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Newly Developed Dopamine D_3 Receptor Antagonists, *R*-VK4-40 and *R*-VK4-116, Do Not Potentiate Cardiovascular Effects of Cocaine or Oxycodone in Rats[S]

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ABSTRACT

Opioid and cocaine abuse are major public health burdens. Existing medications for opioid use disorder are limited by abuse liability and side effects, whereas no treatments are currently approved in the United States for cocaine use disorder. Dopamine D_3 receptor (D_3R) antagonists have shown promise in attenuating opioid and cocaine reward and mitigating relapse in preclinical models. However, translation of D_3R antagonists to the clinic has been hampered by reports that the D_3R antagonists GSK598,809 (5-(5-((3-((1*S*,5*R*)-1-(2-fluoro-4-(trifluoromethyl)phenyl)-3-azabicyclo[3.1.0]hexan-3-yl)propyl)thio)-4-methyl-4*H*-1,2,4-triazol-3-yl)-4-methyloxazole) and SB-277,011A (2-(2-((1*r*,4*r*)-4-(2-oxo-2-(quinolin-4-yl)ethyl)cyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-6-carbonitrile) have adverse cardiovascular effects in the presence of cocaine. Recently, we developed two structurally novel D_3R antagonists, *R*-VK4-40 and *R*-VK4-116, which are highly selective for D_3R and display translational potential for treatment of opioid use disorder. Here, we tested whether *R*-VK4-40 ((*R*)-*N*-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)-3-hydroxybutyl)-1*H*-indole-2-carboxamide) and *R*-VK4-116 ((*R*)-*N*-(4-(4-(3-Chloro-5-ethyl-2-methoxyphenyl) piperazin-1-yl)-3-hydroxybutyl)-1*H*-indole-2-carboxamide) have unwanted cardiovascular effects in the presence of oxycodone, a prescription opioid, or cocaine in freely moving rats fitted with surgically implanted telemetry transmitters. We also examined cardiovascular effects of the D_3R antagonist, SB-277,011A, and L-741,626 (1-((1*H*-indol-3-yl)methyl)-4-(4-chlorophenyl)piperidin-4-ol), a dopamine D_2 receptor–selective antagonist, for comparison. Consistent with prior reports, SB-277,011A increased blood pressure, heart rate, and locomotor activity alone and in the presence of cocaine. L-741,626 increased blood pressure and heart rate. In contrast, *R*-VK4-40 alone dose-dependently reduced blood pressure and heart rate and attenuated oxycodone-induced increases in blood pressure and oxycodone or cocaine-induced increases in heart rate. Similarly, *R*-VK4-116 alone dose-dependently reduced cocaine-induced increases in blood pressure and heart rate. These results highlight the safety of new D_3R antagonists and support the continued development of *R*-VK4-40 and *R*-VK4-116 for the treatment of opioid and cocaine use disorders.

SIGNIFICANCE STATEMENT

Opioid and cocaine abuse are major public health challenges and new treatments that do not adversely impact the cardiovascular system are needed. Here, we show that two structurally novel dopamine D_3 receptor antagonists, *R*-VK4-40 and *R*-VK4-116, do not potentiate, and may even protect against, oxycodone- or cocaine-induced changes in blood pressure and heart rate, supporting their further development for the treatment of opioid and/or cocaine use disorders.

Introduction

Opioid and cocaine abuse both remain major public health challenges, affecting up to 20 million lives in the United States and costing more than $192 billion annually in lost productivity, crime, and healthcare-related expenses (National Drug Intelligence Center; www.justice.gov/archive/ndic/pubs44/44849/44849p.pdf). Opioid overdose deaths have skyrocketed in the last decade, more than doubling between 2007 and 2017, and prescription opioids such as oxycodone were responsible for nearly one-third of opioid overdoses (National Institute on Drug Abuse; https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates). Next to opioids, cocaine abuse remains the second leading cause of drug overdose in the United States and causes more overdose deaths among African Americans than heroin (Frakt, 2018; National Institute on Drug Abuse; https://www.drugabuse.gov/related-

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ABBREVIATIONS: D_2R, dopamine D_2 receptor; D_3R, dopamine D_3 receptor; IRP, Intramural Research Program; NIDA, National Institute on Drug Abuse.

topics/trends-statistics/overdose-death-rates). Existing medications approved by the US Food and Drug Administration for the treatment of opioid use disorder, such as methadone and buprenorphine, are opioid-based and have several limitations, including abuse liability and side effects such as respiratory suppression (Jordan et al., 2019a). Currently, there are no Food and Drug Administration–approved medications for cocaine use disorder. Together, these observations highlight a critical need for new medications and treatment strategies.

The rewarding and euphoric effects of drugs of abuse such as opioids and cocaine are largely attributable to their ability to activate the mesocorticolimbic reward system, where dopamine acts upon five major receptor subtypes: D_1, D_2, D_3, D_4 and D_5 (Volkow et al., 2017). Of these, the dopamine D_3 receptor (D_3R) has received increasing attention as a viable medication target in the treatment of drug use disorders. Compared with other dopamine receptor subtypes the D_3R exhibits restricted distribution in the mesolimbic system and has the highest affinity for endogenous dopamine (Keck et al., 2015; Sokoloff and Le Foll, 2017). As such, pharmacological ligands for the D_3R are anticipated to exert fewer side effects than other dopamine receptor targets (Beaulieu and Gainetdinov, 2011). Preclinical studies indicate that D_3R antagonists reduce opioid and cocaine addiction-related behaviors, including attenuated conditioned place preference, reduced intravenous drug self-administration, and suppressed drug- or cue-primed reinstatement to drug seeking (Song et al., 2012, 2013; Hu et al., 2013; Xi et al., 2013; Galaj et al., 2014, 2015, 2016; Ashby et al., 2015; Boateng et al., 2015; Wager et al., 2017; You et al., 2017, 2019).

Despite their efficacy in attenuating drug reward, the development of D_3R antagonists for the treatment of opioid and cocaine use disorders has been limited by a number of shortcomings, including cardiotoxicity, metabolic instability, and poor absorption, distribution, metabolism, and excretion profiles (Keck et al., 2015). In addition, the D_3R antagonists GSK598,809 (5-(5-((3-((1*S*,5*R*)-1-(2-fluoro-4-(trifluoromethyl) phenyl)-3-azabicyclo[3.1.0]hexan-3-yl)propyl)thio)-4-methyl-4*H*-1,2,4-triazol-3-yl)-4-methyloxazole; Micheli et al., 2010), formerly in clinical trials (see http://clinicaltrials.gov/ct2/results?term=598,809&Search=Search), and SB-277,011A (2-(2-((1*r*,4*r*)-4-(2-oxo-2-(quinolin-4-yl)ethyl)cyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-6-carbonitrile) increased blood pressure, particularly when combined with cocaine, in dogs fitted with telemetry transmitters (Appel et al., 2015; Appel and Acri, 2017). These observations undermined the presumed safety of D_3R antagonists and halted further development of this drug class as putative treatments in a population with known cardiovascular risks (e.g., individuals with cocaine use disorder).

To determine whether all or only certain D_3R antagonists have such unwanted cardiovascular effects, and in efforts to circumvent the limitations barring D_3R antagonists from entering the clinic, our laboratory generated two structurally novel D_3R antagonists, (±)VK4-40 (*N*-(4-(4-(2-chloro-3-ethylphenyl)piperazin-1-yl)-2-hydroxybutyl)-1H-indole-2-carboxamide) and (±)VK4-116 (*N*-(4-(4-(3-chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)-3-hydroxybutyl)-1H-indole-2-carboxamide; Fig. 1; Kumar et al., 2016). These compounds were designed based on structure-activity relationships and the recently available high-resolution crystal structure of D_3R (Keck et al., 2014). Of these, the *R*-enantiomers (Fig. 1), which were both highly D_3R-selective antagonists in vitro, were chosen as lead compounds. Both compounds cross the blood-brain barrier readily and exhibit metabolic stability in mouse, rat, and human liver microsomes as well as following oral gavage in the rat (Kumar et al., 2016; Jordan et al., 2019b; Shaik et al., 2019). Furthermore, *R*-VK4-40 ((*R*)-*N*-(4-(4-(2-chloro-3-ethylphenyl)piperazin-1-yl)-3-hydroxybutyl)-1*H*-indole-2-carboxamide) and/or (±)VK4-116 reduce oxycodone self-administration under fixed and progressive-ratio reinforcement schedules, suppress naloxone-precipitated opioid withdrawal, augment oxycodone-induced analgesia on the hot plate test in rats, and reduce oxycodone-enhanced dopamine-dependent optical brain stimulation reward in mice (Jordan et al., 2019b; You et al., 2019). However, it remains unknown whether *R*-VK4-40 and *R*-VK4-116 ((*R*)-*N*-(4-(4-(3-chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)-3-hydroxybutyl)-1*H*-indole-2-carboxamide) adversely interact with oxycodone or cocaine to impact cardiovascular parameters, as was observed with prior generation D_3R antagonists. Here, we used radiotelemetry in conscious rats to determine the impact of a range of *R*-VK4-40 and *R*-VK4-116 doses (in the presence and absence of oxycodone and cocaine) on blood pressure, heart rate, body temperature, and activity levels. We also tested SB-277,011A, a classic D_3R antagonist, formerly identified to increase blood pressure when combined with cocaine in dogs, and L-741,626,(1-((1*H*-indol-3-yl)methyl)-4-(4-chlorophenyl)piperidin-4-ol) a dopamine D_2 receptor (D_2R)–selective antagonist, for comparison. Figure 1 shows the chemical structures of R-VK4-40, *R*-VK4-116 alongside SB-277,011A, GSK598,809, and L-741,626.

Materials and Methods

Animals and Housing. Eight adult male Long-Evans rats (Charles River) weighing 300–500 g were used. They were individually housed in ventilated racks in a temperature and humidity-controlled room with a 12-hour reverse light/dark cycle (lights off at 7:00 AM). Throughout experimentation, rats were food restricted to ~90% of free-feeding body weight, and water was available ad libitum, except when telemetric measurements were being recorded in daily, 3-hour sessions (as detailed subsequently). All animals used in this study were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All procedures were approved by the Institutional Care and Use Committee of the National Institute on Drug Abuse (NIDA)/Intramural Research Program (IRP) and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Surgical Procedures. Biotelemetry transmitters (HD-S10) were implanted by surgeons at Data Sciences in St. Paul, MN. The transmitters supplied readings for blood pressure (i.e., mean arterial pressure), heart rate (derived from the blood pressure signal), temperature, and motor activity. Motor activity was measured by tracking the strength of the transmitter radio signal as the rat moved about the cage on top of the telemetry receiver; therefore, these measures do not have any units. Briefly, the rats were anesthetized with isoflurane and a midline incision was made in the abdominal wall. The intestines were retracted and the descending aorta isolated. The catheter of the transmitter was then inserted into the descending aorta and glued in place. The transmitter was secured to the abdominal wall and the muscle and skin were sutured closed. Following recovery, the rats were shipped to the NIDA/IRP in Baltimore, MD, where they underwent 7-day quarantine.

Telemetric Measurements. Following release from quarantine, rats were adapted to the training procedures. Rats were transported to the procedure room where food and water were removed from the home cage and the entire home cage was placed on top of a telemetry

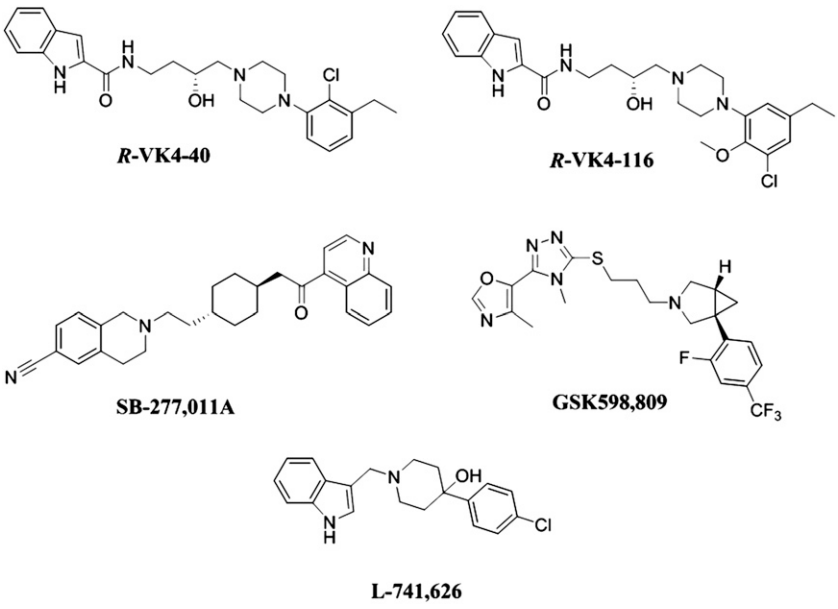

Fig. 1. Chemical structures of the new generation D_3R antagonists, *R*-VK4-40 and *R*-VK4-116, compared with previously reported D_3R antagonists SB-277,011A and GSK598,809, and the D_2R-selective antagonist L-741,626.

receiver (Data Sciences model RPC-1) that was located inside a small acoustical chamber (BRS/LVE, Laurel, MD). Transmitters were only turned on during the experimental sessions. Data for blood pressure, heart rate, and temperature were collected for 10 seconds every 1 minute (DataQuest A.R.T. Gold; Data Sciences) for a total of 3 hours. Activity measures were monitored continuously. Following initial adaptation, the rats were injected with saline (intraperitoneally) 5 minutes prior to the session twice per week until their response following injections was habituated and indistinguishable from noninjection days.

Drug testing began following habituation to the injection procedure. Testing was first conducted with oxycodone (1 mg/kg) and cocaine (10 mg/kg) alone and in combination with the D_3R antagonists *R*-VK4-40 (3, 10, and 20 mg/kg) and *R*-VK4-116 (5, 15, and 25 mg/kg). Following prior publications on cardiovascular responses to cocaine and dopamine D_3 receptor antagonists (Appel et al., 2015), all animals received each treatment, thereby eliminating the potential confound of group differences in baseline (without injection) responses. Doses of oxycodone and cocaine were chosen based on behavioral data indicating their ability to induce reinstatement to drug seeking in rats (Song et al., 2014; You et al., 2019) and dose-effect testing that showed these were the minimal doses to produce increases in blood pressure, allowing for the assessment of both the attenuation and potentiation of the effects of oxycodone or cocaine by the pretreatments. Doses of *R*-VK4-40 and *R*-VK4-116 were chosen based on previously collected data indicating efficacy in attenuating oxycodone reward in rats and mice (Jordan et al., 2019b; You et al., 2019). Oxycodone, cocaine, or vehicle was given 5 minutes prior to the rats being placed in the acoustical chambers, while the D_3R antagonist or vehicle was given 30 minutes prior to oxycodone, cocaine, or vehicle. SB-277,011A (30 mg/kg, administered 30 minutes prior to cocaine; Appel and Acri, 2017) was then tested alone and in combination with cocaine 10 mg/kg. Finally, L-741,626 (3 and 10 mg/kg, administered 5 minutes prior to the session) was tested alone. For all studies, drugs were typically tested at least 3 days apart, with rats placed in the acoustical chamber on intervening days with no prior injections. To ensure consistency of effects, cocaine, oxycodone, and vehicle were tested at least twice throughout the testing. In general, drugs were tested no more frequently than twice per week. Baseline parameters were stable throughout testing and tests with vehicle, cocaine, and oxycodone produced similar results over time during the course of the experiment. The rats were approximately 2–9 months of age during the testing period.

Drugs. Cocaine and oxycodone (NIDA/IRP) were dissolved in saline. *R*-VK4-40, *R*-VK4-116 [synthesized by one of the authors (A.B.S.) and J. Cao according to methods modified from Kumar et al. (2016) and described in Shaik et al. (2019), in the Medicinal Chemistry Section, NIDA/IRP], and SB-277,011A (Sigma-Aldrich) were dissolved in 25% 2-hydroxypropyl β-cyclodextrin and distilled H_2O. L-741,626 (Tocris) was dissolved in 3% cremophor and distilled H_2O. All drugs were administered intraperitoneally in a volume of 1 ml/kg of body weight.

Data Analysis. The averages of the 1-minute samples of blood pressure, heart rate, and temperature and the summed counts for activity are presented for the analyzed time periods. For the time course analysis, data are presented over 10-minute periods and a two-factor (treatment × time) ANOVA was performed with follow-up Bonferroni tests comparing drug treatments to vehicle. Because blood pressure and heart rate effects of cocaine were primarily restricted to the first 70 minutes of the session (see *Results*), subsequent analyses were performed for data from the first hour of the session (1-minute time points averaged) and subjected to ANOVA, followed by Tukey tests for multiple comparisons to determine significant differences between treatment groups (R version 3.4.4).

Results

***R*-VK4-40 and *R*-VK4-116.** Figure 2 shows the time courses of the effects for *R*-VK4-40 (20 mg/kg, i.p.), *R*-VK4-116 (25 mg/kg, i.p.), or their vehicle (administered 30 minutes prior to telemetry sessions) on blood pressure, heart rate, locomotor activity, and body temperature. *R*-VK4-116 produced slight hypertension, whereas *R*-VK4-40 produced hypotension when compared with vehicle treatment (main treatment effect; $F_{2,374} = 30.1$, $P < 0.0001$); however, only the effect of *R*-VK4-40 at the 20-minute time point was significantly different from vehicle ($P < 0.05$). Neither drug produced significant tachycardia or motor stimulation when compared at individual time points, with the exception of the 70-minute time point where R-VK4-40 differed from vehicle in the heart rate measure. Interestingly, both compounds produced sustained hypothermia of about 2°C (interaction effect; $F_{34,374} = 2.3$, $P < 0.001$). The effect of *R*-VK4-40 was

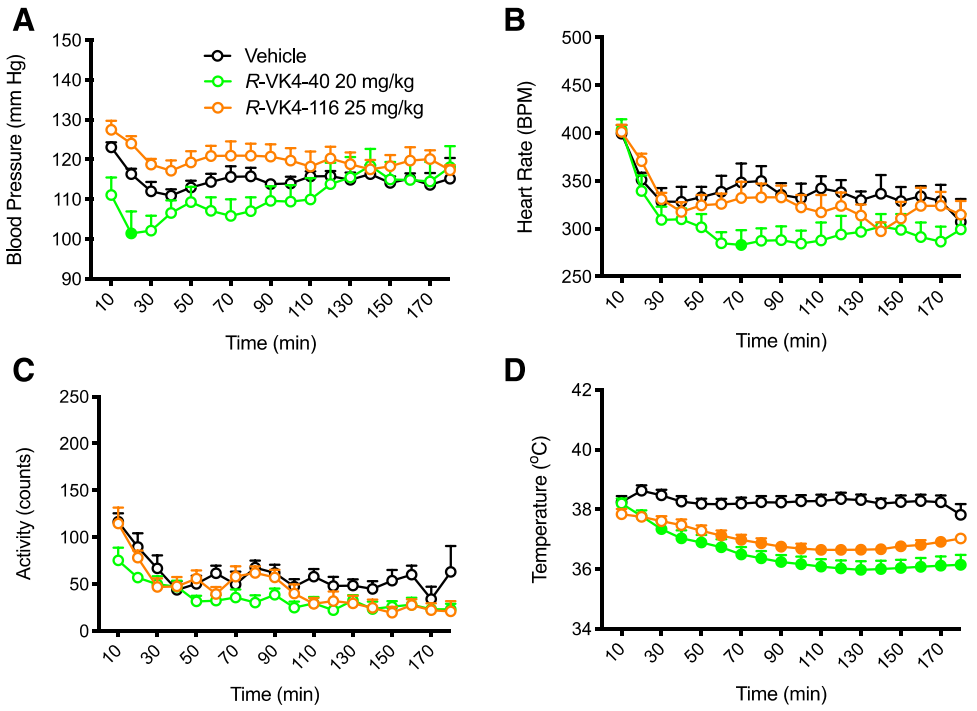

Fig. 2. Time course data showing effects of *R*-VK4-40 (20 mg/kg, i.p.), *R*-VK4-116 (25 mg/kg, i.p.), or their 25% 2-hydroxypropyl β-cyclodextrin vehicle (administered 30 minutes prior to telemetry session) on blood pressure (mean arterial pressure) (A), heart rate (beats per minute) (B), activity levels (C), and body temperature (D). Because the blood pressure and heart rate effects of cocaine were primarily restricted to the first 70 minutes of the session, following analyses of drug interactions were performed on the first hour of the session to ensure that the effects of each compound were maximal. Filled symbols represent statistically significant differences from 25% 2-hydroxypropyl β-cyclodextrin vehicle ($P < 0.05$). Values are mean ± S.E.M.

significantly different from vehicle at every point beyond 20 minutes and for *R*-VK4-116 from 60 to 170 minutes.

Cocaine and Oxycodone. Figure 3 shows the time courses of the effects of oxycodone (1 mg/kg, i.p.), cocaine (10 mg/kg, i.p.), and their vehicle (administered 5 minutes prior to telemetry sessions) on blood pressure, heart rate, locomotor activity, and body temperature. Cocaine and oxycodone both induced sustained elevations over vehicle for blood pressure, heart rate, and activity (interaction effect; $F_{34,366} > 2$, $P < 0.0001$). For oxycodone those effects were significantly different from vehicle at 30–70 minutes and at 90 minutes for blood pressure and 40–70 minutes for heart rate. For cocaine those effects were significantly different from vehicle at 30–70 minutes for blood pressure, 40–90 minutes for heart rate, and 30–70 minutes for activity. Oxycodone increased temperature more than cocaine when compared with vehicle (interaction effect; $F_{34,366} = 3.9$, $P < 0.0001$). Temperature was above vehicle levels for oxycodone from 40 to

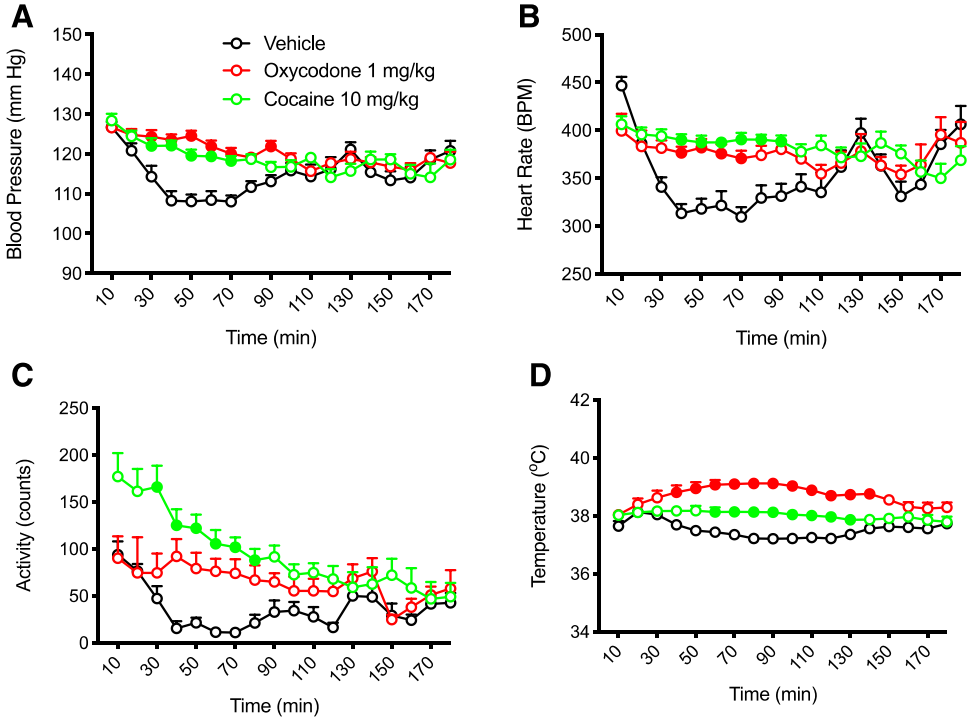

Fig. 3. Time course data showing effects of cocaine (10 mg/kg, i.p.), oxycodone (1 mg/kg, i.p.), or their vehicle (administered 5 minutes prior to telemetry sessions) on blood pressure (mean arterial pressure) (A), heart rate (beats per minute) (B), activity levels (C), and body temperature (D). Because the blood pressure and heart rate effects of cocaine were primarily restricted to the first 70 minutes of the session, following analyses of drug interactions were performed on the first hour of the session to ensure that the effects of each compound were maximal. Filled symbols represent statistically significant differences from vehicle ($P < 0.05$). Values are mean ± S.E.M.

140 minutes and for cocaine from 60 to 130 minutes. Because the blood pressure and heart rate effects of cocaine were primarily restricted to the first 70 minutes of the session, analysis of the vehicle and interaction studies were performed on the first hour of the session to ensure that the effects of both compounds were maximal. Additional analysis of binned 10-minute time points on *R*-VK4-40 and *R*-VK4-116 interactions with oxycodone and cocaine (Supplemental Figs. 1 and 2) confirmed that significant drug interactions on blood pressure were largely restricted to the first hour of the session (see Supplemental Material). Prior telemetry studies on cocaine and D_3R antagonism restricted analyses to the first 5 minutes post-cocaine infusion (Appel et al., 2015).

Baseline versus Vehicle Treatments. Table 1 shows baseline (no injection) values and the effects of saline, 25% 2-hydroxypropyl β-cyclodextrin in distilled H_2O, and 3% cremophor in distilled H_2O injections on blood pressure, heart rate, locomotor activity, and body temperature during the first hour of the session (for comparison with *R*-VK4-40 and *R*-VK4-116 interactions with oxycodone and cocaine, see the previously described results). Overall, only minimal differences were noted for the various vehicles. One-way ANOVA of blood pressure during the first hour revealed a main effect of treatment ($F_{3,27} = 4.69, P < 0.009$). Post-hoc testing indicated that saline differed significantly from preinjection baseline ($P < 0.05$), and that 25% 2-hydroxypropyl β-cyclodextrin significantly differed from saline ($P < 0.03$). One-way ANOVA of heart rate also revealed a significant main effect ($F_{3,27} = 7.14$, $P < 0.001$). Post-hoc testing indicated significant increases in heart rate following saline compared with baseline ($P < 0.003$), and following 3% cremophor injections compared with baseline ($P < 0.003$). One-way ANOVA of activity levels and body temperature failed to reveal significant main effects ($F_{3,27} = 1.64$, $P = 0.2$, and $F_{3,27} = 1.38$, $P = 0.3$).

***R*-VK4-40 and Oxycodone.** Figure 4 shows the mean effects of *R*-VK4-40 (3, 10, and 20 mg/kg, i.p., administered 30 minutes prior to telemetry sessions), in the presence of vehicle or oxycodone (1 mg/kg i.p., administered 5 minutes prior to telemetry sessions), on blood pressure, heart rate, body temperature, and activity levels. Overall, *R*-VK4-40 alone produced significant reduction in blood pressure, heart rate, and body temperature in a dose-dependent manner, while oxycodone alone produced a significant increase in blood pressure and body temperature. Pretreatment with 20 mg/kg *R*-VK4-40 significantly reduced the oxycodone-induced increase in blood pressure and body temperature, while producing a significant increase in locomotor activity at the 3 mg/kg *R*-VK4-40 dose. Specifically, two-way ANOVA of blood pressure revealed significant main effects of oxycodone ($F_{1,56} = 25.4, P < 0.001$) and *R*-VK4-40 pretreatment ($F_{3,56} = 16.8$, $P < 0.001$), and an oxycodone × *R*-VK4-40 interaction ($F_{3,56} = 3.4$, $P < 0.02$). Post-hoc comparisons indicated oxycodone alone and in combination with 3 mg/kg *R*-VK4-40 significantly increased blood pressure compared with vehicle ($P < 0.01$ and $P < 0.002$, respectively). In contrast, 20 mg/kg *R*-VK4-40 alone significantly decreased blood pressure compared with vehicle (Fig. 4A) ($P < 0.006$). This high dose of *R*-VK4-40 also attenuated the oxycodone-induced increases in blood pressure ($P < 0.001$). Two-way ANOVA also revealed a significant main effect of pretreatment ($F_{3,56} = 18.5$, $P < 0.001$) and a pretreatment × oxycodone interaction ($F_{3,56} = 3.6$, $P < 0.02$) on heart rate (Fig. 4B). Post-hoc tests revealed that 10 and 20 mg/kg *R*-VK4-40, alone or in combination with oxycodone, reduced heart rate compared with oxycodone alone ($P < 0.003$).

Two-way ANOVA revealed significant main effects of *R*-VK4-40 ($F_{3,56} = 3.9$, $P < 0.01$) and oxycodone ($F_{1,56} = 6.3$, $P < 0.01$) on locomotor activity, but no interaction (Fig. 4C). Oxycodone did not increase activity to statistically significant levels, except when combined with 3 mg/kg *R*-VK4-40 ($P < 0.003$). Two-way ANOVA revealed significant main effects of *R*-VK4-40 ($F_{3,56} = 35.9, P < 0.001$) and oxycodone ($F_{1,56} = 15.9$, $P < 0.001$), and a *R*-VK4-40 × oxycodone interaction ($F_{3,56} = 5.2$, $P < 0.003$) on temperature (Fig. 4D). Post-hoc testing indicated oxycodone alone and in combination with 3 mg/kg *R*-VK4-40 increased body temperature compared with vehicle (ps < 0.006). *R*-VK4-40 attenuated oxycodone-induced increases in body temperature at 20 mg/kg ($P < 0.001$).

***R*-VK4-116 and Oxycodone.** Figure 5 shows the effects of *R*-VK4-116 (5, 15, and 25 mg/kg, i.p., administered 30 minutes prior to telemetry sessions), in the presence of vehicle or oxycodone (1 mg/kg, i.p., administered 5 minutes prior to telemetry sessions), on blood pressure, heart rate, body temperature, and activity levels, illustrating that *R*-VK4-116 dose-dependently reduced body temperature alone and in combination with oxycodone, whereas oxycodone alone increased blood pressure and body temperature. Two-way ANOVA of blood pressure indicated only a significant main effect of oxycodone ($F_{1,56} = 15.9$, $P < 0.001$). Post-hoc testing revealed that *R*-VK4-116 neither attenuated nor potentiated oxycodone-induced increases in blood pressure at any of the doses tested (Fig. 5A). On heart rate, two-way ANOVA revealed significant main effects of *R*-VK4-116 ($F_{3,56} = 3.9$, $P < 0.01$) and oxycodone ($F_{1,56} = 14.2$, $P < 0.001$), and a *R*-VK4-116 × oxycodone interaction ($F_{3,56} = 3.9$, $P < 0.01$; Fig. 5B). Post-hoc testing revealed that *R*-VK4-116 neither attenuated nor potentiated heart rate in combination with oxycodone, at any of the doses tested.

As was the case with the previous results, two-way ANOVA indicated only a significant main effect of oxycodone ($F_{3,56} = 12.1$, $P < 0.001$) on activity. The combination of 15 mg/kg *R*-VK4-116 with oxycodone significantly increased activity compared with vehicle alone ($P < 0.02$). No other statistically significant effects were observed. Two-way ANOVA revealed

TABLE 1
Comparison of baselines and vehicle treatments (mean ± S.E.M.)

Treatment	Pressure	Heart Rate	Activity	Temperature
Baseline (no injection)	114.1 ± 1.5	377.2 ± 6.9	326.6 ± 67.1	38.1 ± 0.1
Saline	108.4 ± 1.2	339.6 ± 7.1	192.9 ± 17.8	37.8 ± 0.1
25% β-cyclodextrin	114.4 ± 1.2	354.5 ± 5.3	265.4 ± 29.0	37.7 ± 0.1
3% Cremophor	109.5 ± 1.9	339.6 ± 7.3	228.9 ± 50.9	38.0 ± 0.2

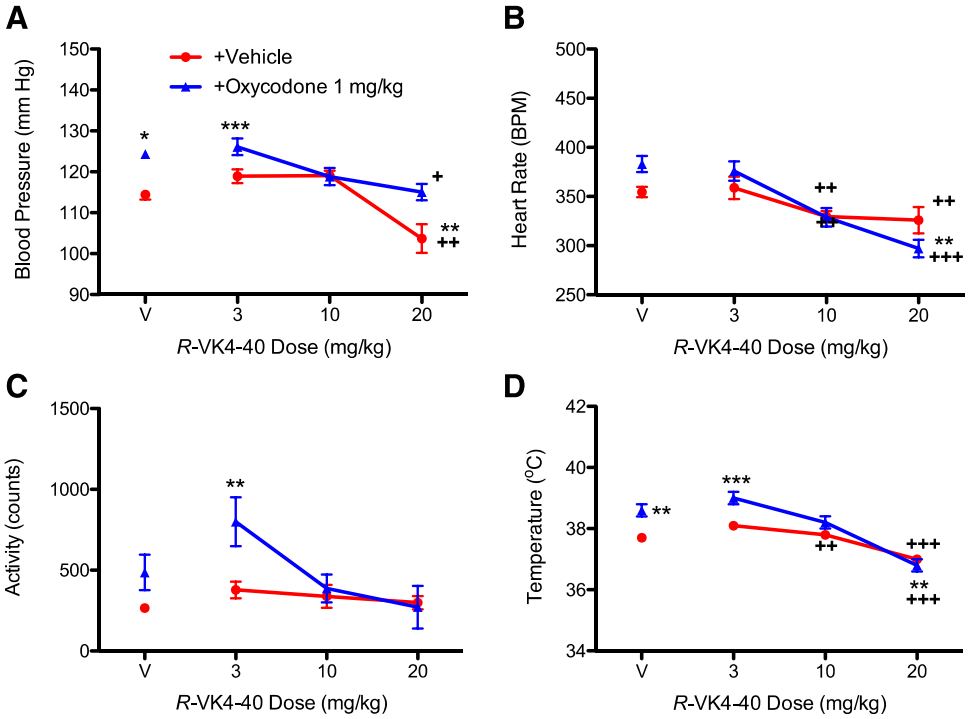

Fig. 4. Effects of oxycodone (1 mg/kg, i.p., administered 5 minutes prior to telemetry sessions) and *R*-VK4-40 (3, 10, and 20 mg/kg, i.p., administered 30 minutes prior to telemetry sessions), or a combination thereof, on blood pressure (mean arterial pressure) (A), heart rate (beats per minute) (B), activity levels (C), and body temperature (D). Analyses were restricted to averages of the data across the first hour of the session to ensure that the effects of each compound were maximal. Vehicle [(V), 2-hydroxypropyl β-cyclodextrin] administered 30 minutes prior to saline (red circle) or oxycodone administered 5 minutes prior to session (blue triangle). $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared with vehicle-vehicle; $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ compared with vehicle-oxycodone. Values are mean ± S.E.M.

significant main effects of pretreatment ($F_{3,56} = 30.9$, $P < 0.001$) and oxycodone ($F_{1,56} = 62$, $P < 0.001$), and a trend toward a pretreatment × oxycodone interaction ($F_{3,56} = 2.6$, $P < 0.06$) on body temperature. Post-hoc testing indicated that at the highest dose tested (25 mg/kg) *R*-VK4-116 attenuated oxycodone-induced increases in body temperature ($P < 0.001$) and reduced temperature when administered alone ($P < 0.004$).

***R*-VK4-40 and Cocaine.** Figure 6 shows the effects of *R*-VK4-40 (3, 10, and 20 mg/kg, i.p., administered 30 minutes prior to telemetry sessions) in the presence of vehicle or cocaine (10 mg/kg, i.p., administered 5 minutes prior to telemetry sessions) on blood pressure, heart rate, body temperature, and activity levels, illustrating that cocaine, at 10 mg/kg, increased blood pressure and activity levels, while *R*-VK4-40, particularly at the high 20 mg/kg dose tested, reduced blood pressure, heart rate, activity, and temperature when combined with cocaine. Two-way ANOVA of blood pressure revealed significant main effects of *R*-VK4-40 pretreatment ($F_{3,56} = 19.1$, $P < 0.001$) and cocaine ($F_{1,56} = 33.5$, $P < 0.001$), but no significant interaction. Post-hoc testing revealed that 3 and 10 mg/kg (but not 20 mg/kg) *R*-VK4-40

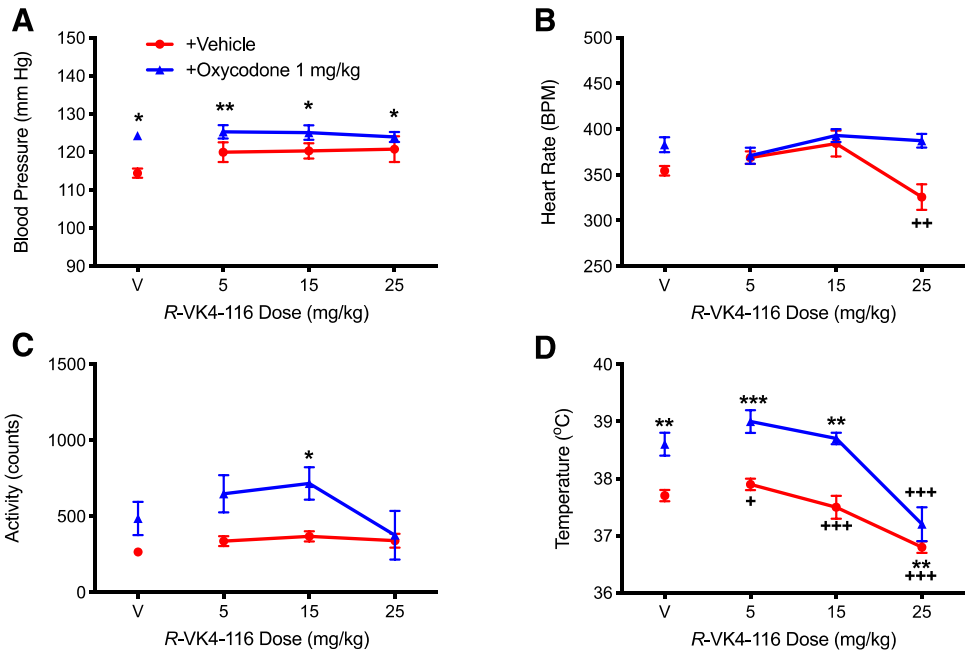

Fig. 5. Effects of oxycodone (1 mg/kg, i.p., administered 5 minutes prior to telemetry sessions) and *R*-VK4-116 (5, 15, and 25 mg/kg, i.p., administered 30 minutes prior to telemetry sessions), or a combination thereof, on blood pressure (mean arterial pressure) (A), heart rate (beats per minute) (B), activity levels (C), and body temperature (D). Analyses were restricted averages of the data across the first hour of the session to ensure that the effects of each compound were maximal. Vehicle [(V), 2-hydroxypropyl β-cyclodextrin] administered 30 minutes prior to saline (red circle) or oxycodone administered 5 minutes prior to session (blue triangle). $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared with vehicle-vehicle; $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ compared with vehicle-oxycodone. Values are mean ± S.E.M.

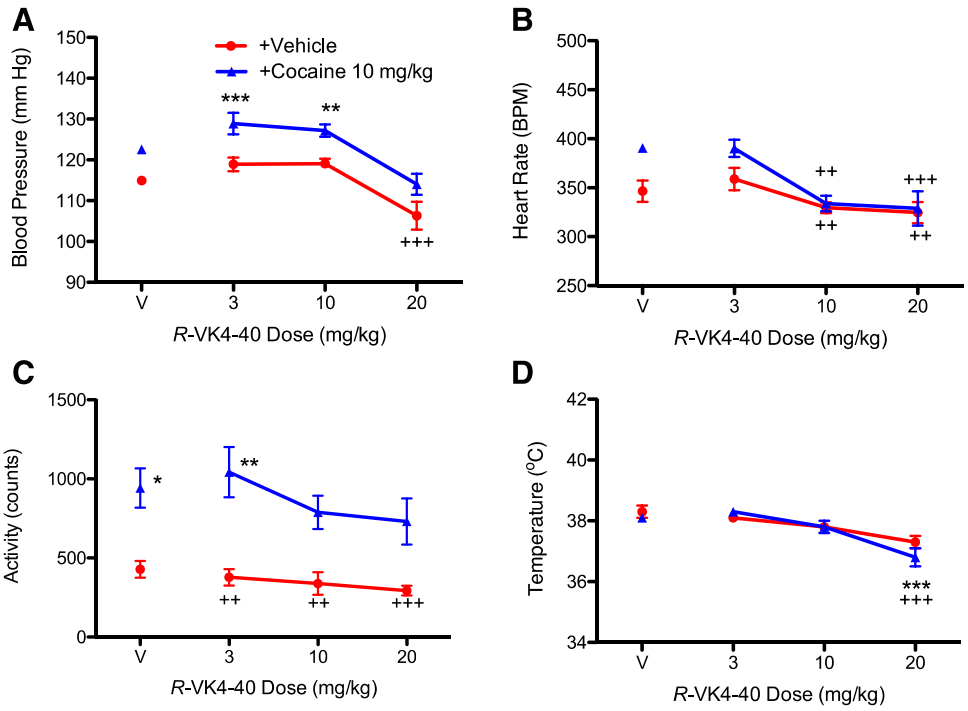

Fig. 6. Effects of cocaine (10 mg/kg, i.p., administered 5 minutes prior to telemetry sessions) and *R*-VK4-40 (3, 10, and 20 mg/kg, i.p., administered 30 minutes prior to telemetry sessions), or a combination thereof, on blood pressure (mean arterial pressure) (A), heart rate (beats per minute) (B), activity levels (C), and body temperature (D). Analyses were restricted to averages of the data across the first hour of the session to ensure that the effects of each compound were maximal. Vehicle [(V), 2-hydroxypropyl β-cyclodextrin] administered 30 minutes prior to saline (red circle) or cocaine administered 5 minutes prior to session (blue triangle) . $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared with vehicle-vehicle; $^{+++}P < 0.001$, $^{++}P < 0.01$ compared with vehicle-cocaine. Values are mean ± S.E.M.

increased blood pressure in the presence of cocaine compared with vehicle alone (ps < 0.002). In contrast, 20 mg/kg *R*-VK4-40 alone significantly reduced blood pressure compared with cocaine alone ($P < 0.001$). Two-way ANOVA revealed significant main effects of pretreatment ($F_{3,56} = 11.3$, $P < .001$) and cocaine ($F_{1,56} = 8.2$, $P < 0.001$) on heart rate, but no interaction. Post-hoc testing indicated that 10 and 20 mg/kg *R*-VK4-40 alone or in the presence of cocaine attenuated heart rate compared with cocaine administered alone (ps < 0.006).

On activity, two-way ANOVA indicated a significant main effect of cocaine only ($F_{1,56} = 74$, $P < 0.001$). Post-hoc tests revealed cocaine increased activity alone and in combination with 3 mg/kg *R*-VK4-40 (ps < 0.01), whereas 10 and 20 mg/kg *R*-VK4-40 blocked cocaine-induced increases in activity (such that there were no significant differences compared with vehicle). *R*-VK4-40 alone, at all doses tested, also significantly suppressed activity compared with cocaine alone (ps < 0.02). Two-way ANOVA revealed only a significant main effect of

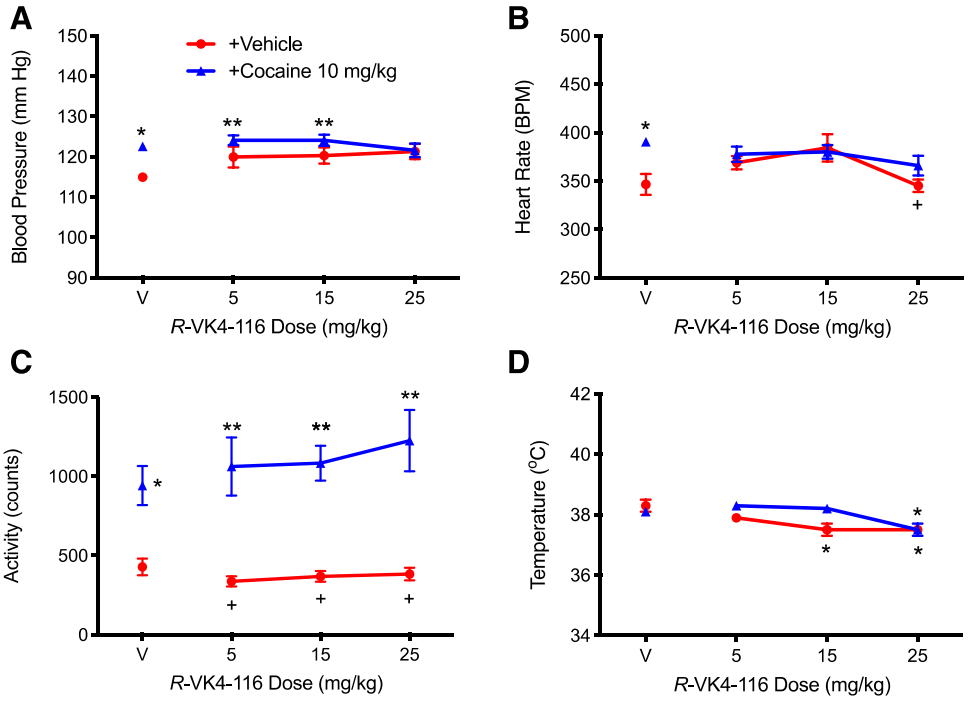

Fig. 7. Effects of cocaine (10 mg/kg, i.p., administered 5 minutes prior to telemetry sessions) and *R*-VK4-116 (5, 15, and 25 mg/kg, i.p., administered 30 minutes prior to telemetry sessions), or a combination thereof, on blood pressure (mean arterial pressure) (A), heart rate (beats per minute) (B), activity levels (C), and body temperature (D). Analyses were restricted to averages of the data across the first hour of the session to ensure that the effects of each compound were maximal. Vehicle [(V), 2-hydroxypropyl β-cyclodextrin] administered 30 minutes prior to saline (red circle) or cocaine administered 5 minutes prior to session (blue triangle). $^{**}P < 0.01$, $^{*}P < 0.05$ compared with vehicle-vehicle; $^{+}P < 0.05$ compared with vehicle-cocaine. Values are mean ± S.E.M.

pretreatment ($F_{3,56} = 17.8$, $P < 0.001$) on temperature. Post-hoc testing suggested that 20 mg/kg *R*-VK4-40 in combination with cocaine reduced body temperature compared with cocaine or vehicle alone (ps < 0.001).

***R*-VK4-116 and Cocaine.** Figure 7 shows the effects of *R*-VK4-116 (5, 15, and 25 mg/kg, i.p., administered 30 minutes prior to telemetry sessions) in the presence of vehicle or cocaine (10 mg/kg, i.p., administered 5 minutes prior to telemetry sessions), on blood pressure, heart rate, body temperature, and activity levels, illustrating that cocaine alone increased blood pressure, heart rate, and activity levels, while *R*-VK4-116 dose-dependently attenuated cocaine's effects on blood pressure and heart rate. Two-way ANOVA of blood pressure revealed only a significant main effect of cocaine ($F_{1,56} = 11.2$, $P < 0.001$). Post-hoc testing showed that compared with vehicle cocaine significantly increased blood pressure when administered alone ($P < 0.04$) or in combination with 5 and 15 mg/kg *R*-VK4-116 ($P < 0.008$ and 0.007, respectively). In contrast, 25 mg/kg *R*-VK4-116 attenuated cocaine-induced increases in blood pressure (such that there was not a significant difference from vehicle). Two-way ANOVA of heart rate revealed significant main effects of pretreatment ($F_{3,56} = 3.1$, $P < 0.03$) and cocaine ($F_{1,56} = 7.6$, $P < 0.008$), and a pretreatment × cocaine interaction ($F_{3,56} = 2.7$, $P < 0.05$). Post-hoc testing indicated cocaine alone significantly increased heart rate compared with vehicle alone ($P < 0.01$). All doses of *R*-VK4-116 blocked cocaine-induced increases in heart rate (such that there were no significant differences from vehicle alone).

For activity, two-way ANOVA revealed significant main effects of pretreatment ($F_{3,56} = 5.7$, $P < 0.001$) and cocaine ($F_{1,56} = 62.5$, $P < 0.001$), and a pretreatment × cocaine interaction ($F_{3,56} = 3.1$, $P < 0.03$). Post-hoc tests indicated *R*-VK4-116 did not attenuate cocaine-induced increases in activity at any dose tested (ps < 0.01 compared with vehicle). Two-way ANOVA indicated significant main effects of pretreatment ($F_{3,56} = 6.7$, $P < 0.001$) and cocaine ($F_{1,56} = 4.4$, $P < 0.04$), and a pretreatment × cocaine interaction ($F_{3,56} = 3.3$, $P < 0.02$) on body temperature. Post-hoc testing revealed cocaine in combination with 25 mg/kg *R*-VK4-116 reduced body temperature compared with vehicle alone ($P < 0.01$). *R*-VK4-116 alone, at 15 and 25 mg/kg doses, also significantly reduced temperature compared with vehicle alone (ps < 0.01).

SB-277,011A and Cocaine. To determine whether the finding that older-generation D_3R antagonists increase blood pressure in dogs (Appel et al., 2015; Appel and Acri, 2017) was replicable in our rodent model, we examined the impact of 30 mg/kg SB-277,011A alone (administered 30 minutes prior to telemetry sessions) and in the presence of 10 mg/kg cocaine (administered 5 minutes prior to telemetry sessions). Figure 8 shows the effects of SB-277,011A in the presence of vehicle or cocaine on blood pressure, heart rate, body temperature, and activity levels, illustrating that SB-277,011A, alone and in the presence of cocaine, increased blood pressure and heart rate. Two-way ANOVA of blood pressure revealed significant main effects of pretreatment ($F_{1,26} = 23.2$, $P < 0.001$) and cocaine ($F_{1,56} = 4.9$, $P < 0.03$), but no interaction. Post-hoc testing indicated SB-277,011A increased blood pressure both alone and in combination with cocaine (ps < 0.001). Two-way ANOVA of heart rate revealed a significant main effect of pretreatment only ($F_{1,26} = 13.7$, $P < 0.001$). Post-hoc testing indicated SB-277,011A increased heart rate both alone and in combination with cocaine (ps < 0.006).

On activity, two-way ANOVA again indicated significant main effects of pretreatment ($F_{1,26} = 6.4$, $P < 0.01$) and cocaine ($F_{1,26} = 24.2$, $P < 0.001$). Post-hoc testing revealed that

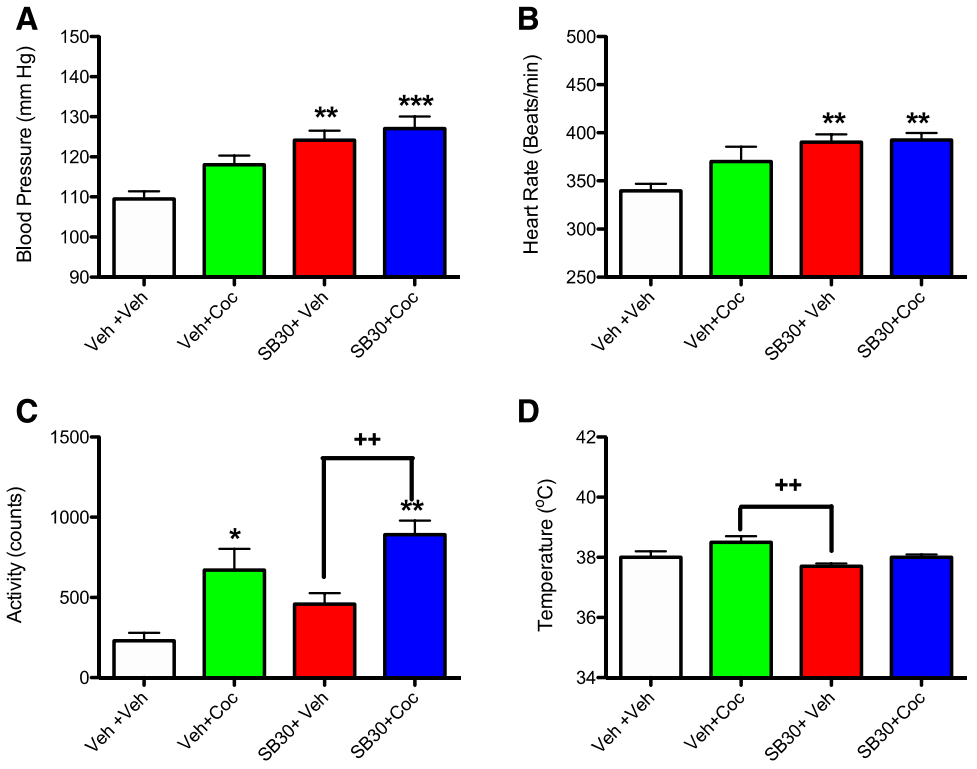

Fig. 8. Effects of SB-277,011A (30 mg/kg, i.p., administered 30 minutes prior to telemetry sessions), an older-generation D_3R antagonist, alone and in the presence of cocaine (10 mg/kg, i.p., administered 5 minutes prior to telemetry sessions) on blood pressure (mean arterial pressure) (A), heart rate (beats per minute) (B), activity levels (C), and body temperature (D). VEH-VEH denotes vehicle alone (2-hydroxypropyl β-cyclodextrin administered 30 minutes prior to saline, 5 minutes prior to session). Analyses were restricted to averages of the data across the first hour of the session to ensure that the effects of each compound were maximal. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared with vehicle-vehicle; $^{++}P < 0.01$ compared with vehicle-cocaine. Values are mean ± S.E.M.

cocaine alone or in combination with SB-277,011A increased activity levels (ps < 0.01). Two-way ANOVA revealed significant main effects of pretreatment ($F_{1,26} = 6.1$, $P < 0.02$) and cocaine ($F_{1,26} = 5.9$, $P < 0.02$) on temperature, but no interaction. Post-hoc testing suggested SB-277,011A alone decreased temperature compared with cocaine alone ($P < 0.008$).

L-741,626 Alone. Finally, to determine whether the effects of *R*-VK4-40, *R*-VK4-116, and SB-277,011A are specific to D_3R antagonism, we administered 3 mg/kg L-741,626 (a D_2R-selective antagonist) 5 minutes prior to telemetry sessions. Figure 9 shows the effects of L-741,626 on blood pressure, heart rate, body temperature, and activity levels. Two-way ANOVA of blood pressure revealed a significant main effect of dose ($F_{2,18} = 5.7$, $P < 0.01$). Post-hoc testing indicated that both 3 and 10 mg/kg L-741,626 increased blood pressure compared with vehicle (ps < 0.03). Two-way ANOVA of heart rate also indicated a significant dose effect ($F_{2,18} = 6$, $P < 0.01$). Post-hoc testing revealed that 3 mg/kg L-741,626 increased heart rate compared with vehicle ($P < 0.009$). There was also a trend toward an increase in heart rate by 10 mg/kg L-741,626 ($P < 0.06$). There were no significant effects of either dose of L-741,626 on activity levels or temperature.

Discussion

The main purpose of the present study was to investigate whether newly developed D_3R antagonists share the adverse cardiovascular effects of previous compounds targeting this site. In contrast to the effects of SB-277,011A, the recently discovered compounds *R*-VK4-40 and *R*-VK4-116 did not potentiate the cardiovascular effects of oxycodone or cocaine. Rather, moderate-to-high doses of *R*-VK4-40 reduced blood pressure and heart rate when administered alone, and attenuated oxycodone-induced increases in blood pressure and oxycodone or cocaine-induced increases in heart rate and body temperature. Similarly, moderate-to-high doses of *R*-VK4-116 reduced body temperature when administered alone, and suppressed oxycodone-induced increases in temperature and cocaine-induced increases in blood pressure and heart rate. Reductions in cardiovascular parameters by *R*-VK4-40 and *R*-VK4-116 may be specific to D_3R, and not D_2R antagonism, because the D_2R antagonist, L-741,626, increased blood pressure and heart rate. Consistent with prior reports using radiotelemetry in dogs receiving intravenously administered cocaine (Appel and Acri, 2017), we found that the older-generation D_3R antagonist, SB-277,011A, increased blood pressure, heart rate, and activity both alone and in the presence of intraperitoneally administered cocaine in rats.

Of note, *R*-VK4-40, *R*-VK4-116, and SB-277,011A were dissolved in 25% 2-hyroxypropyl β-cyclodextrin. Prior studies have suggested that β-cyclodextrin induces phenotypic and functional maturation of dendritic cells and disrupts lipid raft formation, which can also affect blood pressure (Gildea et al., 2011; Kim et al., 2016). However, the doses and routes of administration used to induce changes in cellular maturation and alter lipid rafts markedly differ from those used to dissolve D_3R-targeted compounds in the present study. For example, previous in vivo experimentation involved 3 mg quantities of β-cyclodextrin injected directly into the mouse hindpaw, or 80 μg/kg per minute delivered directly into the renal cortex of Sprague-Dawley rats via osmotic minipumps

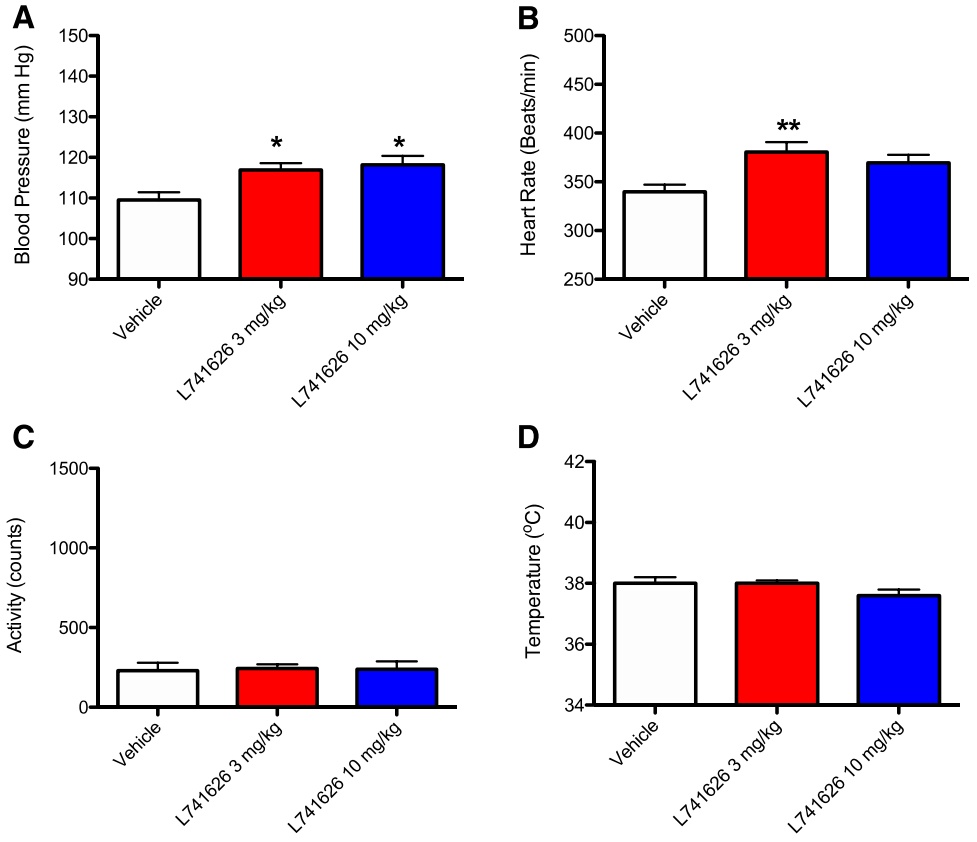

Fig. 9. Effects of vehicle (3% cremophor) or L-741,626 (3 or 10 mg/kg, i.p., administered 5 minutes prior to telemetry sessions), a D_2R-selective antagonist, alone on blood pressure (mean arterial pressure) (A), heart rate (beats per minute) (B), activity levels (C), and body temperature (D). Analyses were restricted to averages of the data across the first hour of the session to ensure that the effects of each compound were maximal. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with vehicle. Values are mean ± S.E.M.

(Kim et al., 2016; Gildea et al., 2011). In contrast, our rats received intraperitoneal doses of 0.25 mg/kg, or approximately 0.11–0.125 mg/treatment of 2-hydroxypropyl β-cyclodextrin. Similarly, cremophor, which was used to dissolve L-741,626, can induce neuropathy, among other effects (Gelderblom et al., 2001). However, doses of up to 30 ml/m^2 as a 3-hour infusion can be safely administered (Gelderblom et al., 2001). In the present study, cremophor was administered in a 3% solution, amounting to approximately 0.015 mg/treatment or 0.03 mg/kg. Considering these differences in dosage and methodology, and because the respective vehicles did not differ in their cardiovascular impact, we do not anticipate changes in blood pressure, heart rate, body temperature, or activity can be attributed to vehicle effects.

In the present study, both intraperitoneal oxycodone (1 mg/kg) and cocaine (10 mg/kg) maintained increased blood pressure and activity levels over the course of the session when compared with their vehicle treatments, consistent with prior reports on acute intravenous and subcutaneous opioid or cocaine treatment (Yeh and Haertzen, 1991; Ambrosio et al., 1996; Tella et al., 1999; Ilbäck et al., 2008; Appel et al., 2015; Appel and Acri, 2016, 2017; Collins et al., 2016; You et al., 2017). Oxycodone further increased body temperature, similar to alternative μ-opioid receptor agonists such as morphine and buprenorphine (Ilbäck et al., 2008; Froger-Colléaux et al., 2011), whereas cocaine augmented heart rate, as observed previously (Ambrosio et al., 1996; Tella et al., 1999). Augmented activity levels by oxycodone and cocaine are presumably mediated by increased dopamine release in the mesocorticolimbic system (Narita et al., 1993; Runegaard et al., 2018). Increases in activity can consequently facilitate increases in heart rate and temperature, and subsequent increases in blood pressure (and vice versa; Ilbäck et al., 2008; Christofaro et al., 2017). However, these four metrics did not consistently overlap in the current experiments. For example, low-to-moderate doses of *R*-VK4-40 and *R*-VK4-116 potentiated oxycodone-related hyperactivity, but attenuated oxycodone-induced increases in heart rate and temperature. These conflicting results suggest changes in activity cannot explain altered cardiovascular function after drug treatment. While cocaine primarily increases blood pressure and heart rate by altering sympathetic outflow through action within the central nervous system (Kiritsy-Roy et al., 1990; Schindler et al., 1992; Tella et al., 1993), cocaine also impacts cardiovascular function via the peripheral sympathetic nervous system, such as through inhibition of norepinephrine reuptake in sympathetic nerve terminals (Muscholl, 1961; Tuncel et al., 2002; Havakuk et al., 2017). In contrast, the cardiovascular and temperature effects of opioids are more directly mediated by μ-opioid receptors within the central nervous system. Brain regions that regulate cardiovascular tone, such as the paraventricular nucleus of the hypothalamus, contain a high density of μ-opioid receptors (Kannan et al., 1989; Zheng et al., 2005), and intracerebral microinjection of μ-receptor agonists (e.g., DAMGO; D-Ala(2)-mephe(4)-gly-ol(5))enkephalin) increases blood pressure and heart rate (Hill-Pryor et al., 2006; Ilbäck et al., 2008).

The new generation D_3R antagonists, *R*-VK4-40 and *R*-VK4-116, attenuated oxycodone and cocaine-induced increases in blood pressure, heart rate, and body temperature to varying degrees. Whether this attenuation is centrally or peripherally mediated remains unclear. Although regions of the brain participating in cardiovascular regulation, such as the paraventricular nucleus of the hypothalamus, express D_2R (as shown by in situ hybridization and Cre reporter lines; Clark et al., 2017), D_3R expression has not been reported in this region. Rather, D_3R distribution in the brain appears relatively constricted to the mesocorticolimbic system (Heidbreder and Newman, 2010; Keck et al., 2015; Sokoloff and Le Foll, 2017), which is unlikely to mediate cardiovascular tone. However, D_3R is also expressed in the kidneys, including the proximal and distal convoluted tubules, cortical collecting ducts, glomeruli, and renal vasculature (O'Connell et al., 1998; Jose et al., 2002; Nürnberger et al., 2004; Zeng et al., 2007). D_3R activation reduces angiotensin II type 1 receptor expression in the renal proximal tubules, an interaction that, when impaired, mediates the pathogenesis of hypertension (Mühlbauer et al., 2000; Luippold et al., 2001; Zeng et al., 2006), and global deletion of the D_3R increases systolic and diastolic blood pressure via augmented renin production and subsequent renal sodium retention (Wang et al., 2015). Together, these observations suggest that *R*-VK4-40 and *R*-VK4-116 attenuation of oxycodone and cocaine cardiovascular effects may be due to peripherally mediated D_3R effects in the kidneys, although additional studies are needed to confirm this hypothesis.

Although expressed at lower levels and in different regions than D_3R in the kidney, D_2R also participates in regulating renal function and blood pressure (Jose et al., 2002; Armando et al., 2011; Han et al., 2015; Konkalmatt et al., 2016). Accordingly, D_2R/D_3R agonists produce vasodilation, bradycardia, and renin-dependent hypotension (Zeng et al., 2007; Tayebati et al., 2011). Our finding that L-741,626 (a D_2R-selective antagonist) and SB,277-011A (a D_3R-selective antagonist) increased blood pressure and heart rate is thus consistent with the role of these receptors in regulating blood pressure. However, the reasons for which *R*-VK4-40 and *R*-VK4116 did not potentiate the adverse cardiovascular effects of oxycodone and cocaine, in contrast to current and previous observations with other D_3R antagonists such as SB,277-011A and GSK598,809, remain unclear. One explanation may be due to relative differences in affinity for D_3R (Table 2). For example, *R*-VK4-40 had the greatest impact on decreasing blood pressure, and also the highest affinity for D_3R among the compounds tested (Jordan et al., 2019b). However, in prior studies GSK598,809 increased blood pressure (Appel et al., 2015; Appel and Acri, 2016, 2017) and exhibited an affinity for D_3R that is similar to *R*-VK4-116. Although relative binding affinities may vary by assay and experimental conditions, these observations nonetheless suggest that differences in D_3R affinity cannot fully explain the current findings. A second explanation may be due to the compounds' relative affinities for $D_3R > D_2R$. However, both SB-277,011A and *R*-VK4-40 are ~200-fold selective for D_3R sites over D_2R sites (Table 2), yet exert opposing effects on blood pressure and heart rate. A third possibility is that these compounds have off-target binding sites that have not yet been described, such as at dopamine D_1 or serotonergic receptors, which also participate in cardiovascular tone (Zeng et al., 2007; Armando et al., 2011). A final possibility is that the structural differences between these compounds (see Fig. 1) preferentially activate different intracellular signaling pathways in a biased manner, as has been reported previously for opioid receptor agonists (Schmid et al., 2017) and recently for

TABLE 2
Binding affinities of D_3R/D_2R antagonists

Compound	Reference	K_i Value		D_2/D_3 Ratio	Blood Pressure Effect
		D_3R	D_2R		
		nM	*nM*		
GSK598,809	Keck et al., 2015	3.15	2110	670	Increased
SB-277,011A	Newman et al., 2005	10.7	2820	263	Increased
R-VK4-40	Jordan et al., 2019b	0.29	75.8	261	Decreased
R-VK4-116	Shaik et al., 2019	5.97	10,200	1709	—
L-741,626	Grundt et al., 2007	163	11.2	0.069	Increased

dopamine receptor ligands (Weïwer et al., 2018; Montgomery et al., 2019). As one example, cariprazine, a D_3R-preferring partial agonist used in the treatment of schizophrenia, may display either antagonist or partial agonist effects depending upon receptor location, G-protein coupling, and dopaminergic tone (Kiss et al., 2010; De Deurwaerdère, 2016). In the kidney, both D_3R and D_2R have multiple splice variants and couple to intracellular signaling pathways ranging from $G_{\alpha i}$, G_o, G_z, G_{q11}, $G_{\alpha q}$, phospholipase D, and other effectors, which have varying downstream effects on adenyl cyclase, mitogen-activated protein kinase (MAPK), and ion channel activity dependent upon their location (Armando et al., 2011). Additional studies will be necessary to determine the relative contributions of off-target binding sites and biased signaling to the cardiovascular impact of various D_3R antagonists.

The finding that both D_3R antagonists produced substantial and prolonged hypothermia might suggest a role for D_3R in the maintenance of body temperature. While there is not abundant evidence for a role of dopamine in temperature control (Madden and Morrison, 2019), previous work has shown that stimulation of dopamine receptors typically produces hypothermia (Cox, 1977; Lipton and Clark, 1986). Alternatively, the decrease in temperature may be due to a decrease in activity, although this seems unlikely given the small changes in activity observed following administration of the D_3R antagonists alone. Further research will be necessary to confirm a role for D_3R in temperature regulation.

Both present and past studies on the cardiovascular effects of dopamine D_3R antagonists and cocaine have been conducted solely in male subjects (Appel et al., 2015, Appel and Acri, 2016, 2017). Likewise, our previous studies on (±)VK4-116 and *R*-VK4-40 attenuation of oxycodone reward and withdrawal and enhancement of analgesia were conducted solely in male rats (Jordan et al., 2019b; You et al., 2019). On this basis, we elected to focus on the cardiovascular effects of these compounds in male rats, although additional studies on opioid reward and analgesia are now underway in female rats. Prior studies in both humans and rodents have shown that females are more resilient to the cardiovascular effects of stimulants than males (Mendelson et al., 1999; Lynch et al., 2008; McClenahan et al., 2019) and neither cardiovascular responses to cocaine nor cocaine pharmacokinetics vary across phases of the menstrual cycle (Mendelson et al., 1999). Although relatively few studies exist on sex differences in the cardiovascular effects of opioids, no sex differences in heart rate responses to acutely administered morphine have been identified (Cruz and Rodríguez-Manzo, 2000). With respect to the D_3R, no sex differences in behavioral (Chang et al., 2010) or dopamine responses (McGinnis et al., 2016) to D_2R/D_3R ligands have been observed in rodents. However, both lean and obese female rats do express higher D_3R in the kidney than males (Wang et al., 2010), and in primates females are less sensitive to quinpirole-induced (a D_3R-preferring agonist) yawning than males (Martelle et al., 2014). Taken together, these observations suggest that females would likely experience either no differences or attenuated cardiovascular responses to cocaine, oxycodone, and dopamine D_3R antagonism compared with males. However, further studies are clearly warranted.

In conclusion, results of the present study challenge the assumption that all D_3R antagonists adversely impact cardiovascular parameters as a class effect. Contrary to current and prior observations with older-generation D_3R antagonists such as SB-277,011A and GSK598,809, *R*-VK4-40 and *R*-VK4-116 do not potentiate oxycodone- or cocaine-induced increases in blood pressure, heart rate, or body temperature, and in some cases reverse the adverse cardiovascular effects of these drugs of abuse. Taken together, these observations support the development of *R*-VK4-40 and *R*-VK4-116 and structurally related compounds for further translation to treating opioid and/or cocaine use disorders.

Acknowledgments

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Authorship Contributions

Participated in research design: Jordan, Schindler, Baumann, Xi, Newman.

Conducted experiments: Jordan, Humburg, Thorndike, Shaik.

Performed data analysis: Jordan, Humburg, Schindler.

Wrote or contributed to the writing of the manuscript: Jordan, Schindler, Humburg, Baumann, Xi, Newman.

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