Keywords

nonhuman primates; choice procedure; reinforcing strength; reinforcing efficacy

Cocaine abuse persists as a major public health problem for which no pharmacotherapy has been approved by the Food and Drug Administration. Although the acute effects of cocaine involve interaction with monoamine transporters to increase extracellular concentrations of dopamine, serotonin and norepinephrine, long-term cocaine use results in widespread changes in multiple neurotransmitter systems and brain circuits known to be critical in the development of addiction (e.g. Koob and Volkow 2010). For example, chronic cocaine has been shown to alter glutamatergic neurotransmission, in particular the activity of group II metabotropic glutamate receptors (mGluR), which include mGluR2 and mGluR3 receptors. Studies in rodents have demonstrated that chronic cocaine exposure alters the function of mGluR2/3 receptors in addiction-relevant brain areas such as the nucleus accumbens and prefrontal cortex (e.g. Baker et al. 2003; Moran et al. 2005; Huang et al. 2007; Hao et al. 2010). Stimulation of these presynaptic receptors decreases glutamate release (Conn and Pin 1997; Anwyl 1999; Schoepp 2001; Xi et al. 2002; Pinheiro and Mulle 2008). In consideration of the pronounced effects of cocaine on brain glutamate function, mGluR2/3 agonists have been assessed as potential pharmacotherapies for cocaine use disorder.

Studies in rodent models have demonstrated that stimulation of mGluR2/3 receptors with the selective agonist (–)-2-oxa-4-aminobicyclo hexane-4,6-dicarboxylic acid (LY379268; Monn et al. 1999) can decrease cocaine self-administration as well as cue- and cocaine-induced reinstatement of extinguished behavior that was previously maintained by cocaine (Baptista et al. 2004; Peters and Kalivas 2006; Cannella et al. 2013; Karkhanis et al. 2016; Shin et al. 2018). Although these results are encouraging, in most studies LY379268 was only slightly more potent in altering cocaine-associated behaviors as it was in altering self-administration and/or reinstatement of behaviors maintained by non-drug reinforcers (Baptista et al. 2004; Peters and Kalivas 2006). In other studies, LY379268 was equi- or more potent in decreasing food-versus cocaine-related behaviors (Lu et al. 2007, 2012; Uejima et al. 2007; Jin et al. 2010). These results, and the extension of this pattern of results to reinstatement of extinguished methamphetamine and heroin self-administration (Bossert et al. 2004, 2006a,b; Crawford et al. 2013; Kufhal et al. 2013) might suggest that LY379268 is capable of reducing use of multiple abused substances. Alternatively, the lack of behavioral selectivity of these effects might also indicate that LY379268 decreases behavior in these models by causing sedation or motor impairment rather than by attenuating the abuse-related effects of drugs.

Nonhuman primate (NHP) models of cocaine use disorder can play an important role in translating encouraging findings from rodent models into human laboratory studies and eventual clinical evaluation. NHPs are the animal model most similar to humans in terms of genetics, neuroanatomy and pharmacokinetics and show similar neurobiological consequences of long-term cocaine use (Weerts et al. 2007; Beveridge et al. 2008; Phillips et al. 2014). The ability of LY379268 to alter the abuse-related effects of cocaine has been examined in three previous NHP studies, all in squirrel monkeys. In the first, LY379286

decreased both cocaine self-administration under a second-order schedule of reinforcement and cocaine-primed reinstatement of extinguished cocaine self-administration (Adewale et al. 2006). These effects were blocked by the mGluR2/3 antagonist LU341495. Doses of LY379268 that attenuated cocaine-related behaviors did not significantly alter locomotion, posture or rates of food-maintained responding. In a second study, although LY379268 attenuated cocaine-induced increases in extracellular dopamine as measured by in vivo microdialysis, cocaine self-administration was only decreased by a single, intermediate dose of LY379268 that also suppressed behavior maintained under a fixed-interval schedule of stimulus termination and induced scratching and emesis; LY379268 did not decrease cocaine-primed reinstatement (Bauzo et al. 2009). Most recently, Justinova et al. (2016) reported that LY379268 did not decrease cocaine self-administration under a fixed-ratio (FR) 10 schedule or cocaine-primed reinstatement, although reinstatement of responding in the presence of cues previously paired with cocaine was attenuated.

In each of the three previous NHP studies, cocaine was self-administered under a second-order or fixed-ratio schedule. Although use of these schedules can determine whether or not a drug serves as a reinforcer, it is often of greater clinical relevance to determine the qualitative degree to which a potential pharmacotherapy alters the reinforcing strength of cocaine using either a progressive-ratio (PR) schedule and a food-drug choice procedure (Woolverton and Nader 1990; Haney and Spealman 2008; Czoty et al. 2016). In the present experiments, we studied cocaine self-administration under a multiple schedule consisting of an initial component in which food reinforcement was available under an FR schedule followed by cocaine availability under a PR schedule of reinforcement. In addition, in another group of monkeys, we assessed the relative reinforcing strength of cocaine compared to food using a choice procedure. The effects of LY379268 on food and cocaine self-administration were assessed under both procedures.

MATERIALS AND METHODS

Subjects: Seven adult male rhesus monkeys (*Macaca mulatta*) served as subjects. Each monkey was fitted with an aluminum collar (Primate Products, Redwood City, CA) and trained to sit calmly in a standard primate chair (Primate Products). To prevent development of obesity and cardiovascular/metabolic problems, monkeys were not fed *ad libitum*. They were also not maintained at a “target weight” set to be an arbitrary percentage below free-feeding weight, because the latter can change with age and other factors, and we did not plan to remove monkeys from the study for periodic re-determination of free-feeding weights. Instead, monkeys were weighed weekly and feed enough food daily (Purina Monkey Chow and fresh fruit and vegetables) to maintain a healthy body weight and appearance as determined by daily inspection and periodic veterinary examinations. Monkeys were individually housed in stainless steel cages with *ad libitum* access to water. Animal housing and handling and all experimental procedures were performed in accordance with the 2011 National Research Council *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* and were approved by the Animal Care and Use Committee of Wake Forest University. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Non-Human Primate Environmental

Enrichment Plan. Three monkeys (R-1661, R-1692 and R-1698) had self-administered cocaine and/or methamphetamine in the context of a food-drug choice procedure for more than three years and has been exposed to acute and 5-day treatments with various drugs that act at dopamine D2/D3 and CB1 receptors (e.g. John et al. 2015a,b). The other four monkeys had more than three years self-administering cocaine under a PR schedule but had not been exposed to other experimental drugs prior to the start of this study.

Catheter implantation.—Each monkey had been prepared with an indwelling venous catheter and subcutaneous vascular access port (VAP; Access Technologies, Skokie, IL) under sterile surgical conditions. An antibiotic (30 mg/kg keftol, i.m.; Cefazolin sodium, Marsam Pharmaceuticals, Inc., Cherry Hill, NJ) was administered 1 hour prior to surgery. Anesthesia was induced with ketamine (15 mg/kg, i.m.) and maintained with ketamine supplements if necessary. A catheter was inserted into a major vein (femoral or internal or external jugular) to the level of the posterior vena cava. The distal end of the catheter was passed subcutaneously to a point slightly off the midline of the back, where an incision was made. The end of the catheter was attached to a VAP, which was placed in a pocket formed by blunt dissection.

Apparatus and general behavioral procedures.—Five days each week, monkeys were seated in a primate chair and transferred from the housing room to the procedure room. The back of the animal was cleaned with 95% ethanol and chlorhexidine, and the VAP was connected to an infusion pump (Cole-Parmer Instrument Co., Niles, IL) located outside the chamber via a 20-gauge Huber Point Needle (Access Technologies) and tubing. The pump was operated for approximately 3 sec to fill the port and catheter with saline or the concentration of cocaine available for the session. The monkey was then placed into a ventilated, sound-attenuating chamber (1.5 × 0.74 × 0.76 m; Med Associates, St. Albans, VT). Two photo-optic switches (Model 117–1007; Stewart Ergonomics, Inc., Furlong, PA) were located on one side of the chamber with a horizontal row of three stimulus lights positioned 14 cm above each switch. The switches were positioned to be easily within reach of the monkey seated in the primate chair. Each monkey had been trained to self-administer food pellets using one of the switches (the “food switch”) and to self-administer cocaine using the other switch (the “drug switch”), with position (left/right) of the food and drug switch counterbalanced across monkeys. A food receptacle, above which was a single white stimulus light, was located between the switches and connected with a Tygon tube to a pellet dispenser (Med Associates) located on the top of the chamber for delivery of 1-g banana-flavored food pellets (Bio-Serv, Frenchtown, NJ). At the conclusion of each behavioral session, catheters were flushed with 100 U/ml heparin and the monkeys were returned to their home cages.

Multiple FR food, PR cocaine schedule of reinforcement.—For monkeys who self-administered cocaine under this schedule (R-1610, R-1717, R-1744 and R-1712), each session began with the illumination of the white light above the food switch. Monkeys first responded under a 20-response fixed-ratio schedule (FR 20); 20 responses on the food switch resulted in the delivery of one food pellet, extinguishing of the white light and illumination of the red light for 5 seconds, during which time responding had no scheduled

consequences. This component lasted until 10 pellets had been delivered or 5 minutes elapsed, whichever came first. Next, the white light above the drug switch was illuminated and monkeys responded under a PR schedule. Under this schedule, emission of 20 responses resulted in the first i.v. injection of cocaine, extinguishing of the white light and a 10-sec TO during which the red light was illuminated. The response requirement for subsequent injections was determined by the equation used by Richardson and Roberts (1996): response requirement = $[5 \times e^{(R \times 0.2)}] \times 5$, where e is the mathematical constant and R is equal to the reinforcer number. For the present studies, the first response requirement (20 responses) corresponds to the 8th value given by this equation and was followed by 25, 32, 40, 50, 62, 77, 95, 117, 144, 177, 218, 267, 328, 402, 492, 602, etc. Sessions ended when 20 min elapsed without an injection or the total session duration reached 4 hours.

Initially, injections of 0.03 mg/kg per injection cocaine were made available for at least 5 days and until responding was stable (number of injections $\pm$ 10% of a 3-day mean with no upward or downward trend). Next, a dose-effect curve was constructed by substituting saline or different doses of cocaine (0.001–0.3 mg/kg per injection) for 0.03 mg/kg per injection until responding stabilized. After the curve was constructed, responding was maintained by injections of 0.03 mg/kg cocaine, which represented the peak of the dose-effect curve for one monkey (R-1744) and was within the dose range that constituted a broader peak in the other three monkeys. When responding was stable, a single dose of LY379268 (0.001–0.56 mg/kg, i.v.) was administered 5 min before the start of the session; this pre-treatment time was based on behavioral data from Bauzo et al. (2009). Most doses were examined at least twice, except for low, ineffective doses.

Food-cocaine choice.—Three monkeys (R-1661, R-1688, R-1692) had been trained to self-administer cocaine under a concurrent FR schedule of food and cocaine availability described previously (Czoty and Nader 2012). Briefly, completion of an FR on the food switch always resulted in delivery of a single food pellet; the yellow light above this switch was illuminated during pellet availability. Completion of an FR on the drug switch resulted in activation of the infusion pump and a cocaine injection (0.003–0.1 mg/kg per injection). Availability of different cocaine doses, administered by varying the duration of pump activation, was associated with illumination of a different set of stimulus lights above the switch (see Czoty and Nader 2012). If a response was emitted on the alternate switch before an FR was completed, the response requirement on the first switch was reset. Delivery of either reinforcer was accompanied by illumination of the red light above the corresponding switch (for 5 sec after a pellet delivery or during an injection) and a subsequent period during which all lights remained off and responding had no scheduled consequences. The total TO duration was 30 seconds.

Each session consisted of 5 components in which monkeys chose between food pellets and ascending doses of cocaine (i.e., no injection, 0.003, 0.01, 0.03 and 0.1 mg/kg per injection cocaine in components 1–5, respectively). Each component ended when 10 total reinforcers had been earned or 20 minutes had elapsed, whichever came first; a 120-sec TO followed each component. Ratio requirements for food and cocaine were adjusted for each monkey such that allocation of responding to the drug switch increased over the session (R-1692: FR10 food, FR 100 drug; R-1689: FR100 food, FR60 drug; R-1688: FR40 food, FR100

drug; R-1661: FR10 food, FR175 drug). Responding was considered stable when 20% of reinforcers were earned on the drug switch during component 1 (when the alternative to food was no injection) and component 2 (when the alternative to a food pellet was an injection of 0.003 mg/kg per injection cocaine) and 80% of reinforcers were earned on the drug switch during component 5 (when the alternative to food was 0.1 mg/kg per injection cocaine). An additional criterion of stability was observation of a dose-related increase in drug choice. A complete dose-effect curve was determined in each monkey each day, typically 5 days per week.

Once responding was stable, vehicle or a single dose of LY379268 (0.01 – 0.17 mg/kg, i.v.) was administered 5 min before the start of a session. Most doses were tested at least twice, except for low, ineffective doses. Next, when responding was stable, vehicle or a single dose of LY379268 (0.01 – 0.1 mg/kg, i.v.) was administered for five consecutive days (Monday-Friday). At least one week of choice sessions with no drug/vehicle treatment separated each 5-day regimen or drug/vehicle administration.

Data analysis.

Multiple FR food, PR cocaine schedule.—Dependent variables of interest were the number of food pellets delivered under the FR 20 schedule and the number of drug injections earned under the PR schedule. For determination of the dose-effect curve, data were averaged across the last 3 days of availability of each dose in each monkey. Because the potency of LY379268 differed across monkeys, drug effects were examined in individual subjects. In each monkey, 95% confidence intervals were calculated for data collected after saline administration (with a minimum of 6 replicates). A LY379268 effect was considered significant when the effect of any dose of LY379268 on food pellets or cocaine injections delivered fell outside the 95% confidence limits of the respective baselines.

Food-cocaine choice procedure.—Dependent variables of interest were cocaine choice (number of reinforcers earned on the drug switch divided by the total reinforcers delivered on both switches, multiplied by 100), the total number of reinforcers delivered and the number of food reinforcers and injections delivered in each component. Because, in this experiment, the potency of LY was similar across subjects, data for each of these variables were averaged across monkeys and analyzed using a two-way analysis of variance with repeated measures, with cocaine dose and LY379286 dose as factors, and post-hoc Tukey's multiple comparisons tests. Sphericity was assumed and no correction was employed. Differences were considered statistically significant at the 95% level of confidence ($p < 0.05$). Statistical analyses were carried out using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA).

Drugs.—(–)-Cocaine was supplied by the National Institute on Drug Abuse (Bethesda, MD). LY379268 was synthesized at Research Triangle Institute, Research Triangle Park, NC. Both drugs were dissolved in sterile 0.9% saline.

RESULTS

Effects of acute LY379268 under the multiple FR food, PR cocaine schedule of reinforcement.

Cocaine self-administration under the PR schedule of self-administration was characterized as an inverted U-shaped function of the available cocaine dose (Fig. 1). Peak numbers of cocaine injections were observed when 0.01–0.1 mg/kg cocaine was available for self-administration; the right y axis depicts the total number of responses emitted. When responding was maintained by injections of 0.03 mg/kg cocaine (Fig. 2), two monkeys received the maximum number of available food pellets and two received approximately 6.5 pellets per session following a pre-session saline injection. There were also individual differences in the number of self-administered injections of 0.03 mg/kg cocaine injections, which ranged from 8.1 to 15.0. Acute injections of LY379268 (0.001–0.56 mg/kg) decreased food-maintained responding and increased or did not affect cocaine-maintained responding.

Effects of acute LY379268 on cocaine-food choice schedule of reinforcement.

Choice of cocaine versus food increased in a manner related to the available cocaine dose (Fig. 3A, open symbols). When cocaine-food choice was stable, saline or a single dose of LY379268 (0.01–0.17 mg/kg) was administered prior to the session. The lowest LY379268 dose (0.01 mg/kg) was without effect; for clarity of presentation these data are not shown in Fig. 3 but are included in the statistical analysis. Acute administration of LY379268 increased the relative reinforcing strength of 0.003 mg/kg cocaine (Fig. 3A, filled symbols). There was a significant main effect of LY379268 dose on cocaine choice ($F_{4,8}=11.92$) as well as a significant effect of cocaine dose ($F_{4,8}=191.17$) and a significant interaction ($F_{16,32}=10.83$). Post-hoc testing revealed that, when an injection of 0.003 mg/kg cocaine was the alternative to a food pellet, the effects of 0.03, 0.1 and 0.17 mg/kg LY379268 were significantly different from saline. Although these doses of LY379268 decreased the total number of reinforcers delivered in the first component (Fig. 3B), there was no significant main effect of LY379268 dose or cocaine dose, and no significant interaction of these factors, on total reinforcers delivered. When analyzed separately, food pellets delivered and injections delivered (Fig. 3C and 3D, respectively) were significantly affected by LY379268 administration in a manner consistent with an increase in the relative reinforcing strength of cocaine. There was a significant main effect of LY379268 dose on food pellets delivered ($F_{4,8}=13.85$) as well as a significant effect of cocaine dose ($F_{4,8}=97.50$) and a significant interaction ($F_{16,32}=7.62$). When choice was between no injection or 0.003 mg/kg cocaine and a food pellet (components 1 and 2), effects of the two higher doses of LY379268 differed from saline. Food pellets were also significantly decreased by 0.03 mg/kg LY379268 when 0.003 mg/kg per injection cocaine was available. Regarding injections delivered, there was a significant main effect of LY379268 dose ($F_{4,8}=6.71$) and cocaine dose ($F_{4,8}=34.79$) and a significant interaction between the two factors ($F_{16,32}=8.65$). As observed for food pellets, when 0.003 mg/kg per injection cocaine was available, effects of the three doses of LY379268 differed from saline.

Effects of five consecutive days of LY379268 administration on food-cocaine choice.

Figure 4 depicts the effects of 5 days of treatment with three different doses of LY379268 (rows) in three monkeys (columns). Acute (i.e., Day 1) administration of 0.01 mg/kg LY379268 had no effect in two monkeys (R-1692 and R-1688, top row) and slightly decreased cocaine choice in the third monkey (R-1688). By Day 5, tolerance had developed to that effect in R-1688 and there were no differences from baseline choice in any monkey. Acute treatment with 0.03 mg/kg LY379268 increased choice of 0.003 mg/kg cocaine to 100% on Day 1 in two monkeys (R-1692 and R-1661, Fig. 4, middle row) and on Day 2 in the third (R-1668). Tolerance developed to these effects, but in only one monkey (R-1692) was choice decreased from baseline after 5 days of treatment. Finally treatment with the highest dose, 0.1 mg/kg LY379268, increased cocaine choice in all three monkeys (Fig. 4, bottom row); tolerance was observed in only 1 of the three monkeys by Day 5 of treatment.

DISCUSSION

This study assessed the effects of the mGluR2/3 receptor agonist LY379268, which is >80-fold selective for mGluR2/3 versus other mGluRs (Monn et al. 1999), on the reinforcing strength of cocaine in male rhesus monkeys. Behavior was studied using two procedures—a PR schedule of reinforcement and a food-drug choice procedure—that assessed whether LY379268 altered the reinforcing strength of cocaine by itself (PR) or relative to food reinforcement (choice procedure). Under both conditions, LY379268 increased cocaine self-administration. With repeated administration of LY379268, tolerance developed to the effect on cocaine choice in a manner related to LY379268 dose. That is, tolerance was more commonly observed when lower doses were administered. The results are consistent in suggesting that LY379268 would not be an effective pharmacotherapy for cocaine use disorder.

Under the multiple FR food, PR cocaine schedule of reinforcement, when 0.03 mg/kg per injection cocaine was available for self-administration, LY379268 decreased food-maintained responding and increased cocaine injections delivered. Only one dose of cocaine was examined under the PR schedule, thus it is possible that self-administration of other cocaine doses may have been affected differently by LY379268. Nonetheless, because the cocaine dose studied (0.03 mg/kg per injection) represented the peak of the dose-effect curve (for R-1744, see Fig. 1) or was within the range of doses that constituted the peak (for R-1610, R-1717, R-1712), the increase in injections delivered is most consistent with an increase in the reinforcing strength of cocaine. Although this result differs from those of the two previous rodent studies that used a PR schedule of cocaine self-administration (Hao et al. 2010; Karkhanis et al. 2016), neither of those assessed behavioral selectivity, leaving open the possibility that decreases in cocaine self-administration observed in those rodent studies were due to sedation, motor impairment or other behavioral effects not directly related to cocaine reinforcement.

To complement studies using the PR schedule, cocaine self-administration was also studied in the context of choice between food and drug. Choice procedures have important translational relevance because they reflect the clinical reality that individuals have the option to allocate their behavior towards obtaining drug versus other environmental stimuli

that could function as reinforcers (e.g. Banks and Negus 2016). Under these conditions, acute treatment with LY379268 increased the reinforcing strength of cocaine relative to food. During the first component of the session, when monkeys chose between food pellets and saline injections, the number of food pellets delivered was decreased by LY379268 without a corresponding increase in injections delivered, indicating some amount of behavioral disruption 5 to 25 minutes after LY379268 administration. Although other NHP studies involving squirrel monkeys primarily used a 30-min pretreatment time (Adewale et al. 2006; Justinova et al. 2016), the present studies are concordant with the results of Bauzo et al. (2009), who demonstrated that LY379268 altered the behavioral-stimulant effects of cocaine when LY379268 was administered 5 minutes, but not 30 minutes, prior to cocaine, in squirrel monkeys. In the present studies, during availability of 0.003 mg/kg per injection cocaine, injection deliveries increased and food pellet deliveries decreased, but no decrease in total reinforcers was observed. This pattern of results is consistent with increases in the reinforcing strength of cocaine relative to food in the absence of non-selective effects like sedation, motor impairment or general decreases in motivation. In agreement with results of studies using the PR schedule, these observations suggest that LY379268 would not be an effective medication in patients with cocaine use disorder.

The conclusion of the present studies in rhesus monkeys appears to contrast with the generally positive findings that have emerged from many rodent studies. It is important to note, however, that the behavioral selectivity of LY379268 in decreasing ongoing self-administration of cocaine (versus a non-drug reinforcer) was not assessed in those studies. That is, it is possible that LY379268 decreased cocaine self-administration due to effects other than attenuation of the reinforcing effects of cocaine. In contrast, some rodent studies have examined whether behavioral effects of LY379268 are selective in the context of reinstatement of extinguished behavior. However, most of those studies found that LY379268 was more potent and effective at decreasing reinstatement of food self-administration (Baptista et al. 2004; Peters and Kalivas 2006; Lu et al. 2007, 2012; Uejima et al. 2007; Jin et al. 2010). Assessment of the effects of mGluR2/3 agonists on food-cocaine choice in rodents, which has not yet been reported, would greatly aid translation of the experiments with LY379268. Regarding the effects of LY379268 in monkeys, the overall conclusion that LY379268 lacks potential as a pharmacotherapy is consistent with two of the three previous studies in NHPs (Bauzo et al. 2009 and Justinova et al. 2016 but not Adewale et al. 2006). Unlike the present study, those studies in squirrel monkeys did not report increases in cocaine self-administration. Regardless, the majority of studies in NHPs support the conclusion that LY379268 would not be an effective pharmacotherapy for cocaine use disorder.

Although the present data are consistently negative regarding the potential for LY379268 as a pharmacotherapy for cocaine use disorder, some limitations of these experiments should be noted. First, as mentioned above, only one dose of self-administered cocaine was studied under the PR schedule. Characterization of effects of LY379268 on self-administration of a range of cocaine doses will provide more precise information regarding the pharmacological interactions of cocaine and mGluR2/3 agonists. Second, although LY379268 did not decrease ongoing cocaine self-administration under either the PR schedule or choice procedure, it is possible that the approach may have utility for relapse prevention, which

would be better assessed with different behavioral procedures. Indeed most of the early, positive data regarding LY379268 arose from studies that examined reinstatement of extinguished responding as a model of relapse (e.g., Baptista et al. 2004; Bossert et al. 2004). Third, of the approximately 30 published studies of the effects of LY379268 on self-administration or reinstatement of abused drugs, only one included female subjects (Adewale et al. 2006, which did not specify how many of the 14 squirrel monkey subjects were female). It is possible that effects of LY379268 on cocaine self-administration may differ in females. Finally, it should be noted that although the effects of LY379268 suggest that direct mGluR2/3 receptor agonists would be ineffective pharmacotherapies, positive allosteric modulators (PAMs) may hold greater potential. For example, in rodent studies in which LY379268 decreased both cocaine and food pellet self-administration, the mGluR2/3 PAM potassium 30-([(2-cyclopentyl-6-7-dimethyl-1-oxo-2,3-dihydro-1H-inden-5-yl)oxy)methyl]biphenyl 1-4-carboxylate (BINA) and its derivatives decreased cocaine self-administration selectively (Jin et al. 2010; Dhanya et al. 2011). In consideration of the extant literature on LY379268 discussed above, it is recommended that future study of mGluR2/3 PAMs be conducted using methods that assess behavioral selectivity, preferably food-cocaine choice procedures, in both rodents and NHPs.

ACKNOWLEDGEMENTS.

The authors wish to acknowledge the technical assistance of Michael Collier, Jill Odom, Phillip Epperly and Susan H. Nader. This research was supported by a grant from the National Institute on Drug Abuse (P50 DA006634).

This research was supported by a grants from the National Institute on Drug Abuse (P50 DA006634). The authors have no financial relationship with the organization that sponsored the research and no other conflicts of interest.

REFERENCES

- Adewale AS, Platt DM, Spealman RD (2006) Pharmacological stimulation of Group II metabotropic glutamate receptors reduces cocaine self-administration and cocaine-induced reinstatement of drug seeking in squirrel monkeys. *J Pharmacol Exp Ther* 318: 922–931 [PubMed: 16675638]
- Anwyl R (1999) Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. *Brain Res Brain Res Rev* 29: 83–120 [PubMed: 9974152]
- Augier E, Dulman RS, Rauffenbart C, Augier G, Cross AJ, Heilig M (2016) ThemGluR2 positive allosteric modulator, AZD8529, and cue-induced relapse to alcohol seeking in rats. *Neuropsychopharmacology* 41: 2932–2940 [PubMed: 27339394]
- Baker DA, McFarland K, Lake RW, Shen H, Tang XC, Toda S, Kalivas PW (2003) Neuroadaptations in cysteine-glutamate exchange underlie cocaine relapse. *Nat Neurosci* 6: 743–749 [PubMed: 12778052]
- Banks ML, Negus SS (2016) Insights from preclinical choice models on treating drug addiction. *Trends Pharmacol Sci* 38: 181–194 [PubMed: 27916279]
- Baptista MA, Martin-Fardon R, Weiss F (2004) Preferential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on conditioned reinstatement versus primary reinforcement: comparison between cocaine and a potent conventional reinforcer. *J Neurosci* 24: 4723–4727 [PubMed: 15152032]
- Bauzo RM, Kimmel HL, Howell LL (2009) Interactions between the mGluR2/3 agonist, LY379268, and cocaine on in vivo neurochemistry and behavior in squirrel monkeys. *Pharmacol Biochem Behav* 94: 204–210 [PubMed: 19703487]
- Beveridge TJ, Gill KE, Hanlon CA, Porrino LJ (2008) Review. Parallel studies of cocaine-related neural and cognitive impairment in humans and monkeys. *Philos Trans R Soc Lond B Biol Sci* 363: 3257–3266 [PubMed: 18640916]

- Bossert JM, Liu SY, Lu L, Shaham Y (2004) A role of ventral tegmental area glutamate in contextual cue-induced relapse to heroin seeking. *J Neurosci* 24: 10726–10730 [PubMed: 15564590]
- Bossert JM, Gray SM, Lu L, Shaham Y (2006a) Activation of Group II metabotropic glutamate receptors in the nucleus accumbens shell attenuates context-induced relapse to heroin seeking. *Neuropsychopharmacology* 31: 2197–2209 [PubMed: 16341024]
- Bossert JM, Poles GC, Sheffler-Collins SI, Ghitza UE (2006b) the mGluR2/3 agonist LY379268 attenuates context- and discrete cue-induced reinstatement of sucrose seeking but not sucrose self-administration in rats. *Behav Brain Res* 173: 148–152 [PubMed: 16834996]
- Cannella N, Halbout B, Uhrig S, Evrard L, Corsi M, Corti C, Deroche-Gamonet V, Hansson AC, Spanagel R (2013) The mGluR2/3 agonist LY379268 induced anti-reinstatement effects in rats exhibiting addiction-like behavior. *Neuropsychopharmacology* 38: 2048–2056 [PubMed: 23624743]
- Conn PJ, Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. *Ann Rev Pharmacol Toxicol* 37: 205–237 [PubMed: 9131252]
- Crawford JT, Roberts DC, Beveridge TJ (2013) The group II metabotropic glutamate receptor agonist, LY379268, decreases methamphetamine self-administration in rats. *Drug Alcohol Depend* 132: 414–419 [PubMed: 23953655]
- Czoty PW, Nader MA (2012) Individual differences in the effects of environmental stimuli on cocaine choice in socially housed male cynomolgus monkeys. *Psychopharmacology* 224: 69–79 [PubMed: 22083591]
- Czoty PW, Stoops WW, Rush CR (2016) Evaluation of the “pipeline” for development of medications for cocaine use disorder: a review of translational preclinical, human laboratory, and clinical trial research. *Pharmacol Rev* 68: 533–562 [PubMed: 27255266]
- Dhanya RP, Sidique S, Sheffler DJ, Nikols HH, Herath A, Yang L, Dahl R, Ardecky R, Semenova S, Markou A, Conn PJ, Cosford ND (2011) Design and synthesis of an orally active metabotropic glutamate receptor subtype-2(mGluR2) positive allosteric modulator (PAM) that decreases cocaine self-administration in rats. *J Med Chem* 54: 342–353 [PubMed: 21155570]
- Haney M, Speelman RD (2008) Controversies in translational research: drug self-administration. *Psychopharmacology* 199: 403–419 [PubMed: 18283437]
- Hao Y, Martin-Fardon R, Weiss F (2010) Behavioral and functional evidence of metabotropic glutamate receptor 2/3 and metabotropic glutamate receptor 5 dysregulation in cocaine-escalated rats: factor in the transition to dependence. *Biol Psychiatry* 68: 240–248 [PubMed: 20416862]
- Huang CC, Yang PC, Lin HJ, Hsu KS (2007) Repeated cocaine administration impairs group II metabotropic glutamate receptor-mediated long-term depression in the medial prefrontal cortex. *J Neurosci* 27: 2958–2968 [PubMed: 17360919]
- Justinova Z, Le Foll B, Redhi GH, Markou A, Goldberg SR (2016) Differential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on nicotine versus cocaine self-administration and relapse in squirrel monkeys. *Psychopharmacology* 233: 1791–1800 [PubMed: 26149611]
- Justinova Z, Panlilio LV, Secci ME, Redhi GH, Schindler CW, Cross AJ, Mrzljak L, Medd A, Shaham Y, Goldberg SR (2015) The novel metabotropic glutamate receptor 2 positive allosteric modulator, AZD8529, decreases nicotine self-administration and relapse in squirrel monkeys. *Biol Psychiatry* 78: 452–462 [PubMed: 25802079]
- Jin X, Semenova S, Yang L, Ardecky R, Sheffler DJ, Dahl R, Conn PJ, Cosford ND, Markou A (2010) The mGluR2 positive allosteric modulator BINA decreases cocaine self-administration and cue-induced cocaine-seeking and counteracts cocaine-induced enhancement of brain reward function in rats. *Neuropsychopharmacology* 35: 2021–2036 [PubMed: 20555310]
- John WS, Banala AK, Newman AH, Nader MA (2015a) Effects of buspiroone and the dopamine D3 receptor compound PG619 on cocaine and methamphetamine self-administration in rhesus monkeys using a food-drug choice paradigm. *Psychopharmacology* 232: 1279–1289 [PubMed: 25327444]
- John WS, Newman AH, Nader MA (2015b) Differential effects of the dopamine D3 receptor antagonist PG01037 on cocaine and methamphetamine self-administration in rhesus monkeys. *Neuropharmacology* 92: 34–43 [PubMed: 25576373]

- Karkhanis AN, Beveridge TJR, Blough BE, Jones SR, Ferris MJ (20016) The individual and combined effects of phenmetrazine and mgluR2/3 agonist LY379268 on the motivation to self-administer cocaine. *Drug Alcohol Depend* 166: 51–60
- Koob GF, Volkow ND (2010) Neurocircuitry of addiction. *Neuropsychopharmacology* 35: 217–235
- Kufahl PR, Watterson LR, Nemirovsky NE, Hood LE, Villa A, Halstengard C, Zautra N, Olive MF (2013) Attenuation of methamphetamine seeking by the mGluR2/3 agonist LY379268 in rats with histories of restricted and escalated self-administration. *Neuropharmacology* 66: 290–301 [PubMed: 22659409]
- Lu L, Uejima JL, Gray SM, Bossert JM, Shaham Y (2007) Systemic and central amygdala injections of the mGluR2/3 agonist LY379268 attenuate the expression of incubation of cocaine craving. *Biol Psychiatry* 61: 591–598 [PubMed: 16893525]
- Lu YL, Xue Y, Steketee JD, Rebec GV, Sun WL (2012) Regulation of cocaine-induced reinstatement by group II metabotropic glutamate receptors in the ventral tegmental area. *Psychopharmacology* 220: 75–85 [PubMed: 21881873]
- Monn JA, Valli MJ, Massey SM, Hansen MM, Kress TJ, Wepsiec JP, Harkness AR, Grutsch JL Jr, Wright RA, Johnson BG, Andis SL, Kingston A, Tomlinson R, Lewis R, Griffey KR, Tizzano JP, Schoepp DD (1999) Synthesis, pharmacological characterization, and molecular modeling of heterobicyclic amino acids related to (+)-2-aminobicyclo[3.1.0] hexane-2,6-dicarboxylic acid (LY354740): identification of two new potent, selective, and systemically active agonists for group II metabotropic glutamate receptors. *J Med Chem* 42: 1027–1040 [PubMed: 10090786]
- Moran MM, McFarland K, Melendez RI, Kalivas PW, Seamans JK (2005) Cysteine/glutamate exchange regulates metabotropic glutamate receptor presynaptic inhibition of excitatory transmission and vulnerability to cocaine seeking. *J Neurosci* 25: 6389–6393 [PubMed: 16000629]
- Peters J, Kalivas PW (2006) The group II metabotropic glutamate receptor agonist, LY 379268, inhibits both cocaine- and food-seeking behavior in rats. *Psychopharmacology* 186: 143–149. [PubMed: 16703399]
- Phillips KA, Bales KL, Capitanio JP, Conley A, Czoty PW, 't Hart BA, Hopkins WD, Hu SL, Miller LA, Nathanielsz PW, Rogers J, Shively CA, Voytko ML (2014) Why primate models matter. *Am J Primatol* 76: 801–827 [PubMed: 24723482]
- Pinheiro PS, Mulle C (2008) Presynaptic glutamate receptors: physiological functions and mechanisms of action. *Nat Rev Neurosci* 9: 423–436 [PubMed: 18464791]
- Richardson NR, Roberts DCS (2006) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Methods* 66: 1–11
- Schoepp DD (2001) Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *J Pharmacol Exp Ther* 299: 12–20 [PubMed: 11561058]
- Shin CB, Templeton TJ, Chiu AS, Kim J, Gable ES, Vieira PA, Kippin TE, Szumlinski KK (2018) Endogenous glutamate within the prelimbic and infralimbic cortices regulates the incubation of cocaine-seeking in rats. *Neuropharmacology* 128: 293–300 [PubMed: 29061508]
- Uejima JL, Bossert JM, Poles GC, Lu L (2007) Systemic and central amygdala injections of the mGluR2/3 agonist LY279368 attenuate the expression of incubation of sucrose craving in rats. *Behav Brain Res* 181: 292–296 [PubMed: 17537525]
- Weerts EM, Fantegrossi WE, Goodwin AK (2007) The value of nonhuman primates in drug abuse research. *Exp Clin Psychopharmacol* 15: 309–327 [PubMed: 17696678]
- Woolverton WL, Nader MA (1990) Experimental evaluation of the reinforcing effects of drugs In: Adler MW, Cowan A (eds) *Testing and Evaluation of Drugs of Abuse*. Wiley-Liss, Inc., New York, NY, pp 165–192
- Xi ZX, Baker DA, Shen H, Carson DS, Kalivas PW (2002) Group II metabotropic glutamate receptors modulate extracellular glutamate in the nucleus accumbens. *300*: 162–171

Fig. 1.

Cocaine self-administration under the multiple FR 20 food, PR cocaine schedule of reinforcement in individual monkeys. Abscissa: saline (SAL) or cocaine dose available for self-administration. Ordinates: left, number of injections delivered; right, number of responses emitted. Data indicate mean $\pm$ SD.

Fig. 2.

Effects of LY379268 on food and cocaine self-administration under the FR 20 food, PR cocaine schedule of reinforcement in individual monkeys. Abscissa: LY379268 dose (or saline [SAL]) administered before the session. Ordinate: number of reinforcers (food pellets or cocaine injections) delivered. Data indicate mean $\pm$ SD. Points above SAL indicate number of reinforcers delivered during saline administration. *: points which fall outside the 95% confidence intervals for the respective reinforcer after saline administration.

Fig. 3.

Effects of two doses of LY379268 (in mg/kg) on food and cocaine self-administration under the choice procedure. Abscissa: cocaine dose available as the alternative to a food pellet. Ordinates: (A) cocaine choice, (B) total reinforcers delivered, (C) number of food pellets delivered, (D) cocaine injections delivered. Data indicate mean $\pm$ SEM ($n=3$). *: significant difference from data obtained after saline treatment during availability of the same cocaine dose

Fig. 4.

Effects of 5 consecutive days of LY379268 administration on cocaine choice. Data are shown for three subjects (rows) and three doses of LY379268 (column). In each graph, data are shown for baseline, Day 1 and Day 5 of treatment. Data from Day 2 is included for R-1688 because the effect observed on Day 1 in the other two subjects was not observed until Day 2 in this monkey. Ordinates and abscissae as in Fig. 3A.