The Serotonin 2C Receptor Antagonist SB 242084 Exhibits Abuse-Related Effects Typical of Stimulants in Squirrel Monkeys

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Received April 2, 2012; accepted June 7, 2012

ABSTRACT

Antagonists of the serotonin (5-hydroxytryptamine; 5-HT) type 2C receptor (5-HT_{2C}R) are being considered as potential pharmacotherapeutics for various affective disorders, but evidence suggests that these compounds enhance the effects of cocaine and related psychostimulants in rodents. However, the effects of selective 5-HT_{2C}R antagonists have not been evaluated in nonhuman primates. The present studies used operant-behavioral and in vivo microdialysis techniques to assess the impact of 5-HT_{2C}R antagonism on the behavioral and neurochemical effects of cocaine in squirrel monkeys. In subjects trained to lever-press on a fixed-interval schedule of stimulus termination, pretreatment with the highly selective 5-HT_{2C}R antagonist 6-chloro-2,3-dihydro-5-methyl-N-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxamide dihydrochloride (SB 242084) (vehicle, 0.01–0.1 mg/kg) produced behavioral-stimulant effects alone and interacted with cocaine in an apparently additive manner. In monkeys trained to self-

administer intravenous cocaine according to a second-order schedule of drug delivery, SB 242084 (vehicle, 0.03–0.1 mg/kg) modulated cocaine-induced reinstatement of previously extinguished responding and maintained self-administration behavior when substituted for cocaine availability. These studies are the first to assess the direct reinforcing effects of a 5-HT_{2C}R-selective antagonist in any species. Finally, in vivo microdialysis studies revealed that pretreatment with SB 242084 (0.1 mg/kg) modulated cocaine-induced dopamine increases within the nucleus accumbens, but not the caudate nucleus, of awake subjects. Taken together, the results suggest that SB 242084 exhibits a behavioral profile that is qualitatively similar to other psychostimulants, although its efficacy is modest compared with cocaine. The observed interactions with cocaine and the substitution for cocaine self-administration may be indicative of some degree of abuse potential in humans.

Introduction

Serotonin (5-hydroxytryptamine; 5-HT) receptors comprise a complex signaling system, with at least 14 distinct receptor subtypes having been identified (for review, see Hoyer et al.,

These studies were funded by the National Institutes of Health National Institute on Drug Abuse [Grants DA12514, DA00517, F31DA026262]; the National Institutes of Health National Center for Research Resources [Grant P51RR165]; the National Institutes of Health Office of the Director Office of Research Infrastructure Program [Grant OD P51OD11132]; and the American Recovery and Reinvestment Act of 2009 [Grant F31DA026262].

These studies represent partial fulfillment of D.F.M.'s Ph.D. dissertation research at Emory University.

Preliminary findings from these experiments were previously presented: Manvich DF and Howell LL (2011) Cocaine-induced reinstatement is differentially modulated by agonism and antagonism of the serotonin 5-HT_{2C} receptor in nonhuman primates, at the *American Society for Pharmacology and Experimental Therapeutics Meeting*; 2011, April 9–13; Washington D.C. American Society for Pharmacology and Experimental Therapeutics, Bethesda, MD.

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.

<http://dx.doi.org/10.1124/jpet.112.195156>.

2002). These receptors, along with the serotonin reuptake transporter protein, serve as pharmacotherapeutic targets for a wide range of physiological and psychiatric disorders (Meltzer, 1999; Jones and Blackburn, 2002; Vaswani et al., 2003; Hensler, 2006). However, a large proportion of clinically available compounds, most notably the selective serotonin reuptake inhibitors, indiscriminately modulate the activity of many or all serotonin receptor subtypes. Consequently, these medications may induce undesirable side effects (Vaswani et al., 2003; Murphy et al., 2008). Over the past decade, the development of compounds demonstrating greater selectivity among the 5-HT receptor subtypes has led to new lines of research investigating their potential use as novel treatments that maintain the therapeutic benefits of their predecessors but limit unwanted side effect profiles.

With the availability of selective agonists and antagonists, the 5-HT_{2C} receptor (5-HT_{2C}R) in particular has been indicated as a novel pharmacotherapeutic target for several psy-

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); 5-HT_{2C}R, 5-HT_{2C} receptor; ANOVA, analysis of variance; DA, dopamine; FI, fixed interval; FR20, fixed-ratio 20; NAc, nucleus accumbens; SB 242084, 6-chloro-2,3-dihydro-5-methyl-N-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxamide dihydrochloride; veh, vehicle; VTA, ventral tegmental area.

chopathological disorders (Lee et al., 2010). For example, in preclinical rodent studies, 5-HT_{2C}R-selective antagonists have demonstrated antidepressant-like effects (Dekeyne et al., 2008), enhanced the antidepressant-like effects of selective serotonin reuptake inhibitors (Cremers et al., 2004), and displayed an anxiolytic-like spectrum of behaviors (Kantor et al., 2005; Harada et al., 2006; Burghardt et al., 2007; Dekeyne et al., 2008). Although the precise neurobiological mechanism of action underlying these effects remains unclear, studies have consistently demonstrated that 5-HT_{2C}Rs exert a modulatory role on dopamine (DA) activity. The mesocorticolimbic system is comprised of DA-releasing neurons originating in the ventral tegmental area (VTA) that send axonal projections to the nucleus accumbens (NAc) and prefrontal cortex. The 5-HT_{2C}R is highly expressed within the mesocorticolimbic DA system of rodents (Alex and Pehek, 2007; for review, Bubar and Cunningham 2008), nonhuman primates (López-Giménez et al., 2001), and humans (Pasqualetti et al., 1999). Functionally, systemic administration of the selective 5-HT_{2C}R antagonist SB 242084 (6-chloro-2,3-dihydro-5-methyl-N-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxamide dihydrochloride) in rats increases the firing rate of VTA dopaminergic neurons and elevates extracellular DA levels at terminal regions within the NAc (Di Matteo et al., 1999), effects that are similar to those of other drugs of abuse (Di Chiara and Imperato 1988). Accordingly, 5-HT_{2C}R antagonism was found to potentiate cocaine-induced elevations of DA within the rat NAc (Navailles et al., 2004) and enhance cocaine-induced hyperlocomotion and cocaine self-administration (Fletcher et al., 2002).

Given that 5-HT_{2C}R antagonists are being investigated as novel medications for the treatment of depression and anxiety disorders in humans, it is critical to note that these conditions may often present as comorbid with substance abuse, which can complicate both diagnosis and treatment (Regier et al., 1990; Brady and Verduin, 2005). One might therefore consider a deleterious situation in which 5-HT_{2C}R antagonists are used clinically to treat one or more affective disorders, but simultaneously exacerbate a concurrent condition of drug abuse or dependence. To address this concern, we sought to characterize the behavioral and neurochemical consequences of 5-HT_{2C}R antagonism by using nonhuman primate operant-conditioning models of stimulant-like behavior, cocaine self-administration, and in vivo microdialysis. In the first experiment, the impact of pretreatment with the highly selective 5-HT_{2C}R antagonist SB 242084 on the behavioral-stimulant effects of cocaine was assessed in squirrel monkeys trained to lever-press according to a fixed-interval (FI) 300-s schedule of stimulus termination. In subsequent experiments, intravenous self-administration and drug-primed reinstatement procedures were used to determine whether SB 242084 exhibited reinforcing effects alone, demonstrated a capacity to induce reinstatement of previously extinguished cocaine-maintained responding, or enhanced the reinstatement effects of cocaine. Finally, in vivo microdialysis techniques were used to characterize the impact of SB 242084 pretreatment on the increase in extracellular DA levels within the NAc and caudate nucleus after cocaine administration. Taken together, the results presented here provide an assessment of the behavioral and neurochemical effects of a selective 5-HT_{2C}R antagonist

alone and in combination with cocaine in nonhuman primates. Furthermore, these findings elaborate further on the potential use of 5-HT_{2C}R antagonists as novel pharmacotherapeutics for affective disorders.

Materials and Methods

Subjects

Fourteen adult male squirrel monkeys (*Saimiri sciureus*) weighing 850 to 1300 g served as subjects. Between experimental sessions, animals were individually housed in a climate-controlled room and fed twice daily (LabDiet 5045 High Protein Monkey Chow, PMI Nutrition International, Brentwood, MO; fresh fruit/vegetables; cereal) with ad libitum access to water. Daily enrichment was provided. Each animal had served in previous behavioral studies involving administration of compounds acting on monoaminergic and/or glutamatergic systems (Ginsburg et al., 2005; Kimmel et al., 2005, 2007, 2009; Banks et al., 2009; Bauzo et al., 2009; Fantegrossi et al., 2009). All studies were conducted in strict accordance with the National Institutes of Health's *Guide for Care and Use of Laboratory Animals* and the American Association for Accreditation of Laboratory Animal Care and were approved by the Institutional Animal Care and Use Committee of Emory University.

Apparatus

During behavioral sessions, animals were comfortably seated in a commercially available Plexiglas chair within a ventilated, sound-attenuating chamber (MED Associates, St. Albans, VT). The chair was equipped with an operant panel consisting of a series of red and white lights, a lever, and a white-noise amplifier, which was activated throughout the duration of all behavioral sessions to further reduce the influence of ambient noise. Med-PC IV software (MED Associates) was interfaced with each chamber to allow for automated output control and lever-press recording. For self-administration and reinstatement studies, a motor-driven syringe pump (model PHD2000; Harvard Apparatus, Inc., Holliston, MA) was mounted on the outer wall of the operant chamber, which held a 35-cc syringe containing appropriate concentrations of cocaine, SB 242084, or their vehicles. Each syringe was connected via stainless-steel adaptors and polyvinyl chloride tubing to the external portion of the subject's catheter during behavioral sessions.

During microdialysis sessions, subjects were seated in Plexiglas chairs supplemented with an adjustable Lexan barrier that was situated slightly above the level of the animal's shoulders to prevent disturbance to microdialysis probes and connective tubing. A motor-driven syringe pump (model 11Plus Dual-Syringe; Harvard Apparatus, Inc.) was mounted on top of the operant chamber for automated delivery of microinfused solutions.

Surgery

For self-administration and reinstatement experiments, subjects were prepared with chronic indwelling venous catheters under aseptic conditions as described previously (Kimmel et al., 2007; Bauzo et al., 2009). For microdialysis experiments, subjects were implanted with bilateral guide cannulae (CMA/11; CMA/Microdialysis, Acton, MA) by using stereotaxic techniques under aseptic conditions as described previously (Czoty et al., 2000). Guide cannulae targeted the caudate nucleus and nucleus accumbens by using the following coordinates from the ear bar: anterior/posterior, +15.0; medial/lateral, $\pm$ 3.0; and dorsal/ventral, -11.0. When not in use, stainless-steel stylets were situated within the cannulae to maintain the integrity of the tissue site. For all surgical procedures, preoperative antibiotics (ceftriaxone) and postoperative analgesics (meloxicam or flunixin) were administered by veterinary staff members who closely monitored the animals.

Procedure

Fixed-Interval Stimulus Termination. Daily sessions were conducted 5 days per week and lasted approximately 90 min. Each session began with the illumination of a pair of red lights. During a 300-s FI, lever presses were recorded but had no programmed consequences. Once the FI elapsed, the schedule progressed into a 3-s limited hold. A single response during the limited hold extinguished the red lights and illuminated a white light for 15 s to signal reinforcement. If the animal failed to press the lever during the limited hold, a mild electrical stimulus (3–6 mA; 300 ms) was delivered to a shaved portion of the tail. A daily session consisted of 15 consecutive fixed-interval components separated by 60-s timeout periods during which all lights were extinguished. Experimental sessions involving drug pretreatments were conducted twice per week (Tuesday and Friday). Cocaine (veh, 0.1–3.0 mg/kg i.m.) was administered 5 s before the onset of the session. SB 242084 (veh, 0.01–0.1 mg/kg i.m.) was administered 30 min before cocaine. The order of dose combinations was randomized within each subject.

Second-Order Cocaine Self-Administration/Substitution. Daily sessions were conducted 5 to 7 days per week and lasted approximately 60 min. Each session began with the illumination of a pair of red lights. During a 600-s FI, a fixed-ratio 20 (FR20) operant schedule was superimposed such that every 20th lever press extinguished the red lights and briefly illuminated a white light for 2 s, followed immediately by reillumination of the red lights. Once the FI elapsed, the schedule progressed into a 200-s limited hold. The first completed FR20 within the limited hold extinguished the red lights and resulted in an intravenous bolus infusion of cocaine (0.1 mg/kg/infusion in 0.5 ml; 25 ml/min flow rate) paired with a 15-s white light, followed by a 60-s timeout during which all lights were extinguished and responses had no programmed consequences. If the animal failed to complete a FR20 during the limited hold, the red lights were extinguished and the schedule moved directly into the timeout. Each daily session consisted of five FI components. Responding was deemed stable when response rates for each session varied <20% across 3 consecutive days. Once responding was stable, the unit dose of cocaine was altered and allowed to stabilize until the maximally effective unit dose of cocaine (ED_{Max} , i.e., the unit dose of cocaine that maintained highest rates of responding) was identified for each individual subject. Before substitution tests, subjects were allowed to self-administer their respective ED_{Max} unit dose of cocaine until responding stabilized as stated above. On substitution test days after stable cocaine self-administration, intravenous SB 242084 (veh, 0.01–0.1 mg/kg/infusion) was substituted for cocaine availability throughout the session. The unit dose of SB 242084 was held constant across consecutive sessions until response rates stabilized as described above. To prevent long-term disruptions in operant behavioral output, responding was considered extinguished when response rates reached $\leq 20\%$ baseline ED_{Max} cocaine self-administration for two consecutive sessions. Animals were allowed to restabilize on ED_{Max} cocaine self-administration between SB 242084 substitution tests. The order of SB 242084 doses substituted was randomized within each subject.

In the second substitution experiment, the reinforcing effects of SB 242084 were assessed when the drug was made available immediately after exposure to saline-extinction sessions. After stable self-administration with ED_{Max} cocaine availability, responding was extinguished by substituting saline for cocaine availability and withholding response-contingent presentations of the conditioned reinforcer (white light) throughout the session. Responding was deemed extinguished when the overall response rate within a single session reached $\leq 20\%$ of the mean response rate of the ED_{Max} cocaine self-administration sessions. Once extinction criteria were satisfied, parameters for the impending session were restored to that of a normal maintenance session with 0.03 mg/kg/infusion SB 242084 available for self-administration. Daily sessions continued until responding was stable across three consecutive sessions. The

dose of SB 242084 used was chosen based on results from the prior multiple-dose substitution experiment.

Cocaine-Primed Reinstatement. For reinstatement experiments, the ED_{Max} unit dose for cocaine self-administration was assessed for each individual animal as described for substitution studies. The reinstatement procedure used consisted of three phases. During “maintenance,” animals were allowed to self-administer their respective ED_{Max} of cocaine until responding stabilized across three consecutive sessions. Subjects then progressed to the “extinction” phase during which responding was extinguished as described above. “Reinstatement” tests occurred on the day immediately after successful extinction of responding. Five minutes before the onset of the session, animals were administered a noncontingent, intravenous bolus infusion (“prime”) of cocaine (veh, 0.03–1.0 mg/kg). Response-contingent conditioned reinforcers were reintroduced, but saline was substituted for cocaine infusions throughout the duration of the session. For each subject, the dose of cocaine prime that induced maximal rates of responding was deemed the ED_{Peak} . The ED_{Peak} for each individual subject was typically one-half log-unit above the ED_{Max} unit dose for maintenance cocaine self-administration sessions. For drug combination studies, SB 242084 (veh, 0.03–0.1 mg/kg i.m.) was administered 30 min before the pre-session prime. Each reinstatement test was separated by a reestablishment of maintenance cocaine self-administration and subsequent extinction. The order of dose combinations was randomized within each subject.

In Vivo Microdialysis. The microdialysis protocols used in the present study have been described previously in detail (Czoty et al., 2000; Kimmel et al., 2005; Bauzo et al., 2009). In brief, a commercially available microdialysis probe with a 14-mm shaft length and 4×0.24 -mm active membrane for caudate access or with a 20-mm shaft length and 2×0.24 -mm active membrane for nucleus accumbens access (CMA/11 MD Probe; CMA Microdialysis, North Chelmsford, MA) was inserted into the guide cannula and perfused with artificial cerebrospinal fluid (1.0 mM Na_2HPO_4 , 150 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, and 0.15 mM ascorbic acid, pH 7.4–7.56) at a rate of 2.0 μ l/min. After a 60-min equilibration period elapsed, three baseline samples were collected at 10-min intervals before drug treatment for determination of basal DA concentrations. After baseline sample collection, SB 242084 (veh, 0.1–0.3 mg/kg i.m.) was administered, and three more 10-min samples were obtained. Cocaine (0.3 mg/kg i.m.) was then administered, and samples were collected at 10-min intervals over a 2-h period. All samples were refrigerated or frozen until immediately before analysis. Probes were tested in vitro both before and immediately after each session to determine probe viability and percentage of recovery (typically 10–15%). The integrity of the tissue site was determined at the conclusion of each session by increasing the concentration of KCl in the perfused artificial cerebrospinal fluid to 100 mM and examining consequent DA release. A single hemisphere served as the unit of analysis for each subject. Each subject was tested twice with at least 2 weeks between experiments. The order of drug dose combinations was randomized within subjects. Samples were analyzed and DA concentrations were determined by using high-performance liquid chromatography with electrochemical detection as described in detail previously (Kimmel et al., 2007; Bauzo et al., 2009).

Drugs

Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) was dissolved in 0.9% saline. SB 242084 2HCl (Tocris Bioscience, Ellisville, MO) was initially dissolved at a concentration of 1.0 mg/ml in a 20:20:60 mixture of 95% ethanol, Tween 80 (Sigma-Aldrich, St. Louis, MO), and 0.9% saline and further diluted to appropriate concentrations by using 0.9% saline. Where intravenous administration is not specified, drugs were administered intramuscularly in the thigh muscle at a volume of 0.2 to 0.6 ml. All drug solutions were passed through a 0.2- μ m-pore polysulfone filter before use. Doses were calculated from the salt weight.

Data Analysis

For fixed-interval stimulus termination experiments, only response rates obtained from the first 10 components (approximately 60 min) of each test session were used for analyses because the behavioral-stimulant effects of cocaine pretreatments alone and in combination with SB 242084 returned to baseline values by this time. For each subject, rates of responding after each drug-combination test were normalized as a percentage of the overall response rate after vehicle pretreatments. Data were analyzed by using repeated-measures ANOVAs with post hoc Dunnett's tests (SB 242084 alone; SB 242084 + 0.1 mg/kg cocaine) or paired *t* test (SB 242084 + 1.0 mg/kg cocaine).

For self-administration and reinstatement experiments, response rates were normalized to the percentage of responding maintained during maintenance cocaine self-administration sessions when the ED_{Max} unit cocaine dose was available. Data were analyzed by using repeated-measures ANOVAs with post hoc Tukey's tests or Dunnett's tests as specified.

For in vivo microdialysis studies, DA levels within each test session were normalized as the percentage of the mean of three baseline values acquired before drug administration. Because the effects of cocaine typically returned to near-baseline levels within 60 min after cocaine administration, samples collected after this time point were excluded from analyses. Data were analyzed by using a two-way repeated-measures ANOVA.

Data were graphically plotted by using GraphPad version 5.01 (GraphPad Software Inc., San Diego, CA) and analyzed by using SigmaStat version 3.0 software (Systat Software Inc., San Jose, CA). For all statistical analyses, significance was accepted at the 95% level of confidence ($\alpha = 0.05$).

Results

Behavioral-Stimulant Effects of SB 242084 Alone and in Combination with Cocaine. The effects of pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 alone and in combination with cocaine on response rates maintained by a fixed-interval 300-s schedule of stimulus termination are shown in Fig. 1. The mean response rate ($\pm$ S.E.M.) after

Fig. 1. Effects of pretreatment with SB 242084 (SB; veh, 0.01–0.1 mg/kg) before administration of saline or cocaine (0.1–3.0 mg/kg) on rates of responding in squirrel monkeys trained to lever-press on a fixed-interval 300-s schedule of stimulus termination ($n = 4$). Data (mean $\pm$ S.E.M.) are expressed as a percentage of responding after administration of the vehicle for both SB 242084 and cocaine. The dotted line represents baseline response rate after vehicle treatment (100%). *, significant difference ($p < 0.05$) for a given data point compared with vehicle treatments of SB 242084 and cocaine. †, significant difference ($p < 0.05$) for a given data point compared with the effects of vehicle SB 242084 before 0.1 mg/kg cocaine. Abscissa, dose of cocaine. Ordinate, normalized response rate.

vehicle pretreatments of both SB 242084 and cocaine was 0.39 ± 0.07 responses/s. When administered alone, one-way ANOVA with post hoc analyses indicated that 0.03 and 0.1 mg/kg SB 242084 significantly increased response rates up to 137 and 154% baseline, respectively ($F_{3,9} = 7.05$, $p = 0.01$; Dunnett's test, $p < 0.05$).

Cocaine administration resulted in a typical inverted U-shape dose-response function, with maximal increases in response rates after pretreatment with 1.0 mg/kg cocaine (Fig. 1). Administration of a low dose of cocaine (0.1 mg/kg) did not significantly increase responding above baseline levels ($\sim 123\%$; $p > 0.05$). However, combined administration of SB 242084 and 0.1 mg/kg cocaine produced behavioral-stimulant effects that were greater than cocaine alone ($F_{4,12} = 17.53$; $p < 0.001$). Post hoc Dunnett's tests revealed a significant effect at dose combinations of 0.03 and 0.1 mg/kg SB 242084 before 0.1 mg/kg cocaine (169 and 198%, respectively) compared with vehicle SB 242084 before 0.1 mg/kg cocaine ($p < 0.05$). The highest dose of SB 242084 tested (0.1 mg/kg) did not significantly alter the effects of the maximally effective 1.0 mg/kg cocaine dose (paired *t* test, $p = 0.30$).

Effects of SB 242084 Pretreatment on Cocaine-Induced Reinstatement. The effects of pretreatment with SB 242084 on cocaine-induced reinstatement are shown in Fig. 2. Data are shown for each individual subject (s175, s191, and s203) along with mean $\pm$ S.E.M. responding across all subjects (group). The mean response rate ($\pm$ S.E.M.) during maintenance ED_{Max} cocaine self-administration sessions was 1.70 ± 0.28 responses/s. After vehicle SB 242084 pretreatment, priming with the ED_{Peak} dose of cocaine reinstated responding to $\sim 102\%$ relative to response rates during maintenance self-administration sessions. Lowering the dose of the cocaine prime by a full log unit in each subject resulted in a dramatic reduction of the reinstatement effect ($\sim 23\%$). Pretreatment with SB 242084 resulted in an apparent upward shift of the ascending limb of the cocaine dose-response curve. Two-way repeated-measures ANOVA indicated significant main effects for cocaine dose ($F_{3,6} = 13.44$; $p = 0.005$) and SB 242084 dose ($F_{2,4} = 15.85$; $p = 0.013$) but not a significant interaction ($F_{6,12} = 0.51$; $p = 0.79$). Post hoc Tukey's tests revealed that priming with either the ED_{Peak} dose of cocaine or a one-half log-unit lower dose (intermediate dose) significantly induced reinstatement compared with a saline prime ($p < 0.05$). Furthermore, post hoc Tukey's tests within the factor of SB 242084 dose indicated that, across all doses of cocaine prime, pretreatment with 0.1 mg/kg SB 242084 resulted in higher reinstatement responding compared with vehicle pretreatment ($p < 0.05$). Pretreatment with 0.1 mg/kg SB 242084 before either an intermediate or low dose of cocaine prime resulted in full reinstatement for each subject; yet this dose of SB 242084 alone produced an appreciable reinstatement in only one of three subjects (s203). Although statistical analysis of the averaged data indicated that SB 242084 failed to induce reinstatement when administered alone ($F_{2,4} = 1.51$; $p = 0.32$), it is clear from the individual-subject data that the 0.1 mg/kg SB 242084 dose induced full reinstatement ($\sim 106\%$) in one subject (s203), but was ineffective in the other two subjects (~ 5 and $\sim 10\%$) as mentioned above. It is noteworthy that for subject s203 the reinstatement effect was dose-dependent because reducing the dose of SB 242084 to 0.03 mg/kg reduced the reinstatement effect to $\sim 38\%$. It is possible that

Fig. 2. Effects of pretreatment with SB 242084 (SB; veh, 0.03–0.1 mg/kg) on cocaine-induced reinstatement in squirrel monkeys ($n = 3$). Data are presented for individual subjects (s175, s191, and s203) and the group mean $\pm$ S.E.M. values. After stable cocaine self-administration behavior, responding was extinguished ($\diamond$; last day of extinction). Presession priming with cocaine reinstated responding in a dose-dependent manner ($\circ$). Pretreatment with SB 242084 caused a dose-dependent upward shift of the ascending limb of the cocaine dose-response function in all subjects (filled symbols). Data (mean $\pm$ S.E.M.) are expressed as the percentage of responding maintained during ED_{Max} cocaine self-administration sessions. The dotted lines represent baseline self-administration rate (100%) and extinction criterion (20%). Abscissae, dose of cocaine prime. Ordinates, normalized response rate.

increasing the dose of SB 242084 to 0.3 mg/kg might have induced reinstatement in the other two subjects. However, the administration of this dose of SB 242084 to one subject in a pilot study induced adverse physiological effects, including emesis and prolonged whole-body scratching, which would likely confound a dependent measure of response rate because these adverse effects might disrupt lever pressing. We therefore did not systematically test doses of SB 242084 above 0.1 mg/kg.

Effects of SB 242084 Substitution for Cocaine Self-Administration. The mean response rate ($\pm$ S.E.M.) during maintenance ED_{Max} cocaine self-administration sessions was 1.59 ± 0.73 responses/s. The effects of SB 242084 substitution on cocaine-maintained self-administration responding are shown in Fig. 3A. When the SB 242084 vehicle was substituted for cocaine, responding decreased to below the 20% extinction criterion within three to four sessions in two subjects and to near-extinction levels ($\sim 40\%$) in a third subject, indicating that the level of responding indeed depended

on the availability of a pharmacological reinforcer. Substituting SB 242084 (0.01–0.1 mg/kg/infusion) for cocaine availability produced an inverted U-shaped dose-response function. For all doses of SB 242084 tested, response rates stabilized within three to six sessions in all subjects. The number of days required to reach stabilization of response rates for all doses tested did not differ significantly (one-way repeated-measures ANOVA: $F_{2,4} = 4.00$; $P = 0.11$). One-way repeated-measures ANOVA followed by post hoc analyses indicated that 0.03 and 0.1 mg/kg/infusion SB 242084 reliably maintained self-administration behavior ($F_{3,6} = 11.417$, $p = 0.007$; Tukey's tests, $p = 0.009$ and 0.013 , respectively) relative to responding maintained during vehicle substitution. It is noteworthy that the rates of responding during self-administration of these effective doses of SB 242084 were nearly equivalent to those maintained during ED_{Max} cocaine self-administration (i.e., 100%).

After the first session of intravenous self-administration of 0.03 mg/kg/infusion SB 242084 we observed whole-body

Fig. 3. An assessment of the reinforcing effects of SB 242084 (SB; veh, 0.01–0.1 mg/kg/infusion) in squirrel monkeys trained to self-administer cocaine (Coc) according to a second-order operant schedule of drug delivery ($n = 3$). A, after several days of cocaine self-administration, SB 242084 availability was substituted for cocaine during daily sessions until responding stabilized. ●, responding during ED_{Max} cocaine self-administration sessions. ■, responding during substitution tests with various doses of SB 242084 or its vehicle. SB 242084 produced an inverted U-shaped dose-response function and maintained maximal rates of responding at levels near those maintained by the ED_{Max} of cocaine. B, substitution of SB 242084 after daily extinction sessions. ●, responding during ED_{Max} cocaine self-administration sessions. ○, response rates on the last day of extinction (Sal Ext., saline extinction). ■, stabilized responding after SB 242084 availability and restoration of maintenance self-administration parameters. The maximally effective dose of SB 242084 (0.03 mg/kg/infusion) identified in the previous experiment was made available for self-administration. Data (mean $\pm$ S.E.M.) are expressed as the percentage of responding maintained during ED_{Max} cocaine self-administration sessions. *, $p < 0.05$; **, $p < 0.01$, significant difference for a given data point compared with the rate of responding maintained by Veh SB 242084. Abscissae, unit dose of drug available for self-administration (A) or session type and drug/dose available (B). Ordinates, normalized response rate.

scratching behavior in one of three subjects (s209). Self-administration of 0.1 mg/kg/infusion SB 242084 also elicited whole-body scratching behavior, but in each of the three subjects tested, and the effect was persistent across all test sessions during which this dose was substituted. In all cases, the scratching behavior seemed to subside within 30 to 60 min of the end of the self-administration session. In addition, self-administration of 0.1 mg/kg/infusion was accompanied by emesis in two of three subjects (s209 and s196), but this effect was observed only on the first day of substitution.

To further test the reinforcing effects of SB 242084, we assessed the capacity of the maximally effective unit dose of SB 242084 (0.03 mg/kg/infusion) to restore and maintain responding after the extinction of cocaine self-administration behavior. The mean response rate ($\pm$ S.E.M.) during maintenance ED_{Max} cocaine self-administration sessions was 1.60 ± 0.44 responses/s. When conditioned reinforcers were withheld and saline was substituted for cocaine availability, responding extinguished below the 20% baseline within two to seven sessions. The effects of subsequent 0.03 mg/kg/infusion SB 242084 availability on responding are shown in Fig. 3B. The mean ($\pm$ S.E.M.) number of sessions required for response rates to stabilize was 7.33 ± 2.4 . Across all subjects, response rates stabilized at $\sim 59\%$ of the ED_{Max} cocaine self-administration rate. Paired t test indicated that this level of responding did not significantly differ from response rates during saline extinction ($p = 0.11$). It should be noted that responding maintained by SB 242084 under these conditions was highly variable across subjects. SB 242084 maintained high rates of responding in one subject ($\sim 92\%$), moderate response rates in a second subject ($\sim 54\%$), and near-extinction levels of responding in a third subject ($\sim 30\%$).

Effects of SB 242084 Pretreatment on Cocaine-Induced Increases in DA. Mean ($\pm$ S.E.M.) basal DA levels uncorrected for probe recovery in the NAc were 2.64 ± 0.89 nM. Administration of 0.3 mg/kg cocaine produced a modest increase in extracellular DA, which peaked at $\sim 133\%$ of basal DA levels at 30 min after cocaine administration. DA

levels subsequently returned to baseline levels soon after the onset of the peak change (Fig. 4A). Combined administration of SB 242084 and cocaine produced a peak increase of $\sim 192\%$ of DA levels within 10 min after cocaine administration. DA levels returned to near baseline by the end of the 60-min sampling period. Two-way repeated-measures ANOVA revealed a significant main effect of time ($F_{11,33} = 3.05$; $p = 0.006$) and for SB 242084 pretreatment ($F_{1,3} = 11.02$; $p = 0.045$). However, the time $\times$ SB 242084 interaction was not significant ($F_{11,33} = 1.25$; $p = 0.29$).

The effects of pretreatment with 0.3 mg/kg SB 242084 on cocaine-induced elevations of DA in the caudate nucleus are shown in Fig. 4B. The 0.3 mg/kg pretreatment dose was chosen for study in this cohort after a pilot study in one subject failed to indicate any modulatory effect of 0.1 mg/kg SB 242084 on cocaine-induced DA overflow within the caudate nucleus. It should be noted that administration of 0.3 mg/kg SB 242084 was not studied in behavioral experiments because of the emergence of effects that would probably disrupt lever-pressing behavior (e.g., emesis, whole-body scratching), but this was not a concern for the present experiment where the dependent measure was a neurochemical effect of cocaine. Mean ($\pm$ S.E.M.) basal DA levels uncorrected for probe recovery in the caudate nucleus were 5.00 ± 1.48 nM. Administration of 0.3 mg/kg cocaine either alone or in combination with 0.3 mg/kg SB 242084 increased extracellular DA in the caudate nucleus within 20 min after cocaine administration that returned to near-baseline levels within 60 min. Two-way repeated-measures ANOVA indicated a significant main effect of time ($F_{11,22} = 13.34$; $p < 0.001$) but not for the main effect of SB 242084 pretreatment ($F_{1,2} = 2.03$; $p = 0.29$) or their interaction ($F_{11,20} = 1.09$; $p = 0.42$).

Discussion

The present studies sought to investigate the effects of combined pretreatment with cocaine and the selective

Fig. 4. Effects of cocaine (0.3 mg/kg) on extracellular levels of DA in the nucleus accumbens (A; $n = 4$) or caudate nucleus (B; $n = 3$) after pretreatment with 0.1 mg/kg SB 242084 (■), 0.3 mg/kg SB 242084 (●), or its vehicle (○) in squirrel monkeys. Data points (mean $\pm$ S.E.M.) are expressed as the percentage of baseline DA levels before drug administration. ptx, pretreatment with vehicle, 0.1, or 0.3 mg/kg SB 242084. coc, administration of 0.3 mg/kg cocaine. Abscissae, time relative to cocaine administration. Ordinates, normalized DA concentration.

5-HT_{2C}R antagonist SB 242084 in nonhuman primates. In the first set of experiments, pretreatment with SB 242084 dose-dependently produced modest behavioral-stimulant effects alone and modulated the behavioral-stimulant effects of a low dose of cocaine in an apparently additive manner in squirrel monkeys. This interactive effect of combined SB 242084 and cocaine administration is consistent with several previous studies in rodents. For example, genetic mutant mice lacking the 5-HT_{2C}R display a greater locomotor response to cocaine relative to wild-type controls (Rocha et al., 2002), and systemic administration of selective 5-HT_{2C}R antagonists enhances cocaine-induced locomotor activity in rats (Fletcher et al., 2002, 2006; Filip et al., 2004). There is also evidence to support our finding that administration of SB 242084 alone induces stimulant-like effects, because administration of a high dose of SB 242084 (1.0 mg/kg) significantly increased basal locomotor activity in rats (Zaniewska et al., 2009). It is noteworthy that pretreatment with SB 242084 did not alter the effects of a dose of cocaine that elicited maximum increases in responding (1.0 mg/kg). One possible interpretation of this finding is that 5-HT_{2C}R antagonism selectively modulates the rate-increasing effects of cocaine and does not alter its rate-decreasing effects, because these results do not indicate a parallel leftward shift of the cocaine dose-response function. It is in fact plausible that SB 242084 pretreatment may have been capable of modulating the rate-increasing effects of 1.0 mg/kg cocaine and was prevented from doing so by a ceiling effect with respect to response rates, but this remains speculative. Although testing the effects of SB 242084 pretreatment in conjunction with higher doses of cocaine would better elucidate the nature of the shift in the cocaine dose-response function, we were hesitant to administer high doses of both drugs simultaneously without clearer foresight of potential adverse reactions. Nevertheless, the present results indicate that 5-HT_{2C}R antagonism produced modest behavioral-stimulant effects alone and modulated the behavioral-stimulant effects of a low dose of cocaine in an apparently additive manner in nonhuman primates.

A similar interaction was observed in the reinstatement experiments, as pretreatment with SB 242084 produced an upward shift of the ascending limb of the cocaine dose-re-

sponse function. This modulation of cocaine-induced reinstatement is in agreement with a previous study demonstrating a dose-dependent potentiation of cocaine-induced reinstatement after SB 242084 pretreatment in rats (Fletcher et al., 2002). It is noteworthy that in that same study pretreatment with SB 242084 alone was insufficient to induce the reinstatement of cocaine-seeking behavior in rats; yet in the present study, SB 242084 administration alone did induce reinstatement at the highest dose tested in one subject. This discrepancy may be accounted for by highlighting the tested dose range within each experiment. For example, the dose of SB 242084 used for reinstatement experiments in the previous rodent study (0.5 mg/kg) also failed to induce significant locomotor effects (Fletcher et al., 2002). However, increasing the dose of SB 242084 to 1.0 mg/kg did produce a modest, but significant, effect on locomotor activity in a separate study (Zaniewska et al., 2009). In our experiments, SB 242084 induced reinstatement in one subject, but only at a dose that also reliably engendered significant behavioral-stimulant effects in a separate group of animals (0.1 mg/kg). Furthermore, decreasing the dose of SB 242084 to 0.03 mg/kg before priming with saline resulted in the absence of any appreciable reinstatement effect in all subjects. It is therefore possible that 5-HT_{2C}R antagonism may have been capable of inducing reinstatement in the previous rodent study, but the dose range tested may have been insufficient to reveal such an effect. Accordingly, it is possible that increasing the dose in the present experiments may have produced more robust reinstatement effects across all subjects. However, we did not perform such tests because of the emergence of adverse physiological side effects after administration of the 0.3 mg/kg dose of SB 242084 that probably would have disrupted operant behavior.

Given that the behavioral-stimulant and reinstatement effects of cocaine are believed to be mediated via increased dopaminergic neurotransmission (Spealman et al., 1989, 1999; Howell and Byrd, 1995), we used *in vivo* microdialysis techniques to determine whether the behavioral effects of SB 242084 were correlated with modulation of cocaine-induced DA increases within the striatum. Because we hypothesized that 5-HT_{2C}R blockade would produce an enhancement,

rather than an attenuation, of cocaine effects, we chose to administer a dose of SB 242084 (0.1 mg/kg) that modulated the behavioral effects of cocaine in earlier experiments in combination with a dose of cocaine (0.3 mg/kg) that produces only modest increases in DA levels within the NAc. Our results indicate that combined pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 and cocaine in monkeys resulted in a greater increase in DA levels within the NAc than those elicited by the administration of cocaine alone. This finding is consistent with a previous study demonstrating that systemic administration of the selective 5-HT_{2C}R antagonist SB 242084 enhanced cocaine-induced increases in DA levels within the NAc in rats (Navailles et al., 2004). It is noteworthy that SB 242084 administration seemed to alter basal DA levels during the 30-min period preceding cocaine administration, a finding that is in agreement with other studies in rodents demonstrating that administration of a 5-HT_{2C}R antagonist or inverse agonist increases basal firing rates of VTA DA neurons and elevates NAc DA levels (Di Giovanni et al., 1999; Di Matteo et al., 1999).

In contrast to the observed effects in the NAc, administration of SB 242084 did not significantly alter cocaine-induced DA increases within the caudate nucleus of nonhuman primates. Indeed, this lack of effect within the dorsal striatum persisted even when the pretreatment dose of SB 242084 was increased to a dose that was higher than that required to produce significant behavioral effects (0.3 mg/kg). This result is in accordance with several previous reports indicating that neither 5-HT_{2C}R activation nor antagonism are effective at modulating DA signaling within dorsal aspects of the striatum in rodents (Di Matteo et al., 1999; Di Giovanni et al., 2000; Marquis et al., 2007), although some studies have provided opposing results (Di Giovanni et al., 1999; Gobert et al., 2000; Deurwaerdere et al., 2004; Navailles et al., 2004; Alex et al., 2005). The reasons for these discrepancies are unknown, but may be related to differences in the drugs administered, drug preparation, dosing, route of drug administration, electrophysiological and neurochemical methodologies, probe/electrode placements, or rodent species, among other variables. Nevertheless, our data indicate that pretreatment with the 5-HT_{2C}R antagonist SB 242084 did not significantly modulate the DA-increasing effects of cocaine within the caudate nucleus of squirrel monkeys and suggest that the nigrostriatal pathway is unaffected by signaling through the 5-HT_{2C}R in nonhuman primates. These results may be explained by a differential pattern of 5-HT_{2C}R expression within striatal territories in monkeys, as the 5-HT_{2C}R mRNA was found within the VTA and NAc, but not the substantia nigra pars compacta or dorsolateral aspects of the striatum, of nonhuman primates (López-Giménez et al., 2001).

Because most psychostimulants function as reinforcers in self-administration procedures, we examined whether intravenous infusions of SB 242084 would maintain responding in squirrel monkeys when substituted for cocaine self-administration. To our knowledge, these studies are the first to assess the direct reinforcing effects of a 5-HT_{2C}R-selective antagonist in any species. Consistent with its suggested behavioral profile, SB 242084 fully substituted for cocaine availability in all subjects tested, maintaining maximal stable rates of responding across three consecutive test sessions that were nearly identical to those maintained by the maxi-

mally effective dose of cocaine. One implication from these results is that 5-HT_{2C}R antagonists may display some degree of abuse potential in humans. However, additional studies are needed to better understand the reinforcing effects of 5-HT_{2C}R antagonists under a variety of experimental conditions before conclusions about its reinforcing strength and abuse potential are made. For example, we only tested the reinforcing effects of SB 242084 in the present study when made available according to a second-order schedule of self-administration, but the effects observed here should be replicated with other 5-HT_{2C}R antagonists and/or inverse agonists and under alternative schedules of reinforcement. Furthermore, the subjects used in the present study each had extensive histories of cocaine self-administration behavior, and it therefore remains to be determined whether drug-naïve subjects will acquire self-administration responding via 5-HT_{2C}R antagonist availability. We did obtain some evidence to suggest that SB 242084 may not be as robust a reinforcer compared with cocaine. When subjects' responding is extinguished, reintroducing cocaine availability reliably restores response rates to baseline levels within one to two sessions. However, after exposure to extinction sessions, the availability of SB 242084 did not result in rapid stabilization of responding, and response rates peaked at only ~60% compared with the maximally effective unit dose of cocaine. These results indicate that the reinforcing effects of SB 242084 may vary across experimental conditions and highlight the need for further study before drawing conclusions regarding abuse potential.

In summary, the present results are the first to demonstrate a modulation of the behavioral and neurochemical effects of cocaine in an apparently additive manner after 5-HT_{2C}R antagonism in nonhuman primates. In addition, the novel finding that SB 242084 maintained intravenous self-administration when substituted for cocaine availability may be indicative of some degree of abuse potential, although further research is needed. As DA systems are understood to mediate the reinforcing and abuse-related effects of many drugs of abuse, it is possible that the capacity for 5-HT_{2C}R antagonists to modulate the effects of cocaine may generalize to other drugs of abuse. Finally, given that 5-HT_{2C}R antagonists are being investigated preclinically as novel pharmacotherapeutics for the treatment of several affective disorders, the present findings suggest that their clinical use may be contraindicated in persons with comorbid substance abuse or dependence.

Acknowledgments

We thank Mi Zhou, Juliet Brown, and Lisa Neidert for expert technical assistance; and the animal care, veterinary care, and facilities staff at Yerkes National Primate Research Center for their exceptional services.

Authorship Contributions

Participated in research design: Manvich, Kimmel, and Howell.

Conducted experiments: Manvich and Cooper.

Performed data analysis: Manvich.

Wrote or contributed to the writing of the manuscript: Manvich, Kimmel, and Howell.

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