Title: Lorcanerin reduces the discriminative stimulus and reinforcing effects of cocaine in rhesus monkeys

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Abstract

Cocaine abuse and obesity are serious public health problems, and studies suggest that both dopamine and serotonin (5-HT) systems are involved in regulating the consumption of drugs and food. Lorcaserin has 5-HT\textsubscript{2C} receptor agonist actions, is approved by the United States Food and Drug Administration (FDA) for treating obesity, and might be effective for treating cocaine abuse. These studies characterized the pharmacokinetic and behavioral profiles of lorcaserin (intra-gastric administration; i.g.) and determined the effectiveness of lorcaserin to alter discriminative stimulus and reinforcing effects of cocaine (intravenous administration; i.v.) in rhesus monkeys. Administered acutely, lorcaserin dose dependently increased the occurrence of yawning while decreasing spontaneous activity and operant responding for food. These effects appeared within 30-60 min of administration and began to dissipate by 240 min, a time course closely matching plasma concentrations of lorcaserin. In monkeys discriminating cocaine from saline, lorcaserin alone did not occasion cocaine-appropriate responding but shifted the cocaine dose-response curve to the right and down in 2 of 3 monkeys. Administered acutely, lorcaserin dose dependently decreased the rate at which monkeys responded for infusions of cocaine. When administered chronically, 3.2 mg/kg lorcaserin reduced the rate of cocaine-maintained responding by 50% for the duration of a 14-day treatment. Together, these results show that lorcaserin attenuates the discriminative stimulus effects of cocaine following acute administration and the reinforcing effects of cocaine following acute and repeated administration, consistent with the view that it might have utility in treating cocaine abuse.
Introduction

Cocaine abuse remains a significant public health problem. The National Survey on Drug Use and Health estimated that, in 2013, 600,000 individuals tried cocaine for the first time, and 1.5 million Americans were considered regular users (Substance Abuse and Mental Health Administration, 2014). Worldwide estimates put the number of regular cocaine users at nearly 20 million (United Nations Office on Drugs and Crime, 2014). Despite longstanding efforts to develop pharmacotherapies (e.g., Dackis and O’Brien, 2003; Grabowski et al, 2004; Mello, 1990; Platt et al, 2002; Roberts and Brebner, 2000; Tanda et al, 2009; Vocci et al, 2005), there are no approved medications for treating cocaine abuse.

Cocaine binds to dopamine, serotonin (5-HT), and norepinephrine transporters with similar affinity, although it is thought that abuse-related effects of cocaine are mediated predominantly by its capacity to increase dopamine neurotransmission (Ritz et al, 1987). One approach for reducing these effects of cocaine is to target neurotransmitter systems and/or specific receptors that modulate (e.g., indirectly decrease or inhibit) dopamine neurotransmission. The ability of 5-HT systems to modulate dopamine activity is well documented, and mounting evidence suggests that these effects are mediated by the 5-HT<sub>2</sub> subfamily of receptors, with agonists acting at 5-HT<sub>2A</sub> receptors stimulating dopamine release, and agonists acting at 5-HT<sub>2C</sub> receptors inhibiting dopamine release within the nucleus accumbens. Conversely, antagonists of 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors are known to decrease or increase dopamine neurotransmission, respectively (for review see Howell and Cunningham, 2015).

Based on these effects, antagonists selective for 5-HT<sub>2A</sub> receptors and agonists selective for 5-HT<sub>2C</sub> receptors have been investigated for their ability to modify the behavioral effects of drugs of abuse, including cocaine. Although 5-HT<sub>2A</sub> receptor antagonists (SR 46349B, M100907) inhibit the effectiveness of non-contingent cocaine injections to reinstate extinguished responding for cocaine (cocaine-primed reinstatement) at doses that do not alter the ongoing
self-administration of cocaine (Filip et al, 2005; Fletcher et al, 2002; Murnane et al, 2013), 5-HT$_{2C}$ receptor agonists (Ro 60-0175, WAY 163909) dose dependently inhibit the effectiveness of cocaine primes and cocaine-associated stimuli (cue-induced reinstatement) to reinstate extinguished responding, as well as the direct reinforcing effects of cocaine in self-administration procedures (Burbassi and Cervo, 2008; Burmeister et al, 2004; Cunningham et al, 2011; Grottick et al, 2000; Manvich et al, 2012; Rüedi-Bettschen et al, 2015). These findings suggest that 5-HT$_{2C}$ receptor-selective agonists might be effective for reducing cocaine abuse and for prolonging abstinence.

Lorcaserin (Belviq®) is a selective 5-HT$_{2C}$ receptor agonist (18- and 100-fold selective for 5-HT$_{2C}$ over 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors, respectively; Thomsen et al, 2008) that was approved in 2012 by the United States Food and Drug Administration (FDA) for the treatment of obesity. In clinical studies, lorcaserin is well tolerated and decreases food intake and body weight (Aronne et al, 2014; Chan et al, 2013; Smith et al, 2009, 2010) at doses that have low potential for producing heart valve disease (Weissman et al, 2013) or abuse (Shram et al, 2011), effects commonly associated with 5-HT$_{2B}$ and 5-HT$_{2A}$ receptor agonists, respectively (Hutcheson et al, 2011; Nichols, 2004). In addition to preclinical studies demonstrating that lorcaserin decreases food intake and body weight (Grottick et al, 2014; Higgins et al, 2013b; Smith et al, 2008; Thomsen et al, 2008), lorcaserin also decreases nicotine self-administration and the reinstatement of responding for nicotine in rats (Higgins et al, 2012, 2013b; Levin et al, 2011).

The current studies evaluated whether lorcaserin might have utility in treating cocaine abuse. Accordingly, the goals of these studies were to characterize the behavioral and pharmacokinetic profiles of lorcaserin and to assess its effectiveness to modify the discriminative stimulus and reinforcing effects of cocaine in rhesus monkeys. Because lorcaserin would be taken orally by cocaine abusers and because drug abuse is a chronic condition, the current studies characterized the effects of intra-gastric (i.g.) lorcaserin. In an attempt to broadly characterize the behavioral effects of lorcaserin, time course data were
collected for the effects of a range of doses of lorcaserin to alter locomotor activity and 24 distinct observable behaviors, as well as food-maintained responding. In order to assess its potential to alter abuse-related effects of cocaine, lorcaserin was also evaluated for its effectiveness to modify plasma concentrations of cocaine, antagonize the discriminative stimulus effects of cocaine, and reduce the self-administration of cocaine; the effects of lorcaserin on cocaine self-administration were assessed following both acute and repeated (14 days) administration. Together, these results provide preclinical evidence that lorcaserin attenuates some abuse-related effects of cocaine following acute and repeated administration and suggest that lorcaserin might be effective in treating cocaine abuse.

Methods

Subjects. A total of 16 adult rhesus monkeys were used in these studies. One male (GO) and 3 female rhesus monkeys (PR, IR, LI), weighing between 6 and 9 kg, were used to characterize changes in locomotor activity and directly observable behaviors produced by lorcaserin. The male monkey (GO) and 2 of the female monkeys (PR, IR), along with one additional male monkey (CO; 9 kg), were used for the pharmacokinetic study. Three other male rhesus monkeys (HU, JO, LA), weighing between 8 and 11 kg, were used to investigate the effects of lorcaserin on food-maintained responding. A separate group of 3 male rhesus monkeys (MU, DU, LR), weighing between 8 and 11 kg, were used for the cocaine discrimination studies. Three male (BO, CH, PE) and 2 female rhesus monkeys (DA, RO), weighing between 6 and 10 kg, were used to evaluate the effectiveness of lorcaserin to inhibit cocaine self-administration. All monkeys were housed individually on a 14-h light and 10-h dark cycle with free access to water in their home cage. Monkeys were fed primate chow (Harlan Teklad, High Protein Monkey Diet, Madison, WI), fresh fruit, and peanuts daily. All monkeys were maintained, and all experiments were performed, in accordance with the Institutional
Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio, and with the Guide for the Care and Use of Laboratory Animals, 8th edition (2011).

**Surgical Preparation.** Monkeys in the drug-discrimination and self-administration studies were surgically prepared with indwelling venous catheters connected to subcutaneous vascular access ports according to methods described elsewhere (Wojnicki et al., 1994). Briefly, monkeys were anesthetized with 10 mg/kg ketamine (subcutaneously; Fort Dodge Laboratories, Fort Dodge, Iowa, USA), intubated, and then maintained on 2 l/min oxygen and isoflurane anesthesia (Butler Animal Health Supply, Grand Prairie, Texas, USA). A polyurethane catheter (SIMS Deltec Inc., St. Paul, Minnesota, USA) was implanted in a vein (e.g., jugular or femoral) and connected to a vascular access port (Access Technologies, Skokie, Illinois, USA) that was positioned subcutaneously in the mid-scapular region.

**Apparatus.** Accelerometers (Actical model number 1081986, Respironics Inc., Murrysville PA) attached to collars were used to measure activity in the home cage. For experiments assessing operant behavior, monkeys were seated in commercially available chairs (Model R001; Primate Products, Miami, Florida, USA) and placed in ventilated, sound-attenuating chambers. Response panels located on the front wall of each chamber contained two response levers and two stimulus lights that could be illuminated red or green. For drug-discrimination studies, drugs were injected directly into the vascular access port which was then flushed with 5 ml of saline. For self-administration studies, cocaine was delivered intravenously (i.v.) by a syringe driver (Razel Scientific Instruments Inc., Stamford, Connecticut, USA) located outside the chamber. The syringe driver contained a 30-ml syringe that was connected to the vascular access port with a 185-cm extension set (Abbott Laboratories, Stone Mountain, Georgia, USA) equipped with a 20-g Huber-point needle (Access Technologies). A computer controlled experimental events and recorded data using Med-PC IV software (Med Associates Inc., St. Albans, Vermont, USA).
Collection and Storage of Samples for Pharmacokinetic Experiments. Monkeys were seated in restraint chairs for i.g. administration of saline or 3.2 mg/kg lorcaserin, i.v. administration of 1 mg/kg cocaine, and collection of 1 ml of blood per sample by acute puncture of the saphenous vein. To monitor plasma concentrations of 3.2 mg/kg lorcaserin alone, blood was collected immediately before and 30, 60, 90, 120, 210 min, and 24 hr after lorcaserin administration. To determine whether lorcaserin altered plasma concentrations of cocaine, monkeys received saline or lorcaserin i.g. and were returned to their home cage; 88 min later, the first blood sample was collected after monkeys were again seated in chairs. Cocaine (1 mg/kg; i.v.) was then administered in the same vein with the interval between i.g. administration of saline or lorcaserin and i.v. administration of cocaine being 90 min. Blood (1 ml) was collected from the opposite saphenous vein 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after cocaine administration. All blood samples were collected into 3 ml EDTA tubes (Vacutainer®, Bectin, Dickson and Company, Franklin Lakes, NJ) and immediately placed on ice and shielded from light. Within 30 min, the tubes were centrifuged at 1000 g for 10 min. Plasma was collected in polypropylene tubes containing 20 µl of a solution of 1 mg/ml sodium fluoride and 60% acetic acid and then stored at -80°C. Pharmacokinetic procedures were based on previous studies in which plasma cocaine levels were assessed in rhesus monkeys (Collins et al, 2012).

Measurement of Lorcaserin and Cocaine in Monkey Plasma. Milli-Q water was used for preparation of all solutions. Lorcaserin, cocaine, and deuterated cocaine (dCOC) super-stock solutions were prepared in methanol at a concentration of 1 mg/ml and stored in aliquots at -80°C. Working stock solutions (10 µg/ml) of each analyte were prepared fresh each experimental day from the super-stock solutions and used to spike the calibrator samples.

The HPLC system consisted of a Shimadzu SCL-10A Controller, LC-10AD pump with a FCV-10AL mixing chamber, SIL-10AD autosampler, and an AB Sciex API 3200 tandem mass spectrometer with turbo ion spray. The analytical column was an Ace Excel 3 Super C18 (3 x 75 mm, 3 µ) purchased from MacMod (Chadds Ford, PA) and was maintained at 60°C during the
chromatographic runs in a Shimadzu CTO-10A column oven. Mobile phase A was 0.1% formic acid dissolved in Milli-Q water and mobile phase B was 0.1% formic acid dissolved in acetonitrile. The flow rate of the mobile phase was 0.6 ml/min. The gradient was as follows: 0 to 2 min, 10%B to 85%B linear; 2 to 3.4 min, 85%B; 3.4 to 3.5 min, 85%B to 10%B linear; 3.5 to 6 min, 10%B. The Q1/Q3 transitions used to quantify the analytes were 196.1→144.1 for lorcaserin, 304.1→182.2 for cocaine, and 307.2→185.3 for dCOC.

*Sample Processing and Quantification of Lorcaserin and Cocaine in Plasma.* Lorcaserin and cocaine were quantified in monkey plasma. Briefly, 300 μl of calibrator and coded plasma samples were mixed with 50 μl of a 1 μg/ml dCOC solution (internal standard) and 2 ml of a mobile phase B (0.1% formic acid dissolved in acetonitrile). The samples were vortexed vigorously for 2 min and then centrifuged at 4925 g for 10 min at 23° C. Supernatants were dried to residue under a stream of nitrogen and then the residues were dissolved in 100 μl of 0.1% formic acid in water:acetonitrile (90:10). Then, 10 μl of the final samples were injected into the LC/MS/MS. The ratio of the peak area of each analyte to that of the internal standard dCOC (response ratio) for each unknown sample was compared against a linear regression of calibrator response ratios at spiked concentrations of 0, 1, 10, 100, 500, and 1000 ng/ml of lorcaserin and cocaine. Final concentrations of all analytes were expressed as ng/ml of plasma.

To test the effect of lorcaserin on the pharmacokinetic profile of cocaine, 4 monkeys received 1 mg/kg cocaine i.v. 90 min after i.g. administration of either saline or 3.2 mg/kg lorcaserin. Area under the curve (AUC) was calculated for the 2-h periods after administration of cocaine, and a two-tailed, paired t-test was performed to determine if lorcaserin altered the pharmacokinetic profile of cocaine. All statistical analyses were performed using GraphPad Prism 6 (La Jolla, CA, USA).

*Activity Monitoring and Directly Observable Behavior.* Each of 4 doses of lorcaserin was examined once in 4 monkeys. Monkeys were seated in primate chairs during the administration of saline or a dose of lorcaserin (0.32-32 mg/kg; i.g.) then immediately returned to their home
cages where activity and directly observable signs were measured. Accelerometers were attached to collars and collected activity counts in 1-min bins continuously until they were removed from the collar. Directly observable behavior was measured 5, 15, 30, 60, 120, 240 min, and 24 hr after lorcaserin administration by 2 observers who were familiar with the behavior of these monkeys; 24 signs (Table 1) were recorded as present or absent during a 30-sec observation period at each time point. One observer administered drug and, therefore, was not blind to treatment; however, both observers were blind to the overall purpose of the experiment and expected outcomes. The level of agreement between the two raters of directly observable signs was determined using the $\kappa$ statistic, which was calculated for signs that were increased by lorcaserin; reliability between observers was considered adequate for making definite conclusions when the value of $\kappa$ exceeded 0.80 (Hallgren, 2012; Landis and Koch, 1977).

Activity counts for the 5 minutes immediately following each observation period (e.g., minutes 6-10 after lorcaserin) were averaged for individuals and then averaged across monkeys ($\pm$ 1 SEM). Two-factor ANOVA with repeated measures and post-hoc Holm-Sidak’s tests were used to determine if activity varied as a function of lorcaserin dose or time. Data for directly observable signs are reported as the proportion of monkeys exhibiting the sign as a function of time since lorcaserin. AUC was also calculated for each time course, and these data are reported as the mean $\pm$ SEM of the AUC for each dosing condition. One-factor ANOVA with repeated measures and post-hoc Holm-Sidak’s tests were used to determine if AUC values derived from the entire 240-min period varied as a function of dose of lorcaserin.

**Food-Maintained Responding.** Each of 3 doses of lorcaserin was examined once in 3 monkeys. Sessions lasted 2 hr and were divided into 15-min cycles. Each cycle began with a 10-min timeout during which chambers were dark and responding had no programmed consequence. During the last 5 min of each cycle, monkeys could respond on 1 lever under a fixed ratio (FR) 10 schedule of food presentation. The response period was signaled by illumination of a green stimulus light; 10 consecutive responses on the lever below the
illuminated light delivered a 300 mg banana-flavored food pellet. The active lever was counterbalanced across monkeys and remained the same for an individual monkey for the entire experiment. The response period ended after delivery of 10 food pellets or 5 min, whichever occurred first; any remaining time before the next cycle was a timeout. On separate occasions, saline or lorcaserin (1-10 mg/kg; i.g.) was administered 0, 60, or 165 min before a 2-hr session.

Control response rates were calculated for individual subjects by averaging response rates across cycles to obtain a mean for a session and then averaging across 10 sessions during which drug was not administered. Data represent the mean (± 1 SEM) rate of responding maintained by food pellets across 19, 5-min response periods. Because these data represent a composite of the effects of lorcaserin across three different experimental sessions with overlapping time points, data from individual subjects were averaged for these time points (i.e., 75, 90, 105, 120 and 180 min) and represents the mean of the two tests, with the grouped data representing a mean across subjects. Because data were collected repeatedly after administration of saline, and because they did not differ significantly as a function of time, data shown in the figure represent the mean (± 1SEM) of the 3 saline tests that preceded each of the 3 lorcaserin tests (i.e., rates for an individual monkey represent the mean of 3 tests). Two-factor ANOVA with repeated measures and post-hoc Holm-Sidak’s tests were used to determine if food-maintained responding varied as a function of lorcaserin dose or time. One-factor ANOVA with repeated measures and post-hoc Holm-Sidak’s tests were used to determine if the AUC values derived from the entire 285-min period varied as a function of the dose of lorcaserin administered.

**Cocaine Discrimination.** Three monkeys discriminated cocaine (0.178 mg/kg; i.v.) from saline while responding under an FR10 schedule of food presentation. Sessions were divided into 15-min cycles that included a 10-min timeout followed by a 5-min response period during which green stimulus lights were illuminated above the two levers. A session could be as short
as 1 cycle and as long as 6 cycles, depending upon the training or testing conditions that were in place. Monkeys received i.v. infusions during the first minute of each cycle, and 10 consecutive responses on the infusion-appropriate lever (1 lever was designated correct for cycles preceded by administration of the training dose of cocaine, whereas the other lever was designated correct for cycles preceded by administration of saline) delivered a food pellet. Response periods ended after delivery of 10 food pellets or 5 min, whichever occurred first; any remaining time before the next cycle was a timeout. During training sessions, cocaine could be administered prior to any of 6 cycles; however, that cycle was always the last cycle of the session. Saline was administered prior to any cycle that preceded administration of cocaine, or prior to all cycles if cocaine was not administered during that training session. Stimulus control was considered adequate for testing when the following criteria were satisfied for 5 consecutive or for 6 of 7 training sessions: at least 80% of the total responses for each cycle occurred on the infusion-appropriate lever and fewer than 10 responses (less than 1 FR) occurred on the incorrect lever before delivery of the first food pellet for each cycle. Thereafter, test sessions were conducted every third day as long as the testing criteria were satisfied during intervening training sessions; otherwise, training continued until the criteria were satisfied for 2 consecutive sessions.

Test sessions were identical to training sessions except that 10 consecutive responses on either lever delivered a food pellet. Either saline or lorcaserin (0.32-10 mg/kg; i.g.) was administered 75 min before 1 of 2 different types of test sessions. First, the time course for lorcaserin was determined by administering saline (i.v.) during each of 6 cycles, thereby examining the effects of lorcaserin 90-165 min after administration. Second, the ability of lorcaserin to alter the discriminative stimulus effects of cocaine was studied by obtaining cocaine dose-effect curves in a single session. During these sessions, saline was always administered during the first cycle, with increasing doses of cocaine administered during subsequent cycles. The first dose of cocaine was 0.032 mg/kg, which was administered during
the second cycle and 90 min after administration of saline or lorcaserin. Thereafter, the cumulative dose of cocaine increased each cycle in \( \frac{1}{4} \) log unit increments until at least 80% of responding occurred on the cocaine-appropriate lever or the rate of responding decreased to less than 20% of the control rate, whichever occurred first.

For individual monkeys discriminating cocaine, the percentage of total responses emitted on the drug lever during response periods was plotted as a function of dose. Control response rates are the mean of 10 training sessions during which only saline was administered and monkeys satisfied the testing criteria. For individual subjects, response rates were averaged first across cycles to obtain a mean for a session and then across sessions to obtain control rates. In subjects DU and LA, dose-response curves for cocaine administered alone or with lorcaserin were determined twice and are reported as the mean (± 1 SEM) whereas in subject MU each dose-response curve was determined once. Although the effects of each dose of lorcaserin alone were obtained 90-165 min after administration, the percentage of cocaine-lever responding obtained during the first cycle (response period occurred 85-90 min after lorcaserin administration) is shown. Discrimination data for an individual monkey were not included when response rates were less than 20% of control.

**Cocaine Self-Administration.** Five monkeys were trained to self-administer cocaine during daily 90-min sessions. Ninety minutes prior to each session, monkeys were briefly seated in chairs and given either saline or 1 of 4 doses of lorcaserin i.g. and returned to their home cage for the duration of the pretreatment period. During sessions, monkeys were placed in dark, ventilated, sound-attenuating chambers. One minute before the start of the session a loading infusion was administered to fill the catheter. The session began with a non-contingent priming infusion of 0.032 mg/kg cocaine which was delivered in conjunction with a 5-sec presentation of the cocaine-associated stimulus (i.e., a red light above the active lever). Immediately following the priming infusion, a green light was illuminated above the active lever signaling drug availability. Responding on the active lever (counterbalanced across monkeys) was reinforced
by infusions of 0.032 mg/kg cocaine and a 5-sec presentation of the associated stimuli according to a FR30 schedule of reinforcement. Each contingent infusion was followed by a 180-sec timeout during which the chamber was dark and responses were recoded but had no scheduled consequence. Once the timeout period had elapsed, the green light was illuminated and cocaine was again available for responding under the FR30:timeout 180-sec schedule of reinforcement. Responding on the inactive lever was recorded but had no scheduled consequence. Saline was occasionally substituted for 0.032 mg/kg/infusion cocaine, in order to ensure that responding was being maintained by the cocaine infusions.

Treatment with lorcaserin was initiated once responding for cocaine satisfied the stability criteria, defined as less than 20% difference in rates of responding across 3 consecutive sessions, with no increasing or decreasing trend. All 5 monkeys received each dose of lorcaserin (0.1-3.2 mg/kg; i.g.) for 14 consecutive days, with treatment occurring 90 min before the start of the self-administration session. Acute effects of lorcaserin were obtained on treatment day 1, with the effects of repeated lorcaserin treatment determined during the subsequent 13 (consecutive) days of treatment. On days in which lorcaserin was not administered, monkeys were treated daily with i.g. saline 90 min before cocaine (0.032 mg/kg/infusion) self-administration sessions. Evaluations of different doses of lorcaserin were separated by a minimum of 7 sessions and until responding satisfied the stability criteria.

Upon completion of the initial dose-response curve for the effects of lorcaserin on responding maintained by 0.032 mg/kg/infusion cocaine, four (BO, CH, PE, DA) of the original five monkeys (3 male and 1 female) were used to determine whether the ability of 3.2 mg/kg lorcaserin to decrease cocaine self-administration could be surmounted by increasing the unit-dose of cocaine available for infusion. Similar to the dose-response for lorcaserin, all monkeys were initially maintained on the FR30:timeout 180-sec schedule of reinforcement for 0.032 mg/kg/infusion cocaine. Treatment with 3.2 mg/kg lorcaserin began once responding satisfied stability criteria and continued for 15 consecutive sessions. During lorcaserin treatment, the
dose of cocaine available for infusion increased every five sessions from 0.032 to 0.1 to 0.32 mg/kg/infusion. Upon completion of the 15-day treatment period, saline replaced lorcaserin, and the dose of cocaine available for infusion decreased every five sessions from 0.32 to 0.1 to 0.032 mg/kg/infusion.

Acute effects of lorcaserin on cocaine self-administration represent the mean (± 1SEM) of the number of infusions and rates of responding obtained during the first day of treatment for each dose of lorcaserin (i.e., treatment day 1 of 14). The mean (± 1 SEM) of the number of infusions and rates of responding were also reported for the first day that saline replaced lorcaserin. Because baseline performance (sessions before lorcaserin treatment began and were preceded by i.g. saline) for individual monkeys did not differ across testing, these data were collapsed across tests, and are reported as the mean (± 1 SEM) for the baseline performance of 5 monkeys. One-factor ANOVA with repeated measures and post-hoc Holm-Sidak’s tests were used to determine if the number of cocaine infusions received or rates of responding varied as a function of lorcaserin dose. Data from repeated administration of lorcaserin represent the mean (± 1 SEM) of the number of infusions and rates of responding obtained for each day of treatment, as well as the 7 sessions that immediately followed lorcaserin treatment, with the gray shaded area representing ± 1 SEM of the mean number of infusions and rates of responding obtained from the 7 sessions that immediately preceded treatment with lorcaserin. Data from the 14-day treatment period were analyzed by one-factor (time) ANOVA with repeated measures and post-hoc Holm-Sidak’s tests to determine if the effect of lorcaserin varied as a function of treatment day. Data from the dose escalation studies represent the mean (± 1 SEM) number of infusions and rates of responding obtained from the 5 sessions comprising each treatment condition (e.g., saline pretreatment with 0.032 mg/kg/infusion cocaine, or lorcaserin pretreatment with 0.032 mg/kg/infusion cocaine). Two-factor ANVOA with repeated measures and post-hoc Holm-Sidak’s tests were used to
determine if the number of cocaine infusions or response rates varied as a function of lorcaserin pretreatment or the unit dose of cocaine.

**Drugs.** For pharmacokinetic studies, lorcaserin standard was obtained from LC Laboratories (Woburn, MA); cocaine and deuterated cocaine (dCOC) standards were purchased from Sigma Chemical Company (St. Louis, MO). HPLC grade methanol and acetonitrile were purchased from Fisher (Fair Lawn, NJ). All other reagents were HPLC grade or higher and were purchased from Sigma Chemical Company (St. Louis, MO). Cocaine HCl was generously provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD), and for behavioral studies was dissolved in physiologic saline and administered i.v. in a volume of 0.1 ml/kg. Lorcaserin HCl was purchased from MedChem Express (Princeton, NJ), dissolved in physiologic saline, and administered i.g. (nasogastric intubation) in a volume of 0.32 ml/kg. Monkeys were seated in restraint chairs and a lubricated nasogastric feeding tube was placed in the nostril and inserted until it reached the stomach at which point saline or lorcaserin was administered; the tube was then flushed with 10 ml of saline to insure the entire dose was administered.

**Results**

**Pharmacokinetics of Lorcaserin and Cocaine.** The inset panel in Figure 1 shows the pharmacokinetic profile of 3.2 mg/kg lorcaserin i.g. The concentration of lorcaserin in plasma reached a maximum of 80 ng/ml 60 min after administration. Plasma levels of lorcaserin were stable from 60 to 120 min. The pharmacokinetic profile of cocaine was evaluated twice in each monkey, once after saline and once after lorcaserin. The main panel in Figure 1 shows the pharmacokinetic profile of 1 mg/kg cocaine administered i.v. 90 min after i.g. administration of either saline or 3.2 mg/kg lorcaserin; lorcaserin did not affect the pharmacokinetic profile of 1 mg/kg cocaine i.v. (two-tailed, paired t-test, p=0.15, n=4).
Activity Monitoring and Directly Observable Behavior. Lorcaserin (i.e.) decreased activity in the home cage in a dose- and time-dependent manner (Figure 2; top, left panel; dose: F[5,15]=5.26; p=0.0055; time: F[5,15]=4.48; p=0.011). Although decreases in activity were apparent within 30 min of administration of 10 or 32 mg/kg lorcaserin, smaller doses (1 and 3.2 mg/kg) decreased activity only at the 240-min time point. A similar dose-dependent decrease in activity was observed when the AUC for the entire 240-min activity time course was analyzed (Figure 2; top, right panel), with activity being significantly lower following doses of 10 or 32 mg/kg lorcaserin compared with activity following administration of saline (dose: F[5,15]=5.49; p=0.0045).

Although most of the directly observable behaviors (22 of 24) were not systematically altered by lorcaserin, yawning and tongue movements were both increased, although the results with tongue movements failed to reach statistical significance. As shown in Figure 2 (middle, left panel), lorcaserin dose dependently increased the proportion of monkeys that yawned (dose: F[5,15]=5.49; p=0.0045). Similar to the effects of lorcaserin on activity, increased yawning was observed within the first 30-60 min after administration of lorcaserin, with all monkeys exhibiting yawning 60 and 120 min after receiving 3.2 mg/kg. Analysis of the AUCs derived from the entire 240-min yawning time-course assessment showed a similar dose-dependent increase in yawning (dose: F[5,15]=5.34; p=0.0051), with a dose of 3.2 mg/kg lorcaserin significantly increasing the proportion of monkeys yawning, compared with saline. Although failing to reach statistical significance, lorcaserin also induced unusual tongue movements (e.g., rolling, poking cheeks, etc.); this effect appeared to be dose-related in 2 monkeys, with 3 of 4 monkeys exhibiting some form of unusual tongue movement at larger doses. Reliability between raters of directly observable signs was determined using the κ statistic; for the two signs that were increased by lorcaserin (i.e., yawning and tongue movement), agreement between observers was found to be adequate with κ=0.89.
**Food-Maintained Responding.** In monkeys responding under an FR10 schedule of food presentation, control responding (± 1 SEM), averaged across 3 monkeys, was 1.57 ± 0.19 responses/sec. Lorcaserin decreased rates of responding in a dose- and time-dependent manner across the 19, 5-min response periods comprising the 285-min time course (Figure 3; left panel; dose: F[3,6]=21.0; p=0.0014; time: F[18,36]=2.34; p=0.015). A dose of 10 mg/kg produced a sustained decrease in responding from 45-285 min after drug administration whereas a dose of 3.2 mg/kg decreased responding only from 135-210 min after drug administration. Analysis of AUC values for the entire 285-min period revealed a dose-dependent decrease in response rate (F[3,6]=18.7; p=0.0019), with significant reductions observed after administration of 3.2 and 10 mg/kg lorcaserin (Figure 3; right panel).

**Cocaine Discrimination.** Mean control response rate was 1.17 ± 0.23 responses/sec in 3 monkeys discriminating cocaine while responding under an FR10 schedule of food presentation. All 3 monkeys responded exclusively on the saline-associated lever after receiving saline and responded progressively more on the drug-associated lever with increasing doses of cocaine (Figure 4). A cumulative dose of 0.1 mg/kg of cocaine produced responding exclusively on the cocaine-associated lever in one monkey (LA) and increased response rates in that monkey (data not shown). A larger cumulative dose of cocaine (0.32 mg/kg) was needed to produce responding exclusively on the cocaine-associated lever in the other two monkeys (MU and DU), and this dose decreased response rates in both monkeys. Figure 4 also shows data for lorcaserin alone (0.32, 1, 3.2, and 5.6 mg/kg; i.g.) administered 75 min before the first of 6 test cycles (85 min before the start and 90 min before the end of the first response period); in subsequent cycles monkeys responded predominantly on the saline-associated lever (105-165 min) with the average cocaine-lever responding never exceeding 35%. When administered 75 min before the first cycle, the smallest dose of lorcaserin increased response rates to 130% and 117% of control in DU and MU, respectively, and decreased response rates to 68% of control in LA; the effects of larger doses on response rates varied across monkeys and across doses.
(data not shown). In subsequent cycles, response rates varied as time since lorcaserin administration increased, although the changes were not consistent across monkeys or across cycles.

When administered 75 min before a cocaine test session (90 min before the first dose of cocaine), 1 mg/kg lorcaserin shifted the cocaine dose-response curve downward or rightward in 2 of the 3 monkeys (MU and LA) without affecting the curve in a third monkey (DU); in DU, evidence for attenuation of the discriminative stimulus effects of cocaine by this dose of lorcaserin was apparent only at the largest dose of cocaine (i.e., the dose that produced exclusive choice of the cocaine-appropriate lever under control conditions). A dose of 1 mg/kg lorcaserin decreased responding in DU and had little effect on response rates in the other two monkeys; under these treatment conditions, cocaine decreased rates in MU and DU and did not change rates in LA up to the dose that produced responding on the cocaine-associated lever. A larger dose of lorcaserin (3.2 mg/kg; data not shown) markedly decreased response rates in all monkeys; the rate-decreasing effect was most evident in MU and on 1 of 2 occasions in DU, when response rates were decreased to <20% of control. In LA, 3.2 mg/kg lorcaserin decreased responding to 0.32 responses/sec (67% of control), and in the presence of this dose of lorcaserin, cocaine produced no more than 24% responding on the cocaine-associated lever up to a dose that eliminated responding (0.56 mg/kg).

Cocaine Self-Administration. During baseline sessions preceded by saline (i.g.), 0.032 mg/kg/infusion cocaine maintained high rates of responding (average of 1.31 ± 0.27 responses/sec) in all monkeys, with monkeys receiving an average of 25.9 ± 0.9 infusions per session (Figure 5; shaded areas). Upon substituting saline for cocaine, responding decreased in all monkeys (0.09 ± 0.03 responses/sec) with an average of 9.8 ± 1.8 infusions of saline received during the first session in which saline was available (Figure 5; area between dotted lines). When administered acutely 90 min before the session, lorcaserin decreased the number of cocaine infusions received (F[4,16]=11.37; p=0.0001) and rates of responding (F[4,16]=7.03;
p=0.0018) in a dose-dependent manner, with significant decreases in both measures observed at 3.2 mg/kg lorcaserin (filled symbols, Figure 5).

Similar to the effects obtained with the acute administration of lorcaserin, daily administration of 3.2 mg/kg lorcaserin decreased the number of cocaine infusions received as well as response rate across a 14-day treatment period (Figure 6, bottom two panels); daily administration of smaller doses of lorcaserin (0.1-1.0 mg/kg/day) did not systematically alter cocaine self-administration (Figure 6, top six panels). One-factor ANOVA failed to reveal any significant difference in the number of infusions received or response rates across the 14-day treatment period for any dose of lorcaserin. Upon discontinuation of daily treatment (i.e., 24 hr after the last dose of lorcaserin), both rates of responding and the number of cocaine infusions received returned to control (i.e. prior to lorcaserin treatment) values where they remained for at least 7 sessions (Figure 6, filled symbols).

To assess whether the effects of lorcaserin in decreasing cocaine self-administration could be surmounted by larger unit doses of cocaine, on a separate occasion, monkeys were again treated with 3.2 mg/kg lorcaserin 90 min before daily sessions for 15 days. However, unlike the initial study that examined only 0.032 mg/kg/infusion cocaine, the unit dose of cocaine increased by ½ log unit every 5 sessions, from 0.032 to 0.1 to 0.32 mg/kg/infusion. When saline was administered 90 min before sessions, increasing the unit dose of cocaine produced a dose-dependent decrease in the number of infusions and response rates (gray circles, Figure 7).

There was a main effect of lorcaserin on the number of cocaine infusions received, and this effect was dependent on the unit dose of cocaine (Figure 7; lorcaserin dose: F[1,3]=89.57; p=0.0025; cocaine dose: F[2,6]=15.09; p=0.046). Although lorcaserin decreased average rates of responding maintained by 0.032 or 0.1 mg/kg/infusion cocaine, these decreases were not statistically significant (possibly due to the variability among monkeys in rates of cocaine-maintained responding under baseline conditions).
Discussion

The results of the present studies demonstrate that lorcaserin decreases ongoing cocaine self-administration, that the effects of lorcaserin on cocaine self-administration are not surmountable when larger unit doses of cocaine are made available, that tolerance to lorcaserin does not develop over 14 days of once-daily treatment, and that attenuation of the effects of cocaine by lorcaserin is likely due to pharmacodynamic, and not pharmacokinetic, mechanisms. Importantly, these effects of lorcaserin were observed at a dose that also produced yawning, a putative 5-HT$_{2C}$ receptor-mediated effect, but smaller than doses that were required to produce large disruptions in food-maintained responding or spontaneous activity. Taken together, these findings in nonhuman primates suggest that lorcaserin, a drug with agonist activity at 5-HT$_{2C}$ receptors that was recently approved by the FDA for the treatment of obesity, might also be effective for treating cocaine abuse and addiction.

One goal of these studies was to characterize the behavioral effects of lorcaserin administered i.g., from ineffective doses to doses that significantly alter behavior. Lorcaserin was well tolerated up to a dose of 32 mg/kg and produced dose- and time-dependent effects on spontaneous activity, yawning, tongue movements, and food-maintained responding. Yawning began to emerge within 30 min of administration of 3.2 mg/kg, with yawning observed in all monkeys 60 and 120 min after drug administration. The time-course for yawning induced by 3.2 mg/kg lorcaserin closely matched plasma drug concentrations after administration of this dose, including the lack of behavioral effect and disappearance of lorcaserin in blood 24 hr after administration. These findings are consistent with a role for 5-HT$_{2C}$ receptors in mediating the induction of yawning by drugs acting on 5-HT mechanisms (Collins and Eguibar, 2010; Pomerantz et al, 1993). In addition to yawning, larger doses of lorcaserin also decreased spontaneous activity and food-maintained responding in all monkeys and produced oral-facial stereotypies (i.e., abnormal tongue movements) in a subset of monkeys. Taken together, these findings suggest that i.g. administration of 3.2 mg/kg lorcaserin results in plasma drug...
concentrations that are sufficient to produce putative 5-HT$_{2C}$ receptor-mediated effects without markedly disrupting spontaneous or operant behavior.

Based on the behavioral and pharmacokinetic profiles of lorcaserin, a 90-min pretreatment time was used to evaluate the effectiveness of lorcaserin to alter behavioral effects of cocaine that are related to its abuse, including discriminative stimulus effects and reinforcing effects. When administered alone, over a wide range of doses lorcaserin failed to substantially increase responding on the cocaine-associated lever. Although these are the first studies to evaluate lorcaserin in animals trained to discriminate cocaine, they are in agreement with previous studies in rats discriminating MK 212, a less selective 5-HT$_{3/2C2B}$ receptor agonist (Callahan and Cunningham, 1995) and suggest that 5-HT$_{2C}$ receptor agonists, such as lorcaserin, do not share discriminative stimulus effects with cocaine. When administered before cocaine, 1 mg/kg lorcaserin shifted the cocaine dose-response curve downward or rightward in 2 of 3 monkeys. Although modest, these shifts in dose-response functions are similar in magnitude to results reported for combinations of MK 212 and cocaine in rats and suggest that lorcaserin does not enhance, but rather attenuates, the discriminative stimulus effects of cocaine after i.g. administration. Although these studies were not designed to elucidate the mechanism(s) by which lorcaserin inhibits the effects of cocaine, given that lorcaserin did not alter the pharmacokinetic properties of cocaine, it is likely that these effects are mediated by its actions at 5-HT$_{2C}$ receptors. Consistent with this notion is the finding that 5-HT$_{2C}$ receptor antagonists (e.g., SDZ SER-082) can enhance the discriminative stimulus effects of cocaine (Filip et al, 2006) and that 5-HT$_{2C}$ receptor agonists and antagonists appear to modify the effects of cocaine by inhibiting and enhancing dopamine transmission, respectively (for reviews see Bubar and Cunningham, 2006, 2008).

Unlike typical pharmacotherapies for drug abuse, which often target the primary site of action of the abused drug (e.g., methadone and naltrexone, both acting at mu opioid receptors, for treating opioid addiction), it is also possible to reduce abuse-related effects through actions
at sites other than the primary site of action of the abused drug. Because of their role in modulating dopamine neurotransmission, 5-HT$_{2C}$ receptors have received considerable attention as a potential target for treating cocaine abuse (Higgins and Fletcher, 2015; Higgins et al, 2013a; Howell and Cunningham, 2015); however, the relative lack of highly selective compounds has limited a full characterization of the effectiveness of 5-HT$_{2C}$ receptor agonists to reduce cocaine self-administration. Lorcaserin is 18- and 100-fold selective for 5-HT$_{2C}$ over 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors, respectively (Thomsen et al, 2008). In the present studies, lorcaserin, dose dependently decreased rates of responding and number of cocaine infusions received, with significant decreases observed for both measures following i.g. administration of 3.2 mg/kg lorcaserin. It is unlikely that this suppression of cocaine self-administration reflects a leftward shift in the cocaine dose-response curve insofar as lorcaserin shifts the cocaine self-administration dose-response curve to the right in monkeys responding under a progressive ratio schedule (unpublished observation). Taken together, these findings are consistent with the acute effects of the 5-HT$_{2C}$ receptor agonists WAY 163909 and Ro 60-0175 in rats and squirrel monkeys, respectively (Cunningham et al, 2011; Manvich et al, 2012; Rüedi-Bettschen et al., 2015), and provide further support for the notion that targeting 5-HT$_{2C}$ receptors could provide an effective strategy for treating cocaine addiction.

In addition to assessing the acute effects of lorcaserin, the current studies also evaluated the effectiveness of lorcaserin across 14 days of repeated treatment, as well as whether the effectiveness of lorcaserin is surmounted when larger unit doses of cocaine are made available. Lorcaserin not only retained its effectiveness to decrease cocaine self-administration across the entire 14-day treatment period, but it also continued to decrease cocaine self-administration even when larger unit doses of cocaine were available. A similar downward shift in the dose-response curve for cocaine self-administration has been reported for squirrel monkeys treated acutely with Ro 60-0175 (Manvich et al, 2012). These findings are important because they suggest that individuals would not become tolerant to the effects of
lorcaserin during long-term treatment, and that they would be unlikely to overcome the effects of lorcaserin by taking more cocaine.

In summary, the current studies characterized the behavioral and pharmacokinetic profile of the clinically used 5-HT\textsubscript{2C} receptor agonist, lorcaserin, and evaluated its effectiveness to reduce the discriminative stimulus and reinforcing effects of cocaine in rhesus monkeys. The main findings of these studies are as follows: 1) lorcaserin dose dependently increased 5-HT\textsubscript{2C} receptor-mediated behaviors over a time course that closely matched its pharmacokinetic profile and at doses smaller than those that disrupted other spontaneous or operant behavior; 2) acute administration of lorcaserin attenuated the discriminative stimulus effects of cocaine in 2 of 3 monkeys and dose dependently inhibited cocaine self-administration in all monkeys, at doses smaller than those that produced comparable reductions in food-maintained responding; and 3) lorcaserin retained its effectiveness to decrease cocaine self-administration over a range of cocaine doses and over 14 days of once-daily treatment. Together, these findings provide further support for the hypothesis that activation of 5-HT\textsubscript{2C} receptors can decrease abuse-related effects of cocaine and suggest that lorcaserin, a drug with 5-HT\textsubscript{2C} receptor agonist properties that is approved by the FDA, should be evaluated further as a candidate medication for cocaine abuse and addiction.
Acknowledgments

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Authorship Contributions

Participated in research design: Collins, Gerak, Javors, France

Conducted experiments: Gerak, Javors

Contributed new reagents or analytic tools:

Performed data analysis: Collins, Gerak, Javors

Wrote or contributed to the writing of the manuscript: Collins, Gerak, Javors, France
References:


Footnotes

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Legends for Figures

Figure 1. Plasma concentration of lorcaserin alone, cocaine alone, and cocaine in combination with lorcaserin. Lorcaserin and cocaine were administered at doses of 3.2 mg/kg i.g. and 1 mg/kg i.v., respectively. The inset panel shows the pharmacokinetic profile of lorcaserin, administered alone and immediately after the collection of the first blood sample (0 min), with each filled circle representing the mean (± 1 SEM) plasma concentration of lorcaserin in ng/ml (n=4). The main panel shows the pharmacokinetic profile of cocaine 90 min after administration of either saline (gray circles) or lorcaserin (open squares); cocaine was administered immediately after the collection of the first blood sample (0 min). The symbols represent the mean (± 1 SEM) plasma concentration of cocaine in ng/ml (n=3).

Figure 2. Acute effects of lorcaserin on activity and directly observable behaviors. A total of 24 directly observable behaviors were monitored (see Table 1) before as well as 5, 15, 30, 60, 120, and 240 min after i.g. administration of lorcaserin (0.32-32 mg/kg) or saline (n=4). Activity data (top left panel) represent the mean (± 1 SEM) number of activity counts observed during 5-min immediately following each observation period. For yawning (middle left panel) and tongue movements (bottom left panel), the data represent the proportion of monkeys exhibiting each behavior during the 30-sec period beginning at each time point. AUC was calculated for each time course, with the mean (± 1 SEM) shown in the right panels. Data obtained following saline (mean ± 1 SEM) are represented by the gray-shaded areas. Black-filled symbols represent points that were significantly different from saline (p<0.05), as determined by post-hoc Holm-Sidak’s tests.

Figure 3. Acute effects of lorcaserin on food-maintained responding. On different occasions, saline or lorcaserin (1-10 mg/kg; i.g.) was administered 0, 60, or 165 min before 2-hr sessions
comprising 15-min cycles (n=3). The mean (± 1 SEM) rates of responding during the last 5 min of each cycle are shown in the left panel, with the mean (± 1 SEM) AUC for each time course shown in the right panel. Data obtained following saline (mean ± SEM) are represented by the gray-shaded areas. Black-filled symbols represent points that were significantly different from saline (p<0.05), as determined by post-hoc Holm-Sidak’s tests.

Figure 4. Acute effects of lorcaserin in 3 monkeys (MU, DU, and LA) discriminating 0.178 mg/kg cocaine i.v. from saline. Gray-filled circles represent the mean (± 1 SEM) percent of responding that occurred on the cocaine-appropriate lever during test sessions in which saline was administered 90 min before saline (S) or the first dose of cocaine. Open squares represent the percent responding on the cocaine-associated lever during tests in which lorcaserin was administered before sessions (see Methods for details). Open circles represent the mean (± 1 SEM) percent responding on the cocaine-associated lever during tests in which 1 mg/kg lorcaserin was administered 75 min before cumulative doses of cocaine.

Figure 5. Acute effects of lorcaserin on cocaine self-administration. Plotted are the mean (± 1 SEM) number of infusions received of 0.032 mg/kg cocaine (left panel) and mean (± 1 SEM) rates of responding (right panel) during sessions in which lorcaserin (0.1-3.2 mg/kg; i.g.) was administered as a 90-min pretreatment (n=5). Data obtained during baseline cocaine self-administration sessions preceded by saline (mean ± SEM) are represented by the gray-shaded areas. Black-filled symbols represent values that are significantly different from baseline (p<0.05), as determined by post-hoc Holm-Sidak’s tests. Dotted lines represent the mean (± 1 SEM) of the number of infusions and the rate of responding during a single session in which saline was substituted for cocaine.
Figure 6. Effects of repeated lorcaserin administration on cocaine self-administration. Plotted are the mean (± 1 SEM) number of infusions received of 0.032 mg/kg cocaine (left panels; n=5) and the mean (± 1 SEM) rates of responding (right panels) during 14 consecutive sessions in which lorcaserin (0.1-3.2 mg/kg; i.g.) was administered as a 90-min pretreatment (open symbols); gray-filled symbols represent data obtained during the block of 7 consecutive sessions that immediately followed lorcaserin treatment for which saline was administered 90 min beforehand. Gray-shaded areas represent the mean (± 1 SEM) for the average number of cocaine infusions (left panels) and rates of responding (right panels) during the 7 baseline sessions that immediately preceded lorcaserin treatment.

Figure 7. Effects of increasing the unit-dose of cocaine available for self-administration during 15 consecutive sessions, each preceded by administration of 3.2 mg/kg lorcaserin i.g. Gray-filled circles represent the mean (± 1 SEM) for the average number of cocaine infusions received and rates of responding during 5 consecutive sessions in which saline was administered as a 90- min pretreatment (n=4). Open squares represent the mean (± 1 SEM) for the average number of cocaine infusions and rates of responding during 5 consecutive sessions preceded by 3.2 mg/kg lorcaserin (90-min pretreatment). Black-filled symbols represent points that were significantly different from baseline (p<0.05), as determined by post-hoc Holm-Sidak’s tests.
### Table 1. Descriptions of directly observable behaviors.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>eyes closed</td>
<td>3 second minimum</td>
</tr>
<tr>
<td>stereotypy</td>
<td>repetitive movements (entire body, just head, often hand licking/gnawing)</td>
</tr>
<tr>
<td>chewing</td>
<td>chewing movements of the mouth</td>
</tr>
<tr>
<td>licking</td>
<td>repetitively lick cage or themselves</td>
</tr>
<tr>
<td>tongue movements</td>
<td>tongue rolling inside mouth from cheek to cheek</td>
</tr>
<tr>
<td>tongue protrusion</td>
<td>sticking tongue out of the mouth minimum of 3 sec</td>
</tr>
<tr>
<td>biting</td>
<td>on cage</td>
</tr>
<tr>
<td>vocalization</td>
<td>barking or grunting</td>
</tr>
<tr>
<td>tremor (body)</td>
<td>shaking or trembling of body</td>
</tr>
<tr>
<td>tremor (extremities)</td>
<td>shaking or trembling digits or limbs</td>
</tr>
<tr>
<td>ptosis</td>
<td>drooping eyelid</td>
</tr>
<tr>
<td>hypersalivation</td>
<td>excessive salivation</td>
</tr>
<tr>
<td>retching</td>
<td>dry heaving, not actually vomiting</td>
</tr>
<tr>
<td>emesis</td>
<td>food that has been eaten comes out from the mouth</td>
</tr>
<tr>
<td>rhinorrhea</td>
<td>runny nose</td>
</tr>
<tr>
<td>lacrimation</td>
<td>secretion of tears</td>
</tr>
<tr>
<td>piloerection</td>
<td>hair standing up</td>
</tr>
<tr>
<td>scratching</td>
<td>fingers or toes rapidly contacting and sliding on a part of the body</td>
</tr>
<tr>
<td>yawning</td>
<td>mouth open widely and vertically without making a sound</td>
</tr>
<tr>
<td>tail wagging</td>
<td>movement of the tail</td>
</tr>
<tr>
<td>loss of balance</td>
<td>unstable movement</td>
</tr>
<tr>
<td>aggressiveness</td>
<td>threaten observer or parts of cage, vocalize by barking or grunting</td>
</tr>
<tr>
<td>skin color</td>
<td>change from normal skin color</td>
</tr>
<tr>
<td>posture</td>
<td>contorted and neck extension</td>
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</tbody>
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Figure 1

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Figure 3

Saline

Lorcaserin (mg/kg; IG)

○ 1  □ 3.2  △ 10

Rate (Resp / Sec)

Time (min)

AUC

Lorcaserin (mg/kg; IG)
Figure 4

- MU
- DU
- LA

% Cocaine-Appropriate Responding

Dose (mg/kg)

○ Cocaine (IV)
□ Lorcaserin (IG)
○ 1 mg/kg Lorcaserin (IG) + Cocaine (IV)
<table>
<thead>
<tr>
<th>Saline</th>
<th>Lorcaserin (mg/kg; IG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○ 0.1</td>
</tr>
<tr>
<td></td>
<td>□ 0.32</td>
</tr>
<tr>
<td></td>
<td>○ 1</td>
</tr>
<tr>
<td></td>
<td>□ 3.2</td>
</tr>
</tbody>
</table>

Figure 5

Infusions / 90 min

Rate (Resp / Sec)

Lorcaserin (mg/kg; IG)
Figure 6

- **0.1 mg/kg/day Lorcaserin**
  - Infusions / 90 min
  - Baseline (saline PT)

- **0.32 mg/kg/day Lorcaserin**
  - Infusions / 90 min
  - Baseline (saline PT)

- **1.0 mg/kg/day Lorcaserin**
  - Infusions / 90 min
  - Baseline (saline PT)

- **3.2 mg/kg/day Lorcaserin**
  - Infusions / 90 min
  - Baseline (saline PT)

Rate (Resp / Sec)

Consecutive Sessions

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Figure 7

<table>
<thead>
<tr>
<th>Pretreatment (IG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (○)</td>
</tr>
<tr>
<td>3.2 mg/kg Lorcaserin (□)</td>
</tr>
</tbody>
</table>

- Infusions / 90 min
- Rate (Resp / Sec)

Cocaine (mg/kg/inf)