Suppression of cocaine relapse-like behaviors upon pimavanserin and lorcaserin co-administration

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HIGHLIGHTS

- Lorcaserin, but not pimavanserin, suppresses cocaine self-administration in rats.
- Repeated pimavanserin or lorcaserin sustains reduced cocaine-seeking behavior.
- Low doses of pimavanserin *plus* lorcaserin reduce cocaine-seeking during abstinence.

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ABSTRACT

Cocaine use disorder (CUD) is a major public health challenge for which there are no pharmacotherapeutics approved by the United States Food and Drug Administration (FDA). The propensity to relapse in CUD involves several vulnerability factors including sensitivity to cues associated with cocaine-taking. Serotonin (5-hydroxytryptamine, 5-HT) neurotransmission, particularly through the 5-HT$_{2A}$ receptor (5-HT$_{2A}$R) and 5-HT$_{2C}$ receptor (5-HT$_{2C}$R), is mechanistically linked to cocaine-seeking in preclinical models. In the present experiments, we employed self-administration assays in male rats to investigate whether acute and/or repeated administration of the FDA-approved selective 5-HT$_{2A}$R antagonist/inverse agonist pimavanserin, selective 5-HT$_{2C}$R agonist lorcaserin or their combination would alter cocaine intake and/or cocaine-seeking behavior. We found that acute administration of lorcaserin, but not pimavanserin, attenuated cocaine intake while pimavanserin *plus* lorcaserin did not impact cocaine self-administration. In contrast, 10-days of repeated administration of pimavanserin, lorcaserin, or pimavanserin *plus* lorcaserin during forced abstinence from cocaine self-administration, blunted cocaine-seeking, similar to the acute administration of each ligand. Taken together, these data reveal the efficacy of repeated treatment with pimavanserin plus lorcaserin to attenuate factors important to relapse-like behaviors in rodent models of CUD.

1. Introduction

Rising numbers of cocaine overdose deaths are linked to the surge in cocaine availability and the ongoing opioid overdose crisis (McCall Jones et al., 2017), with the alarming news of a 61% increase in young adults (18–25 yo) initiating cocaine use in the United States (2013–2015) and the highest cocaine overdoses reported since 2006 (Hughes et al., 2016). In 2017, an estimated 2.2 million persons aged 12 or older were current users of cocaine with one million new initiates; ~966,000 were diagnosed with cocaine use disorder (CUD) (Center for Behavioral Health Statistics and Quality, 2018), a progressively debilitating acquired disorder characterized by disruption of drug and natural reward processing and higher order cognitive function (Koob and Volkow, 2016). Core deficits in the ability to inhibit behavior, whether a premorbid neurodevelopmental trait or an acquired consequence of chronic cocaine use, coupled with increased attentional bias toward drug reward stimuli factor into relapse vulnerability in CUD individuals (Anastasio et al., 2014a; Liu et al., 2012).

Cocaine generates its reinforcing effects via stimulation of dopamine (DA) mesolimbic efflux; DA and glutamate transmission have

well-described roles in encoding drug reward and saliency for drug-associated cues at the level of mesocorticolimbic circuit (Koob and Volkow, 2016). In light of unsuccessful clinical trials for direct dopaminergic agents to reduce relapse (Indave et al., 2016; Kohut and Bergman, 2017; Minozzi et al., 2015), modulation of other neurotransmitters that may themselves regulate DA or otherwise regulate behavior are of interest in the development of pharmacotherapies for CUD. Importantly, preclinical studies implicate serotonin (5-HT) as an influential regulator of neural circuit dynamics involved in the substance use disorder (SUD) cycle, in part via direct and/or indirect interactions with DA systems (Lucki, 1998; Soubrié, 1986). The actions of 5-HT in neurons are transduced by 14 subtypes of 5-HT receptors (5-HT$_X$Rs) in which the 5-HT$_{2A}$R and 5-HT$_{2C}$R support a modulatory role in relapse-like behaviors in rodents (for reviews, (Cunningham and Anastasio, 2014; Howell and Cunningham, 2015)). Selective inhibition of the 5-HT$_{2A}$R (e.g., by M100907) or activation of the 5-HT$_{2C}$R (e.g., by WAY163909), acutely, consistently suppresses both cue- and cocaine-evoked reinstatement (cocaine-seeking) after a period of cocaine self-administration and extinction (Cunningham et al., 2011; Fletcher et al., 2008; Neisewander and Acosta, 2007; Nic Dhonnchadha et al., 2009). Moreover, acute administration of M100907 *plus* WAY163909 curbs cocaine-induced hyperactivity (replicated by an independent laboratory) (Pockros et al., 2012), as well as cue-evoked and cocaine-primed reinstatement (Cunningham et al., 2013). These provocative outcomes prompted the hypothesis that 5-HT$_{2A}$R and 5-HT$_{2C}$R exhibit a special relationship, rooted in key nodes of SUD neurocircuitry, which control cocaine-associated behaviors (for reviews, (Cunningham and Anastasio, 2014; Howell and Cunningham, 2015)).

The emergence of clinically available, selective 5-HT$_{2A}$R and 5-HT$_{2C}$R ligands presents new pharmacotherapeutic prospects for the treatment of CUD. Pimavanserin (Nuplazid®) is an FDA-approved, potent and selective 5-HT$_{2A}$R antagonist (Vanover et al., 2006) approved for treatment of psychosis in Parkinson's disease (Sahli and Tarazi, 2018) and also displays inverse agonist activity in functional assays (Vanover et al., 2006), a property inherent in many such ligands that affect basal and constitutive activity of the 5-HT$_{2A}$R (Aloyo et al., 2009). Pimavanserin exhibits robust activity in a variety of preclinical models of CNS disease (McFarland et al., 2011; Vanover et al., 2006, 2008) including cocaine associated relapse-like behaviors (Sholler et al., 2019). Lorcaserin (Belviq®) is a 5-HT$_{2C}$R agonist that was until recently FDA-approved for the treatment of obesity (Gustafson et al., 2013). Lorcaserin suppresses cocaine self-administration (Collins et al., 2016) and reinstatement of drug-seeking for cocaine in preclinical models (Gerak et al., 2016, 2019; Harvey-Lewis et al., 2016), effects reversed by pretreatment with the selective 5-HT$_{2C}$R antagonist SB242084 (Collins et al., 2016; Gerak et al., 2016, 2019; Harvey-Lewis et al., 2016). In the present study, we tested the hypothesis that pimavanserin, lorcaserin, or their combination would suppress cocaine self-administration and cocaine-seeking behavior in rats and coupled these analyses with investigation of potential pharmacokinetic interactions between pimavanserin and lorcaserin. In addition, we tested the hypothesis that repeated, daily treatment with combined administration of pimavanserin *plus* lorcaserin would blunt cocaine-seeking behavior similar to acute administration. Such findings would support the novel pharmacotherapeutic prospect that pimavanserin alone or in combination with lorcaserin may support behavioral recovery in CUD.

2. Materials and methods

2.1. Animals

Behaviorally and surgically-naïve male Sprague-Dawley rats (n = 156 total; Envigo, Indianapolis, IN) weighing 250–325 g at the start of experiments were acclimated for seven days in a colony room maintained at a constant temperature (21–23 °C) and humidity (45–50%) on a 12-h light-dark cycle (lights on 0600–1800 h). All rats

for behavioral analyses were implanted with jugular catheters in our laboratory. For pharmacokinetic analyses, an additional cohort of male Sprague-Dawley rats (n = 63) were obtained with jugular vein catheters fitted with a PinPort™ (Instech Laboratory, Plymouth Meeting, PA) implanted by the supplier. Rats were housed two/cage, except for PinPort™ surgically prepared rats which were housed one/cage; all rats were handled daily throughout the study. Food and water were available *ad libitum* throughout all phases of the studies. All experiments were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (2011) and with approval from the University of Texas Medical Branch Institutional Animal Care and Use Committee.

2.2. Drugs

(−)-Cocaine (National Institute on Drug Abuse, Research Triangle Park, NC) and lorcaserin hydrochloride (Trylead Chemical Technology Co., Ltd., Hangzhou, China) were dissolved in 0.9% NaCl (vehicle employed for comparison to cocaine and lorcaserin). Pimavanserin (Trylead Chemical Technology, Hangzhou, China) was dissolved in 0.9% NaCl with 1M HCl and brought to a pH of 5.5 using 1M NaOH (vehicle employed for comparison to pimavanserin). SB242084 [6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy) pyrid-5-yl]carbamoyl] indolinedihydrochloride; Sigma Chemical Co., St. Louis, MO] was dissolved in 0.9% NaCl containing 10 mM citric acid (Sigma Chemical Co.) and 8% 2-hydroxypropyl-β-cyclodextrin (Trappsol® Hydroxpropyl Beta Cyclodextrin, Pharmaceutical Grade, Cyclodextrin Technologies Development Inc., High Springs, FL) with the final pH of the solution adjusted to 5.6.

2.3. Assessment of cocaine self-administration and cocaine-seeking behavior

2.3.1. Surgery

Rats (n = 156) were implanted with intravenous catheters with back mounts under anesthesia with a cocktail containing 8.6 mg/kg of xylazine, 1.5 mg/kg of acepromazine, and 43 mg/kg of ketamine in bacteriostatic saline and allowed to recover for 5–7 days before initiation of self-administration training (Anastasio et al., 2014a, 2014b, 2013; Neelakantan et al., 2017; Sholler et al., 2019). Catheter patency was maintained by daily flushes with a solution of 0.1 ml bacteriostatic saline containing heparin sodium (10 U/ml; American Pharmaceutical Partners, East Schaumburg, IL), streptokinase (0.67 mg/ml; Sigma Chemical), and ticarcillin disodium (66.67 mg/ml; Research Products International, Mt. Prospect, IL) immediately following daily cocaine self-administration sessions.

2.3.2. Self-administration training

Standard operant conditioning chambers (Med-Associates, Inc., St. Albans, VT) housed in ventilated, sound-attenuating cubicles with fans (Med-Associates, Inc.) were utilized for cocaine self-administration studies. Each chamber was equipped with a pellet receptacle flanked by two retractable response levers, a stimulus light above each response lever, and a house light opposite the levers. Cocaine infusions were delivered via syringes attached to infusion pumps (Med-Associates, Inc.) located outside the cubicles. The infusion pumps were connected to liquid swivels (Instech, Plymouth Meeting, PA) fastened to the catheters via polyethylene 20 tubing encased inside a metal spring leash (Plastics One, Roanoke, VA).

Freely-fed rats were trained to lever press for cocaine infusions using established methods (Anastasio et al., 2014a, 2014b, 2013; Neelakantan et al., 2017; Sholler et al., 2019). Cocaine self-administration training consisted of daily 180-min sessions during which rats were trained to press the active lever to obtain an infusion on a fixed ratio (FR) 1 schedule of reinforcement before progressing to a final FR 5 schedule of reinforcement. Schedule completions on the active lever resulted in delivery of a cocaine infusion over a 6-sec period paired

simultaneously with illumination of the house and stimulus lights and activation of the infusion pump (discrete cue complex); inactive lever presses produced no scheduled consequences. Following reinforcer delivery, the stimulus light and infusion pump were inactivated; the house light remained on for an additional 20 s to indicate a timeout period during which lever presses had no scheduled consequences.

2.3.3. Assessment of pimavanserin, lorcaserin, or the combination on cocaine self-administration

The efficacy of pimavanserin, lorcaserin, or their combination to alter cocaine intake was assessed in rats (n = 48) trained to self-administer cocaine (0.75 mg/kg/0.1 ml infusion) to stability. Once acquired, rats were transitioned to a lower dose of cocaine (0.25 mg/0.1 ml infusion) and maintained on this dose throughout testing. Rats readily acquired cocaine self-administration in daily 180 min sessions to stability and displayed < 10% variation in the number of infusions received (i.e., cocaine intake) (data not shown) per our previous publications (Anastasio et al., 2014a, 2014b, 2013; Sholler et al., 2019). Tests were administered in a pseudorandomized order with a minimum of two intervening sessions of cocaine self-administration to assure stability of baseline responding. Fourteen rats failed to meet acquisition/maintenance criteria or lost catheter patency and were excluded from subsequent analyses (pimavanserin efficacy: n = 9/12 analyzed; lorcaserin efficacy: n = 13–14/24 analyzed; pimavanserin *plus* lorcaserin efficacy: n = 12/12 analyzed).

Pretreatment with the 5-HT$_{2C}$R antagonist SB242084 was employed to confirm that the effect of lorcaserin on cocaine self-administration was 5-HT$_{2C}$R specific. Pimavanserin and lorcaserin were administered s.c. and SB242084 was administered i.p.; vehicles were dosed at 1 ml/kg. Pretreatment times were consistent with numerous rat cocaine self-administration and reinstatement studies employing a 15 min pretreatment for lorcaserin and other 5-HT$_{2C}$R agonists (Cunningham et al., 2011, 2013; Fletcher et al., 2008; Neisewander and Acosta, 2007) and 30 min pretreatment for pimavanserin and other 5-HT$_{2A}$R antagonists/inverse agonists (Cunningham et al., 2013; Nic Dhonnchadha et al., 2009; Sholler et al., 2019).

 i. Rats were injected with vehicle or pimavanserin (0.3–3 mg/kg; 30 min) prior to the start of the cocaine self-administration session in a within-subjects design.
 ii. Rats were injected with vehicle, lorcaserin (0.25–1 mg/kg; 15 min), or the 5-HT$_{2C}$R antagonist SB242084 (0.5 mg/kg; 30 min) *plus* lorcaserin (1 mg/kg; 15 min) prior to the start of the cocaine self-administration session in a within-subjects design.
 iii. Rats were injected with vehicle, pimavanserin (0.5 mg/kg; 30 min), lorcaserin (0.5 mg/kg; 15 min) or the combination of pimavanserin *plus* lorcaserin prior to the start of the cocaine self-administration session in a within-subjects design.

2.3.4. Assessment of pimavanserin, lorcaserin, or the combination on cocaine-seeking behavior

The efficacy of acute or repeated pimavanserin, lorcaserin, or their combination to alter cocaine-seeking behavior during a period of forced abstinence (FA) was assessed in a separate cohort of rats (n = 108) trained to self-administer cocaine (0.75 mg/kg/0.1 ml infusion) to stability (as described above) prior to initiation of FA and similar to previous publications (Anastasio et al., 2014a, 2014b, 2013; Sholler et al., 2019). We assessed cocaine-seeking behavior in an abstinence model; in brief, lever presses during the test sessions which occurred during abstinence from cocaine self-administration were reinforced with the discrete cue complex which had previously been paired with cocaine delivery. Once the criterion for stable cocaine self-administration was attained and maintained, rats were subjected to FA from cocaine self-administration for 10 days. During the FA period, rats were returned to their home cages, weighed, and handled daily. Following the assigned FA period, rats were evaluated in a cocaine-seeking

behavior test session (60 min) in which lever presses were reinforced by delivery of the discrete cue complex (stimulus light illuminated, infusion pump activated) on a FR1 schedule. Inactive lever presses were recorded but had no scheduled consequences. Seventeen rats failed to meet acquisition/maintenance criteria or lost catheter patency during the course of the study, and were excluded from subsequent analyses (vehicle: n = 13/15 analyzed; pimavanserin efficacy: n = 26/27 analyzed; lorcaserin efficacy: n = 25/25 analyzed; pimavanserin *plus* lorcaserin efficacy: n = 24/24 analyzed). Route of administration and pretreatment times were as described above.

 i. For acute treatment efficacy experiments, rats were injected with vehicle, pimavanserin (3 mg/kg), lorcaserin (1 mg/kg) or the combination of pimavanserin (0.5 mg/kg) *plus* lorcaserin (0.5 mg/kg) prior to the 60 min drug-seeking test session on FA day 10 in a between-subjects design.
 ii. For repeated treatment efficacy experiments, rats were injected with vehicle, pimavanserin (3 mg/kg), lorcaserin (1 mg/kg) or the combination of pimavanserin (0.5 mg/kg) *plus* lorcaserin (0.5 mg/kg) twice daily for 10 days during abstinence in a between-subjects design.

2.4. Assessment of pharmacokinetics (PK) of pimavanserin, lorcaserin, or the combination

Blood samples were collected from the jugular vein for determination of the plasma concentrations following administration of pimavanserin, lorcaserin, or the combination of pimavanserin *plus* lorcaserin. Upon receipt at the animal facility at UTMB, the jugular vein catheter implanted in each animal was checked for patency. Once blood was withdrawn through the PinPort™, heparinized 0.9% NaCl (10 IU heparin/ml diluted in saline) was infused into the lumen to clear the line. On the morning of dosing and 30 min prior to drug administration, the patency of each jugular vein catheter was again confirmed. Pre-dose blood samples (~0.25 ml) were collected at this point. Following dosing, blood (~0.25 ml) was collected from each animal in each treatment group via the PinPort™ at each of the following timepoints: 0, 15, 30, 60, 120, 180, 240, 360 min. Treatment groups included pimavanserin (0.5–10 mg/kg; s.c.), lorcaserin (0.5–3 mg/kg; s.c.) or pimavanserin *plus* lorcaserin (0.5 mg/kg + 0.5 mg/kg; 1 mg/kg + 1 mg/kg; s.c.). After each blood collection, ~0.25 ml of heparinized saline (10 IU/ml) was flushed through each rat via the catheter. Each blood sample (~0.25 ml) was collected into a heparinized test tube. Blood samples were centrifuged at approximately 1,800 × *g* for 5 min. The plasma was transferred to new tubes and stored frozen at −20 °C until analysis. The PK analyses were performed by SRI International (Menlo Park, CA) under contract to the National Institute on Drug Abuse.

2.5. Statistical analyses

The data from the cocaine self-administration and cocaine-seeking experiments were analyzed separately. A one-way analysis of variance (ANOVA) for a within-subjects design was employed to assess (a) cocaine infusions, (b) active lever presses, and (c) inactive lever responses during self-administration. A one-way ANOVA for a between-subjects design was employed to assess (a) cue-reinforced lever presses, (b) inactive lever presses, and (c) first response latency on the cue-reinforced lever during cocaine-seeking test sessions. *A priori* comparisons were defined prior to the start of experimentation and conducted by Dunnett's procedure or Bonferroni's method as appropriate (Keppel, 1973). Investigators who performed ligand administration and endpoint analyses were blinded to group assignments. All statistical analyses were conducted with GraphPad Prism (version 7.05) with an experiment-wise error rate set at α = 0.05.
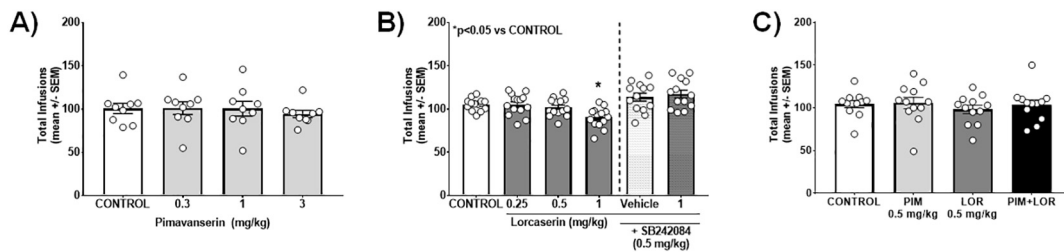
Fig. 1. Acute administration of pimavanserin *plus* lorcaserin does not suppress cocaine self-administration. **(A)** Effects of acute pimavanserin (0.3, 1, 3 mg/kg) on total cocaine infusions (mean ± SEM) are presented (n.s. vs. control). **(B)** Effects of acute lorcaserin (0.25, 0.5, 1 mg/kg) to alter total cocaine infusions (mean ± SEM) are presented. Lorcaserin (1.0 mg/kg) suppresses cocaine intake relative to control (*p < 0.05 vs. control; left panel); this effect is prevented by the selective 5-HT$_{2C}$R antagonist SB242084 (right panel). **(C)** Effects of subthreshold doses of pimavanserin (PIM; 0.5 mg/kg) plus lorcaserin (LOR; 0.5 mg/kg) on total cocaine infusions (mean ± SEM) are presented (n.s. vs. control).

3. Results

3.1. Lorcaserin, but not pimavanserin, suppresses cocaine self-administration

Acute administration of lorcaserin and investigatory 5-HT$_{2C}$R agonists, but not selective 5-HT$_{2A}$R antagonists (like M100907), dose-dependently decrease cocaine intake (for reviews, Cunningham and Anastasio, 2014; Higgins et al., 2020). Here we assessed acute administration of doses of pimavanserin and lorcaserin to block intake of a relatively low dose (0.25 mg/kg/inf) cocaine self-administration to establish doses for combination testing. No main effect of pimavanserin treatment on infusions (F(3,32) = 0.2315, p = 0.8738; Fig. 1A) active lever presses (F(3,32) = 0.2039, p = 0.8929; Table 1) or inactive lever presses (F(3,32) = 0.5564, p = 0.6476; Table 1) was observed. These are the first data to suggest that pimavanserin at these doses lacks efficacy to alter cocaine self-administration, similar to previous studies with M100907.

A main effect of lorcaserin treatment on infusions (F(5,76) = 7.539, p < 0.0001; Fig. 1B) was observed; *a priori* comparisons indicated lorcaserin (1 mg/kg) suppressed cocaine intake vs. control (p < 0.05). The 5-HT$_{2C}$R antagonist SB242084 prevented this lorcaserin-evoked suppression of cocaine intake (p < 0.05; Fig. 1B). A main effect of lorcaserin treatment on active lever presses [F(5,72) = 5.826, p = 0.0001], but not inactive lever presses [F(5,72) = 1.943, p = 0.0977], was observed (Table 1). These data replicate previous observations that 5-HT$_{2C}$R agonists exhibit efficacy to suppress cocaine intake in the rat self-administration model (Burbassi and Cervo, 2008; Cunningham et al., 2011; Fletcher et al., 2008; Grottick et al., 2000;

Higgins et al., 2020; Neisewander and Acosta, 2007).

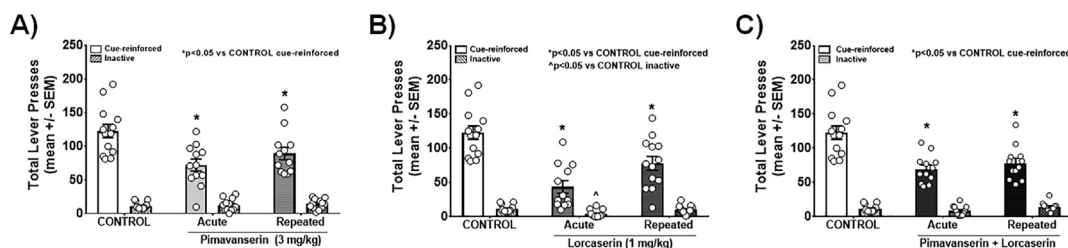### 3.1.1. Pimavanserin plus lorcaserin does not suppress cocaine self-administration

No main effect of combination treatment on infusions [F (3,36) = 1.221, p = 0.3162; Fig. 1C], active lever presses [F (3,36) = 0.1151, p = 0.9507; Table 1] or inactive lever presses [F (3,36) = 0.4227, p = 0.7379; Table 1] was observed. These data indicate that subthreshold doses of pimavanserin *plus* lorcaserin do not alter cocaine intake.

3.2. Acute and repeated pimavanserin or lorcaserin suppress cocaine-seeking behavior

A main effect of pimavanserin treatment on cue-reinforced lever presses during a cocaine-seeking test on FA day 10 was observed (F (2,34) = 7.708, p = 0.0017; Fig. 2A); *a priori* comparisons demonstrated that both acute (~41.4% decrease, p < 0.05) and repeated pimavanserin (~27.1% decrease, p < 0.05) suppressed cue-reinforced lever presses vs. control. No main effect of pimavanserin treatment on inactive lever presses was observed (F(2,34) = 0.4201, p = 0.6603; Fig. 2A). A main effect of pimavanserin treatment on latency to the first response [F(2,34) = 3.91, p = 0.0296] was observed; *a priori* comparisons demonstrated that repeated (35.25 ± 7.81 s; ~2.5-fold increase, p < 0.05), but not acute (29.83 ± 5.43 s; n.s.), pimavanserin treatment increased latency to the first response vs. control (14.39 ± 2.41 s). These data suggest that acute and repeated treatment with pimavanserin alone attenuates cocaine-seeking behavior on FA day 10, with minor behavioral inhibition initially.

A main effect of lorcaserin treatment on cue-reinforced lever presses during a cocaine-seeking test on FA day 10 was observed (F (2,35) = 16.02, p < 0.0001; Fig. 2B); *a priori* comparisons demonstrate acute (~64.4% decrease, p < 0.05) and repeated lorcaserin (~36.4% decrease, p < 0.05) suppressed cue-reinforced lever presses vs. control. A main effect of lorcaserin treatment on inactive lever presses (F(2,35) = 5.673, p = 0.0073; Fig. 2B) was observed; *a priori* comparisons demonstrate acute (~64.6% suppression; p < 0.05), but not repeated, lorcaserin (n.s.) suppressed inactive lever presses vs. control. A main effect of lorcaserin treatment on latency to the first response [F(2,35) = 4.615, p = 0.0166] was observed; *a priori* comparisons demonstrate acute (95.83 ± 35.78 s; ~6.7-fold increase; p < 0.05), but not repeated (30.15 ± 5.52 s; n.s.), lorcaserin treatment increased latency to the first response vs. control (14.39 ± 2.41 s). These data suggest that acute and repeated treatment with lorcaserin blunts cocaine-seeking behavior on FA day 10, with a moderate impact on the latency to first response only following acute administration.

Table 1
Effects of pimavanserin, lorcaserin, or their combination on active and inactive lever presses during cocaine self-administration (mean ± SEM).

	Active Lever Presses	Inactive Lever Presses
Pimavanserin (mg/kg)		
CONTROL	564.94 ± 62.31	2.87 ± 0.74
0.3	587.11 ± 72.80	3.67 ± 2.11
1	540.33 ± 57.42	1.22 ± 0.86
3	523.00 ± 54.57	3.44 ± 1.74
Lorcaserin (mg/kg)		
CONTROL	575.33 ± 11.89	3.10 ± 0.98
0.25	547.46 ± 16.57	1.77 ± 0.43
0.5	536.23 ± 16.03	2.08 ± 0.40
1	492.46 ± 18.99	3.00 ± 0.91
Lorcaserin (mg/kg) + SB242084 (mg/kg)		
CONTROL+ 0.5	663.92 ± 49.87	7.54 ± 3.59
1 + 0.5	636.15 ± 27.34	1.62 ± 0.49
Pimavanserin (mg/kg) + Lorcaserin (mg/kg)		
CONTROL	558.63 ± 12.56	2.67 ± 0.75
0.5 +CONTROL	562.60 ± 17.06	2.00 ± 0.65
CONTROL + 0.5	562.50 ± 18.89	2.40 ± 0.65
0.5 + 0.5	550.80 ± 16.13	3.60 ± 1.72

Fig. 2. Acute and repeated administration of pimavanserin *plus* lorcaserin suppress cocaine-seeking behavior during abstinence. **(A)** Effects of acute and repeated pimavanserin (3 mg/kg) on cue-reinforced or inactive lever presses (mean ± SEM) on FA 10 are presented (*p < 0.05 vs. control). **(B)** Effects of acute and repeated lorcaserin (1 mg/kg) on cue-reinforced or inactive lever presses (mean ± SEM) are presented (*p < 0.05 vs. control cue-reinforced; ^p < 0.05 vs. control inactive). **(C)** Effects of acute and repeated combination of pimavanserin (0.5 mg/kg) *plus* lorcaserin (0.5 mg/kg) on cue-reinforced or inactive lever presses (mean ± SEM) on FA 10 are presented (*p < 0.05 vs. control cue-reinforced).

3.2.1. Acute and repeated pimavanserin plus lorcaserin suppresses cocaine-seeking behavior

A main effect of pimavanserin *plus* lorcaserin treatment on cue-reinforced lever presses was observed [$F(2,34) = 13.02$, $p < 0.0001$; Fig. 2C]; *a priori* comparisons demonstrate acute (~56.7% decrease, $p < 0.05$) and repeated pimavanserin *plus* lorcaserin (~63.7% decrease, $p < 0.05$) suppressed cue-reinforced lever presses vs. control. Multiple comparisons assessed using Bonferroni's method revealed no difference in cue-reinforced lever presses between rats receiving acute pimavanserin vs. acute pimavanserin *plus* lorcaserin (n.s.), acute lorcaserin vs. acute pimavanserin *plus* lorcaserin (n.s.), repeated pimavanserin vs. repeated pimavanserin *plus* lorcaserin (n.s.), or repeated lorcaserin vs. repeated pimavanserin *plus* lorcaserin (n.s.). No main effect of pimavanserin *plus* lorcaserin treatment on inactive lever presses was observed [$F(2,34) = 2.154$, $p = 0.1316$I; Fig. 2C]. A main effect of pimavanserin *plus* lorcaserin treatment on latency to the first response was observed [$F(2,34) = 6.131$, $p = 0.0053$]; *a priori* comparisons demonstrate acute (40.17 ± 4.20 s; ~2.8-fold increase; $p < 0.05$) and repeated pimavanserin *plus* lorcaserin (37.33 ± 9.04 s; ~2.5-fold increase; $p < 0.05$) increased latency to the first response vs. control (14.39 ± 2.41 s). These data suggest that acute and repeated treatment with pimavanserin *plus* lorcaserin attenuates cocaine-seeking behavior on FA day 10, with a small impact on the latency to first response.

3.3. Pharmacokinetic analyses of pimavanserin, lorcaserin or the combination

We assessed *in vivo* PK interactions for subcutaneously administered pimavanserin, lorcaserin or pimavanserin *plus* lorcaserin in naïve rats (Tables 2 and 3). Mean PK profiles after a single dose of pimavanserin indicated C_{MAX} increased from ~12 to 213 ng/ml, T_{MAX} ranged from ~15 to 60 min and AUC_{0-inf} increased from ~15 to 1290 ng h/ml post-dosing that was dose-related (Table 2). The clearance (Cl/F) and half-life ($t_{1/2}$) of pimavanserin were dose-related; $t_{1/2}$ of pimavanserin ranged between ~53 and 218 min (Table 2). All doses of lorcaserin presented the relatively same metabolic profile (Table 2). Mean PK profiles after a single dose of lorcaserin indicated C_{MAX} increased from ~15 to 106 ng/ml, T_{MAX} ranged from ~15 to 25 min and AUC_{0-inf} increased from ~33 to 199 ng h/ml post-dosing (Table 2). The clearance and $t_{1/2}$ of lorcaserin were not dose-related; $t_{1/2}$ of lorcaserin ranged between ~108 and 143 min (Table 2). Mean PK profiles after single dosing of pimavanserin *plus* lorcaserin demonstrated no pharmacological interaction relative to single dosing of pimavanserin or lorcaserin alone (Table 2). Plasma concentrations of pimavanserin and lorcaserin at various timepoints are provided; there was no interactive effect of lorcaserin *plus* pimavanserin on metabolism of the medications (Table 3).

4. Discussion

We employed pimavanserin (0.3–3 mg/kg) (Hubbard et al., 2013; McFarland et al., 2011) and lorcaserin (0.25–1 mg/kg) (Higgins et al., 2012; Levin et al., 2011) at doses that do not alter spontaneous locomotor activity when administered alone or in combination, as reported previously. We demonstrate here that lorcaserin alone (1.0 mg/kg) given acutely suppresses cocaine intake in a self-administration model, while treatment with pimavanserin alone or the combination of lower, inactive doses of pimavanserin (0.5 mg/kg) *plus* lorcaserin (0.5 mg/kg) is insufficient to alter cocaine intake. The finding that the 5-HT$_{2A}$R antagonist pimavanserin acutely does not alter cocaine intake is consistent with previous findings utilizing investigational 5-HT$_{2A}$R antagonists in rats (e.g., M10907; (Filip, 2005; Fletcher et al., 2002; Nic Dhonnchadha et al., 2009). Conversely, we found that acute and repeated pimavanserin (3 mg/kg) or lorcaserin (1 mg/kg) each suppress cocaine-seeking after 10 days of forced abstinence without evidence for sensitization or tolerance upon repeated administration. Further, the combination of subthreshold doses of pimavanserin (0.5 mg/kg) *plus* lorcaserin (0.5 mg/kg) suppressed cocaine-seeking behavior on FA day 10. Finally, PK analyses indicate these medications may not share a common elimination mechanism. This series of findings suggests that this combination of 5-HT$_2$R interventions might have greater utility for relapse-prevention than for cessation.

Plasma exposure of pimavanserin and lorcaserin alone or in combination following subcutaneous administration was similar to previous single dose studies in rodents (Higgins et al., 2017, 2020; Vanover et al., 2006). Pimavanserin (0.5–10 mg/kg) produced a dose-related increase in serum drug concentration, as did lorcaserin (0.5–1 mg/kg), which corresponded to effects on cocaine-taking and -seeking behaviors. The dose ranges employed for both pimavanserin and lorcaserin preclinically correlate to behavioral efficacy (Collins et al., 2016; Higgins et al., 2017; Price et al., 2018; Sholler et al., 2019; Vanover et al., 2007) as well as clinical steady-state plasma exposure levels (Higgins et al., 2020; Smith et al., 2008; Thomsen et al., 2008; Vanover et al., 2007). Of note, pimavanserin dose-dependently and non-competitively inhibited clearance, suggesting that repeated pimavanserin dosing may progressively reduce clearance, increasing plasma levels of pimavanserin, especially at higher doses (Vanover et al., 2007). Metabolism of pimavanserin occurs via cytochrome P450 enzymes, e.g., CYP3A4/A5 (major), CYP2J2 (minor), and CYP2D6 (minor) (Bozymski et al., 2017), while lorcaserin engages multiple cytochrome P450 enzymes at μM concentrations, e.g. CYP1A2 (minor), CYP2C8 (minor), CYP2C9 (minor), CYP2C19 (minor), CYP2D6 (major), and CYP3A4 (minor), with the major metabolite of lorcaserin, a sulfamate, produced independent of induction of the cytochrome P450 enzymes (Higgins et al., 2020). Thus, our findings of a lack of significant drug-drug interaction following pimavanserin *plus* lorcaserin is as expected (Bozymski et al., 2017; Gustafson et al., 2013). Taken together, these data support future studies for a combinatorial paradigm of

Table 2

Pharmacokinetic parameters of pimavanserin or lorcaserin following a single dose of each medication alone or in combination (mean ± SEM).

	C_{MAX} (ng/ml)	T_{MAX} (hr)	AUC_{0-t}(ng·hr/ml)	AUC_{0-inf}(ng·hr/ml)	AUC_{Extrap}(%)	λ(1/hr)	Half-life, $t_{1/2}$(hr)	Cl/F(L/hr/kg)
				Pimavanserin				
Pimavanserin(mg/kg; s.c.)								
0.5	12.17 ± 0.38	0.29 ± 0.04	14.40 ± 0.31	15.02 ± 0.32	3.89 ± 1.42	0.76 ± 0.14	1.29 ± 0.45	33.40 ± 0.73
1	30.13 ± 2.17	0.25 ± 0.00	41.27 ± 2.64	41.72 ± 2.7	1.09 ± 0.28	0.79 ± 0.04	0.89 ± 0.05	24.55 ± 1.81
3	79.52 ± 3.76	0.38 ± 0.06	189.33 ± 7.75	197.5 ± 8.33	4.26 ± 0.68	0.59 ± 0.03	1.18 ± 0.05	15.28 ± 0.65
10	213.17 ± 15.86	0.96 ± 0.25	857.50 ± 54.72	1290.17 ± 112.09	32.22 ± 4.18	0.21 ± 0.02	3.63 ± 0.54	8.10 ± 0.83
Pim + Lor[a](mg/kg; s.c.)								
0.5 + 0.5	12.03 ± 1.97	0.25 ± 0.00	13.88 ± 3.32	14.34 ± 3.47	2.90 ± 0.69	0.80 ± 0.09	0.93 ± 0.12	41.15 ± 5.30
1 + 1	26.87 ± 5.04	0.25 ± 0.00	38.18 ± 11.19	39.05 ± 11.56	1.90 ± 0.44	0.68 ± 0.05	1.05 ± 0.08	28.00 ± 7.26
				Lorcaserin				
Lorcaserin(mg/kg; s.c.)								
0.5	15.63 ± 0.74	0.29 ± 0.04	29.03 ± 0.53	33.43 ± 1.31	12.68 ± 2.45	0.31 ± 0.03	2.39 ± 0.33	15.07 ± 0.56
1	34.35 ± 3.19	0.25 ± 0.00	58.52 ± 3.41	63.70 ± 3.91	8.06 ± 0.47	0.39 ± 0.02	1.80 ± 0.09	15.97 ± 0.85
3	106.33 ± 14.74	0.42 ± 0.05	183.50 ± 7.71	199.17 ± 8.24	7.97 ± 0.50	0.38 ± 0.01	1.83 ± 0.07	15.18 ± 0.66
Pim + Lor[a](mg/kg; s.c.)								
0.5 + 0.5	14.74 ± 0.71	0.30 ± 0.05	26.46 ± 1.80	28.48 ± 1.88	7.14 ± 0.75	0.40 ± 0.02	1.74 ± 0.11	17.9 ± 1.29
1 + 1	26.62 ± 1.69	0.35 ± 0.06	48.88 ± 2.71	52.82 ± 2.84	7.55 ± 0.52	0.41 ± 0.02	1.71 ± 0.07	19.12 ± 0.99

[a] Pimavanserin administered 15 min prior to lorcaserin; C_{MAX}, maximum serum concentration; T_{MAX}, time to reach C_{MAX}; AUC_{0-t}, area under plasma drug concentration-time curve (AUC) up to time 't'; AUC_{0-inf}, extrapolated AUC to infinity; AUC_{Extrap}, effect of extrapolated AUC on AUC_{0-inf} estimation; λ, individual estimate of terminal elimination rate constant; $t_{1/2}$, elimination half-life; Cl/F, apparent total body clearance.

pimavanserin *plus* lorcaserin in a therapeutic setting.

The most consistent evidence of a role for 5-HT receptors in cocaine relapse-like behaviors emerges from evaluation of 5-HT$_{2A}$R and 5-HT$_{2C}$R systemic manipulations. Pretreatment with 5-HT$_{2A}$R antagonists, (e.g., selective 5-HT$_{2A}$R antagonist/inverse agonists M100907 and pimavanserin; 5-HT$_{2A/2C}$R antagonists/inverse agonists ketanserin and ritanserin), indicate that the 5-HT$_{2A}$R does not evidently mediate intake of cocaine in the self-administration paradigm in rats (Fletcher et al., 2002; Lacosta and Roberts, 1993; Nic Dhonnchadha et al., 2009; Peltier and Schenk, 1993; Pockros et al., 2011) or rhesus monkeys (Murnane et al., 2013). Conversely, 5-HT$_{2C}$R knockout mice exhibit heightened cocaine intake (Rocha et al., 2002), consistent with studies employing systemic administration of selective 5-HT$_{2C}$R agonists (e.g., selective 5-HT$_{2C}$R agonists WAY163909 and lorcaserin; 5-HT$_{2B/2C}$R agonist Ro60-0175) in which the reinforcing effects of cocaine in animals is blunted (Collins and France, 2018; Collins et al., 2016; Cunningham et al., 2011; Fletcher et al., 2008; Grottick et al., 2000; Harvey-Lewis et al., 2016) and prevented by the 5-HT$_{2C}$R antagonist SB242084 (present study) (Cunningham et al., 2011; Manvich et al., 2012). Future experiments are required to extend these observations to a broader dose range for pimavanserin and lorcaserin treatment as well as analysis of the effects of these ligands on intake following repeated administration.

Medications that attenuate sensitivity to cocaine-associated cues may afford CUD individuals heightened control over relapse-triggering stimuli. From a therapeutic perspective, recruitment of the 5-HT$_{2A}$R and/or 5-HT$_{2C}$R during and/or after abstinence from cocaine may differ relative to the goal to suppress ongoing intake. The control afforded by both acute and repeated administration of pimavanserin over cue-evoked cocaine-seeking, but not cocaine self-administration, supports other lines of evidence that the neural mechanisms underlying the incentive-motivational aspects of drugs and drug-associated cues are distinct (Everitt and Wolf, 2002; Kruzich et al., 2001; Robinson and Berridge, 2003). The findings presented here extend previous reports that M100907 suppresses cue-evoked reinstatement of cocaine seeking following extinction training from cocaine self-administration (Fletcher et al., 2002; Nic Dhonnchadha et al., 2009; Pockros et al., 2011). Furthermore, M100907 was most efficacious in suppressing cue-evoked reinstatement in those rats with the highest extinction responsiveness (Nic Dhonnchadha et al., 2009). Selective 5-HT$_{2C}$R agonists (Ro60-0175, WAY163909, lorcaserin) not only reduce cocaine self-administration, but also cocaine-seeking behavior (Anastasio et al., 2014a; Burbassi and Cervo, 2008; Cunningham et al., 2011; Fletcher et al., 2008; Grottick et al., 2000; Higgins et al., 2020; Neisewander and Acosta, 2007; Swinford-Jackson et al., 2016). Higgins and Fletcher

Table 3

Plasma concentration of pimavanserin or lorcaserin following a single dose of each medication alone or in combination (mean ± SEM).

	0 h	15 min	30 min	60 min	120 min	180 min	240 min	360 min
				Pimavanserin (ng/ml)				
Pimavanserin(mg/kg; s.c.)								
0.5	0.08 ± 0.05	12.15 ± 0.39	9.93 ± 0.50	5.40 ± 0.23	2.09 ± 0.13	0.86 ± 0.11	0.45 ± 0.10	0.13 ± 0.06
1	0.05 ± 0.03	30.13 ± 2.17	24.50 ± 1.59	16.8 ± 1.02	6.34 ± 0.39	3.55 ± 0.81	1.12 ± 0.14	0.35 ± 0.08
3	0.02 ± 0.02	71.13 ± 5.89	74.48 ± 5.10	63.40 ± 3.59	39.83 ± 2.40	27.80 ± 2.37	15.66 ± 1.48	4.85 ± 0.62
10	0.09 ± 0.41	169.67 ± 18.84	190.50 ± 19.86	208.17 ± 15.62	179.17 ± 11.60	154.17 ± 15.09	116.15 ± 7.99	79.87 ± 6.24
Pim + Lor[a](mg/kg; s.c.)								
0.5 + 0.5	0.09 ± 0.06	12.03 ± 1.97	8.20 ± 1.99	5.49 ± 1.34	1.87 ± 0.52	0.91 ± 0.29	0.43 ± 0.16	0.28 ± 0.10
1 + 1	0.08 ± 0.04	26.87 ± 5.04	19.70 ± 4.29	14.27 ± 3.94	7.07 ± 2.74	3.19 ± 1.32	1.72 ± 0.78	0.53 ± 0.23
				Lorcaserin (ng/ml)				
Lorcaserin(mg/kg; s.c.)								
0.5	0.00 ± 0.00	15.55 ± 0.76	13.97 ± 0.50	10.37 ± 0.26	4.83 ± 0.13	3.03 ± 0.18	2.05 ± 0.07	1.20 ± 0.12
1	0.00 ± 0.00	34.35 ± 3.19	27.52 ± 1.10	21.13 ± 1.06	9.88 ± 0.60	6.11 ± 0.48	3.86 ± 0.39	1.98 ± 0.16
3	0.00 ± 0.00	99.2 ± 15.97	97.47 ± 7.97	63.48 ± 4.38	32.35 ± 1.07	17.63 ± 0.77	12.35 ± 0.48	5.97 ± 0.28
Pim + Lor[a](mg/kg; s.c.)								
0.5 + 0.5	0.00 ± 0.00	14.68 ± 0.72	13.48 ± 0.92	9.49 ± 0.91	4.74 ± 0.34	2.58 ± 0.22	1.66 ± 0.10	0.80 ± 0.06
1 + 1	0.00 ± 0.00	25.46 ± 2.05	24.32 ± 2.34	17.14 ± 1.12	8.85 ± 0.80	5.08 ± 0.13	3.33 ± 0.17	1.60 ± 0.07

[a] Pimavanserin administered 15 min prior to lorcaserin.

(Higgins et al., 2012) demonstrated that 1 mg/kg of lorcaserin did not impair measures of balance, coordination, or motor planning in the rotarod test. Rotarod performance is proposed as a stronger indicator of skilled performance (e.g., operant responding) than spontaneous locomotor activity (Stromberg, 1988). Taken together, the efficacy of 1 mg/kg of lorcaserin to suppress cocaine intake and cocaine-seeking is not definitively attributable to a non-specific inhibition in motor behavior. Nonetheless, it is worth noting that a moderate impact on the latency to the first response was detected following lorcaserin treatment, which was dampened in the presence of pimavanserin, suggesting the potential for non-specific inhibition of spontaneous locomotor activity. Thus, once distinguished pharmacologically, the $5\text{-HT}_{2A}R$ and $5\text{-HT}_{2C}R$ play an excitatory and inhibitory role, respectively, over cocaine-seeking during abstinence. The added value of repeated administration of pimavanserin or lorcaserin to suppress the incentive motivational properties of these cocaine-paired cues, without evidence for the development of sensitization or tolerance to the medications, ascribes a further dimension of likely pharmacotherapeutic potential for CUD.

The possibility that the $5\text{-HT}_{2A}R$ and $5\text{-HT}_{2C}R$ may act in concert is suggested by the fact that both cue- and cocaine-primed drug-seeking were suppressed by the combination of low, singly ineffective doses of a selective $5\text{-HT}_{2A}R$ antagonist (M100907, pimavanserin) *plus* a selective $5\text{-HT}_{2C}R$ agonist (WAY163909, lorcaserin) (present study; (Cunningham et al., 2013). Further, growing evidence from biochemical, behavioral and pharmacological studies indicate that the $5\text{-HT}_{2A}R$ and $5\text{-HT}_{2C}R$ interact functionally at the cellular, brain region, and circuit level (Anastasio et al., 2015; Bazovkina et al., 2015; Burton et al., 2013; Cunningham et al., 2013; Moutkine et al., 2017; Pockros et al., 2012). Of note, the $5\text{-HT}_{2A}R$ and $5\text{-HT}_{2C}R$ are oppositional in some *in vivo* analyses (for review, Bubar and Cunningham, 2008) and therefore results observed following administration of nonselective $5\text{-HT}_{2A/2C}R$ antagonists are difficult to interpret as simultaneous blockade of the $5\text{-HT}_{2C}R$ would be expected to have opposing actions to $5\text{-HT}_{2A}R$ antagonism. Thus, the ineffectiveness of non-selective $5\text{-HT}_{2A/2C}R$ antagonists to attenuate cocaine-seeking in animals (Burmeister et al., 2004; Filip, 2005; Schenk, 2000) or humans (Ehrman et al., 1996) or influence self-reported euphoric effects of cocaine (Newton et al., 2001) or craving (De La Garza et al., 2005; Loebl et al., 2008) in clinical studies is not surprising. Nonetheless, our findings support the hypothesis that 5-HT neurotransmission can critically influence aspects of cocaine-mediated behaviors via the $5\text{-HT}_{2A}R$ and $5\text{-HT}_{2C}R$ systems and that these receptors act in concert to regulate the neural bases for behavior (for reviews, (Cunningham and Anastasio, 2014; Howell and Cunningham, 2015).

Preclinical models have greatly enhanced our understanding of the neurobiology of the rewarding and reinforcing properties of cocaine and cocaine-associated cues and have advanced the goal to identify medications that lack abuse liability, yet exhibit efficacy to enhance abstinence, reduce craving, and prevent relapse. The identification of efficacy following pimavanserin *plus* lorcaserin (but see) (Banks and Negus, 2017) indicate the promise of a combinational strategy of these two targeted 5-HT_2R family medications to modulate relapse-related behaviors. While dosing and routes of administration could be further optimized, we propose strategies to couple $5\text{-HT}_{2A}R$ antagonist and $5\text{-HT}_{2C}R$ agonist pharmacotherapy (Booth et al., 2009; Chen et al., 2017; Gilbertson et al., 2018; Rowland et al., 2008; Shashack et al., 2011; Soto et al., 2018) or co-administration of otherwise ineffective doses of pimavanserin and lorcaserin presents the opportunity to yield therapeutic efficacy for each agent while reducing the potential for adverse effects (Morphy et al., 2004). In addition, our findings presented herein will guide future experiment to probe the neurobiology of the 5-HT_{2A} and $5\text{-HT}_{2C}R$ systems in cocaine relapse-related behaviors.

Authorship contributions

NCA, FGM, and KAC conceptualized and oversaw the project. NCA,

JMB, FGM and KAC participated in research design. SJS and RGF conducted experiments. NCA, DJS, SJS, RGF, and CRM performed data analyses. NCA, DJS, CRM, JMB, FGM, and KAC wrote or contributed to the writing of the manuscript. All authors provided final approval of the submitted manuscript.

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