Antagonism of 5-Hydroxytryptamine\textsubscript{4} Receptors Attenuates Hyperactivity Induced by Cocaine: Putative Role for 5-Hydroxytryptamine\textsubscript{4} Receptors in the Nucleus Accumbens Shell\textsuperscript{1}

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ABSTRACT
The localization of 5-hydroxytryptamine \textsubscript{4} (5-HT\textsubscript{4}) receptors suggests their role in the regulation of dopamine (DA) neurotransmission, a speculation that has been supported by neurochemical studies. Mesolimbic DA systems play a prominent role in mediating the behavioral effects of the abused psychostimulant cocaine, and the intent of the present study was to assess the role of 5-HT\textsubscript{4} receptors in the control of spontaneous and cocaine-induced activity. Systemic administration of the 5-HT\textsubscript{4} receptor partial agonist 1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]1-propanone hydrochloride (RS 67333; 0.0001–1 mg/kg) or the 5-HT\textsubscript{4} receptor antagonist 4-amino-5-chloro-2-methoxy-benzoic acid-(diethylamino)ethyl ester hydrochloride (SDZ 205,557; 0.0001–1 mg/kg) did not significantly alter spontaneous activity, whereas SDZ 205,557 significantly attenuated cocaine-induced horizontal activity and rearing. To test the hypothesis that cocaine-elicited behaviors were modulated by 5-HT\textsubscript{4} receptors in the nucleus accumbens (NAc) shell, two separate groups of male rats were implanted with bilateral cannulas aimed at the NAc shell. Intra-NAc shell microinjections of either RS 67333 (1 or 3 \textmu g/0.2 \textmu l/side) or SDZ 205,557 (1–5 \textmu g/0.2 \textmu l/side) did not alter spontaneous activity observed after a systemic saline injection but did significantly attenuate the hyperactivity induced by systemic cocaine injection (10 mg/kg). These results support an involvement of 5-HT\textsubscript{4} receptors, particularly those in the NAc shell, in the locomotor stimulatory effects of cocaine. Furthermore, these data suggest that 5-HT\textsubscript{4} receptors may regulate behavioral processes dependent on mesolimbic DA pathways and may provide a novel target for the development of medications useful in the treatment of both drug dependence and psychiatric disorders.

Cocaine abuse remains a significant social and medical dilemma in many communities throughout the United States and the world. Increasing efforts to identify effective pharmacotherapies for cocaine dependence have focused to a large extent on dopamine (DA) ligands, however, their clinical use has been impeded by adverse side effect profiles (Kleber, 1995). In addition to its action as a DA reuptake inhibitor, cocaine is also an inhibitor of serotonin (5-hydroxytryptamine (5-HT)) reuptake (Koe, 1976). Although the behavioral effects of cocaine are thought to be predominantly mediated by mesocorticlimbic DA pathways, particularly those that project from DA cell bodies of the ventral tegmental area to the nucleus accumbens (NAc) (Kelley and Iversen, 1976; Delfs et al., 1990; Callahan et al., 1994), 5-HT pathways also innervate these DA mesocorticolimbic circuits (Herve et al., 1987; Van Bockstaele et al., 1993), and 5-HT control of DA pathways may provide a target to modify behaviors induced by cocaine with fewer side effects.

Acute cocaine administration dose dependently increases horizontal motor activity and rearing in the rat and, at high doses, elicits stereotyped behaviors (SheeKrugter et al., 1976). Depletion of DA (Kelley and Iversen, 1976) and blockade of DA D\textsubscript{1}- and D\textsubscript{2}-like receptors attenuates cocaine-elicited hyperactivity (Kita et al., 1999), and microinfusion studies have verified the NAc as a key neural site involved in the generation of the locomotor stimulatory effects of this psychostimulant (Kelley and Iversen, 1976; Delfs et al., 1990). Although a specific role for designated subnuclei of the NAc, which includes the rostrally located core and the caudomedially located shell (Zahm and Brog, 1992), in the be-

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ABBREVIATIONS: DA, dopamine; 5-HT, 5-hydroxytryptamine (serotonin); NAc, nucleus accumbens; RS 67333, 1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]1-propanone hydrochloride; SDZ 205,557, 4-amino-5-chloro-2-methoxy-benzoic acid-(diethylamino)ethyl ester hydrochloride.
havioral effects of cocaine has not yet been thoroughly assessed, recent neurochemical studies suggest that cocaine administration preferentially enhances DA levels in the NAc shell relative to the core (Pontieri et al., 1995) and that the NAc shell is more sensitive to the motor activating effects of DA agonists (Swanson et al., 1997). Thus, it appears that the NAc shell may play a critical role in the locomotor stimulatory actions of cocaine.

Manipulations of 5-HT have been shown to modulate the locomotor stimulatory actions of cocaine. For example, enhancement of brain 5-HT levels with the 5-HT precursor 5-hydroxytryptophan attenuated cocaine-induced hyperactivity, and depletion of brain 5-HT levels with the 5-HT synthesis inhibitor p-chlorophenylalanine potentiated cocaine-induced hyperactivity (Scheel-Kruger et al., 1976). Recent advances in the pharmacology of 5-HT receptor subtypes have enabled the identification of specific 5-HT receptors that may be involved in the behavioral properties of cocaine. Of the 14 5-HT receptor subtypes described to date (Hoyer et al., 1994), hyperactivity induced by cocaine has been shown to be modulated by ligands selective for 5-HT1A (R. De La Garza and K.A.C., unpublished observations), 5-HT1B (Lucas et al., 1997), 5-HT2A (L.R.M. and K.A.C., unpublished observation), and 5-HT3 receptors (Reith, 1990; Svingos and Hitzemann, 1992). A role for the more novel 5-HT5 receptors, in particular those that are resident in mesocorticolimbic pathways, has not been addressed.

One such receptor is the 5-HT4 subtype, which is a member of the G protein-coupled receptor superfamily and is linked to stimulation of adenyl cyclase in the brain (Hoyer et al., 1994). High levels of 5-HT4 receptor protein have been localized in the olfactory system, striatum, NAc, hippocampus, and substantia nigra (Waerber et al., 1994). A number of studies have suggested that the activation of 5-HT4 receptors facilitates neurotransmitter release in the brain. For example, i.c.v. microinjection of relatively selective 5-HT4 agonists facilitated acetylcholine overflow in rat frontal cortex as measured with microdialysis (Consolo et al., 1994), and local microinjection of 5-HT4 agonists facilitated in vivo efflux of 5-HT in rat hippocampus (Ge and Barnes, 1996). Interestingly, the local application of 5-HT4 agonists enhanced in vitro and in vivo release of DA in rat striatum, an effect that was reversed by the coadministration of selective 5-HT4 antagonists (Steward et al., 1996).

Given the excitatory relationship between 5-HT4 receptor stimulation and dopamine release, as well as the localization of 5-HT4 receptors in the NAc shell, experiments were conducted to investigate whether 5-HT4 receptors play a modulatory role in cocaine-induced hyperactivity. The 5-HT4 partial agonist 1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propanone hydrochloride (RS 67333; KI = 2 nM) and the 5-HT4 antagonist 4-amino-5-chloro-2-methoxy-benzoic acid-(diethylamino)ethyl ester hydrochloride (SDZ 205,557; KI = 10.6 nM) were chosen based on their high affinity for 5-HT4 receptors, their documented use in other behavioral paradigms, their ability to cross the blood-brain barrier, and their commercial availability (Eglen et al., 1993, 1995; Schiavi et al., 1994). These two ligands were administered systematically or into the NAc shell to test the hypothesis that pharmacological manipulation of 5-HT4 receptors would alter either spontaneous or cocaine-stimulated activity. The NAc shell was chosen as a microinjection site because higher levels of 5-HT4 receptors were found in the NAc shell relative to the NAc core (Compan et al., 1996).

Materials and Methods

Animals. The study included 53 male Sprague-Dawley albino rats (Harlan, Indianapolis, IN) weighing 250 to 300 g at the beginning of the experiment. The rats were housed three to a cage in standard plastic rodent cages in a colony room maintained at 21 ± 2°C and at 40 to 50% humidity under a 12-h light/dark cycle (lights on at 7 AM). Once implanted with indwelling bilateral guide canulas, the rats were housed individually. Each rat was provided with continuous access to tap water and rodent chow throughout the experiment except during experimental sessions.

Apparatus. Locomotor activity was monitored and quantified using an open field activity system (San Diego Instruments, San Diego, CA). Each clear Plexiglas chamber (40 × 40 × 40 cm) was housed within a sound-attenuating enclosure and was surrounded with a 4 × 4 photobeam matrix located 4 cm from the floor surface. Interruptions of the photobeams resulted in counts of activity in the peripheral and central fields of the chamber. Another horizontal row of 16 photobeams, located 16 cm from the floor surface, provided each chamber with a measurement of vertical activity (rearing). Video cameras positioned above the chambers permitted continuous observation of behavior without disruption. Separate counts of peripheral, central, and vertical activity counts were made with the control software (Photobeam Activity Software; San Diego Instruments) and stored for subsequent statistical evaluation. Peripheral and central activity counts were summed to provide a single measure of total horizontal activity.

Guide Cannula Surgery. Thirty-two rats underwent surgical implantation of a fused, 22-gauge stainless steel bilateral guide cannula (Small Parts Inc., Miami Lakes, FL) aimed 2 mm above the NAc shell. Each rat was anesthetized with an i.m. injection of 8.5% xylazine, 14% acepromazine, and 43% ketamine in physiological saline (0.9% NaCl). With the upper incisor bar of a Kopf stereotaxic instrument positioned at −3.8 mm below the interaural line, the ventral surface of the bilateral guide cannulas was positioned 1.4 mm anterior to bregma, 0.75 mm lateral to the longitudinal suture, and 6.0 mm below the surface of the skull (Paxinos and Watson, 1998). The guide cannulas were fastened to the skull with stainless steel screws (Small Parts Inc.) and cranioplast cement (Plastics One Inc., Roanoke, VA). Each guide cannula was fitted with a 28-gauge stainless steel bilateral obturator (Small Parts Inc.). Each rat received a single i.m. injection of 300,000 U of sodium ampicillin after surgery and was handled and weighed daily during a 1-week recovery period.

Behavioral Procedures: Systemic Injections. All rats were maintained in the colony room for a minimum of 1 week before behavioral testing for acclimation to daily handling procedures. Rats were habituated to the test environment for 2 h/day on each of the 2 days before the start of the experiment. On each of the test days, rats were habituated to the activity monitors for 1 h before the administration of drugs. Using a repeated measures design and eight test sessions, one group of rats (n = 11) received an i.p. injection of either saline (1 ml/kg) or RS 67333 (0.0001, 0.01, or 1 mg/kg), followed 45 min later by an injection of either saline (1 ml/kg) or cocaine (10 mg/kg). Using the same design, a separate group of rats (n = 10) received an i.p. injection of either saline (1 ml/kg) or SDZ 205,557 (0.0001, 0.01, or 1 mg/kg), followed 45 min later by an injection of either saline (1 ml/kg) or cocaine (10 mg/kg). Measurement of locomotor activity counts began immediately after the second injection and was divided into 5-min bins for a total of 1 h. Test sessions were conducted every other day, and the order of drug tests was assigned randomly to each rat with the caveat that the four cocaine tests were spaced 4 days apart.

An observational time-sampling procedure was also used to record the frequency of various behaviors by an investigator blind to the
treatment assignments (Swanson et al., 1997). The presence or absence of a given behavior was scored during a 1 min time bin 0, 15, and 30 min after saline or cocaine injection for a total of 3 min for each rat during the 1-h observation period. The presence of ambulation (forward locomotion or turning) and rearing was determined; the maximum possible score for ambulation and rearing was 3. Measurements of “head-up sniffing,” “head-down sniffing,” and “head bob/sway” (Swanson et al., 1997) were combined as “sniffing”; the maximum possible score for sniffing was 9.

Behavioral Procedures: Intracranial Microinjections. All rats were habituated to the colony room and test environment as described for systemic drug treatments. In addition, each rat was habituated to the brief confinement associated with the intracranial microinjection technique by removing the 28-gauge internal obturator, gently restraining the rat for approximately 3 min, and replacing the obturator. Using a repeated measures design and six test sessions, one group of rats (n = 16) received an intra-NAc shell microinjection of either saline (0.2 μl/side) or RS 67333 (1 or 3 μg/0.2 μl/side), followed immediately by an i.p. injection of either saline (1 ml/kg) or cocaine (10 mg/kg). Using a repeated measures design and eight test sessions, a separate group of rats (n = 16) received an intra-NAc shell microinjection of either saline (0.2 μl/side) or SDZ 205,557 (0.5, 1, or 5 μg/0.2 μl/side), followed immediately by an i.p. injection of either saline (1 ml/kg) or cocaine (10 mg/kg). For each intra-NAc shell microinjection, the 28-gauge bilateral obturator was removed, and a 33-gauge stainless steel bilateral internal cannula (Small Parts Inc.) was positioned to extend 2 mm ventral to the bilateral guide cannula tip. The bilateral internal cannulas were attached to two 5-μl Hamilton syringes via PE-20 tubing. A microsyringe drive (Baby Bee; BAS, West Lafayette, IN) driven by a programmable controller (Bee Hive Controller; BAS) delivered a volume of 0.2 μl/side at a rate of 0.1 μl/min. After each microinjection, the bilateral internal cannula was left in place for 1 min, and the obturator was replaced. Measurement of locomotor activity counts began immediately after the systemic injection and was divided into 5-min bins for a total of 1 h. Test sessions were conducted every 3 days, and the order of microinjections of the 5-HT₄ ligand was counterbalanced for each rat. The tests with cocaine (three or four per rat) were spaced every 6 days.

Histology. At the end of the experiment, rats implanted with bilateral guide cannulas were overdosed with sodium pentobarbital (50 mg/kg i.p.). The brains were removed and stored in 10% sucrose/10% formalin solution for at least 3 days before sectioning on a cryostat. Sections (50 μm) were mounted onto gelatin-coated glass slides, defatted, stained with cresyl violet, cleared with xylene, and coverslipped. Guide cannula placements were verified using a light microscope. Only rats identified with bilateral cannula placements in the NAc shell were included in the analyses (Paxinos and Watson, 1988).

Data Analysis. Data were analyzed as total horizontal (peripheral plus central activity counts) and vertical activity counts over the first and second 30-min intervals of the 1-h test session. Observational scores of behaviors, including sniffing, rearing, ambulation, and the total of all observed behaviors, were analyzed and presented as counts/1-min bin sampled at 0, 15, and 30 min post-treatment injection for a total of 3 min during the 1-h observation period. The main effect of drug combinations on total horizontal and vertical activity as well as total observed behaviors, were analyzed and presented as counts/1-min bin sampled at 0, 15, and 30 min post-treatment injection for a total of 3 min during the 1-h observation period. The presence of ambulation (forward locomotion or turning) and rearing was determined; the maximum possible score for ambulation and rearing was 9. Measurements of “head-up sniffing,” “head-down sniffing,” and “head bob/sway” (Swanson et al., 1997) were combined as “sniffing%; the maximum possible score for sniffing was 9.

Systemic Injections of 5-HT₄ Partial Agonist RS 205,557. A significant main effect of drug was observed for total horizontal activity during the first (F₁,₁₆ = 8.55, p < .001) and second 30-min intervals (F₁,₁₆ = 14.87, p < .001). In addition, a significant main effect of drug was observed for total vertical activity during the first (F₁,₁₆ = 5.28, p < .001) and second 30-min intervals (F₁,₁₆ = 7.83, p < .001). For the first and second 30-min intervals, RS 67333 (0.0001, 0.01, or 1 mg/kg) did not alter horizontal or vertical hyperactivity induced by cocaine compared with saline-cocaine controls (p > .05; data not shown). The administration of RS 67333 alone did not significantly alter either basal horizontal or vertical activity in either time period (p > .05; data not shown).

As measured by an observation-based time-sampling procedure, pretreatment with RS 67333 (0.0001, 0.01, or 1 mg/kg) did not significantly alter sniffing, ambulation, or rearing observed after treatment with saline or cocaine (p > .05; data not shown).

Systemic Injections of 5-HT₄ Antagonist SDZ 205,557. Horizontal and vertical activity counts were assessed after i.p. injection of saline or SDZ 205,557 (0.0001, 0.01, or 1 mg/kg) before an i.p. injection of saline or cocaine (10 mg/kg; Figs 1 and 2). A significant main effect of drug was observed for total horizontal activity during the first (F₁,₁₆ = 6.47, p < .001) and second 30-min intervals (F₁,₁₆ = 6.29, p < .001). For the first 30-min interval (Fig. 1A), horizontal activity was significantly increased by cocaine in rats pretreated with saline or 0.0001 mg/kg SDZ 205,557 compared with saline-

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**Fig. 1.** Total horizontal activity after systemic pretreatment with the 5-HT₄ antagonist SDZ 205,557. Mean total horizontal activity (counts/30 min) ± S.E.M. after i.p. injection of saline (Sal) or SDZ 205,557 (SDZ, 0.0001, 0.01, 1 mg/kg) followed by treatment with saline or cocaine (Coc; 10 mg/kg) during the (A) first and (B) second 30-min intervals of the 60-min test session. *, activity levels that were significantly different from saline-saline controls. \*, activity levels that were significantly different from saline-cocaine controls based on a Student-Newman-Keuls procedure (p < .05).
saline controls (p < .05). However, pretreatment with 0.01 or 1 mg/kg SDZ 205,557 significantly attenuated cocaine-induced horizontal activity (p < .05). For the second 30-min interval (Fig. 2B), horizontal activity was significantly increased by cocaine after pretreatment with saline or 0.0001 or 0.01 mg/kg SDZ 205,557 compared with saline-saline controls (p < .05). The administration of doses of SDZ 205,557 alone did not significantly alter basal horizontal activity in either time period (p > .05).

A significant main effect of drug was revealed for total vertical activity during the first (F(7,63) = 4.16, p < .001) and second 30-min intervals (F(7,63) = 3.81, p < .01). For the first 30-min interval (Fig. 2A), only cocaine alone significantly elevated vertical activity over saline-saline controls (p < .05). Pretreatment with 0.01 or 1 mg/kg SDZ 205,557 significantly reduced cocaine-induced vertical activity (p < .05). For the second 30-min interval (Fig. 2B), only cocaine significantly increased vertical activity compared with saline-saline controls (p < .05). The administration of SDZ 205,557 alone did not significantly alter basal vertical activity in either time period (p > .05).

Behavioral time-sampling procedures were also used to assess rearing (Fig. 3A), sniffing (Fig. 3B), ambulation (Fig. 3C), and a composite score of all 3 behaviors (Fig. 3D) during automated assessments of locomotor activity after saline or SDZ 205,557 (0.0001, 0.01, or 1 mg/kg) plus saline or cocaine (10 mg/kg). A significant main effect of drug was observed for rearing (F(7,63) = 4.15, p < .001), sniffing (F(7,63) = 4.79, p < .001), ambulation (F(7,63) = 5.50, p < .001), and the composite score for all three behaviors (F(7,63) = 6.36, p < .001). Cocaine significantly increased rearing, sniffing, ambulation, and the composite score compared with saline-saline controls (p < .05). Pretreatment with 0.0001, 0.01, or 1 mg/kg SDZ 205,557 significantly attenuated the increase in ambulation scores induced by cocaine (p < .05), whereas pretreatment with 1 mg/kg SDZ 205,557 significantly attenuated the increase in rearing scores observed after cocaine (p < .05). Although a trend toward a reduction in the cocaine-induced increase in sniffing was observed after pretreatment with 0.01 or 1 mg/kg SDZ 205,557, this trend did not reach statistical significance. Cocaine-induced increases in the composite behavior scores were significantly attenuated by pretreatment with 0.0001, 0.01, or 1 mg/kg SDZ 205,557 (p < .05). The administration of doses of SDZ 205,557 alone did not significantly alter rearing, sniffing, ambulation, and composite behavior scores (p > .05).

Intra-NAc Shell Microinjections of 5-HT4 Partial Agonist RS 67333. Of the 16 rats originally cannulated and tested, 8 rats exhibited cannulas placements bilaterally po-
sitioned in the center of the NAc shell; only data from these 8 rats were included in the analyses. Horizontal and vertical activity counts were determined after pretreatment with intra-NAc shell saline or RS 67333 (1 or 3 μg/0.2 μl/side) and systemic treatment with saline or cocaine (10 mg/kg; Figs. 4 and 5). A significant main effect of drug was observed for total horizontal activity during the first ($F_{5,35} = 20.55, p < .001$) and second 30-min intervals ($F_{5,35} = 20.28, p < .001$). For both the first (Fig. 4A) and second 30-min intervals (Fig. 4B), systemic cocaine significantly increased horizontal activity after intra-NAc shell saline ($p < .05$). Intra-NAc shell pretreatment with either 1 or 3 μg of RS 67333 significantly attenuated cocaine-evoked horizontal activity ($p < .05$), although horizontal activity during these tests was still greater than that for saline-saline controls during both intervals ($p < .05$). Intra-NAc shell pretreatment with RS 67333 (1 or 3 μg) alone did not alter basal horizontal activity ($p > .05$).

A significant main effect of drug was revealed for total vertical activity during the second ($F_{5,35} = 3.30, p < .05$), but not the first, 30-min interval ($F_{5,35} = 1.44, p = .23$). For the second 30-min interval (Fig. 5B), Student-Newman-Keuls procedure revealed no significant differences in vertical activity among treatments ($p > .05$).

**Intra-NAc Shell Microinjections of 5-HT<sub>4</sub> Antagonist SDZ 205,557.** Of the 16 rats originally cannulated and tested, 8 rats exhibited cannulas placements that were bilaterally positioned in the center of the NAc shell; only data from these 8 rats were included in the analyses. Horizontal and vertical activity counts were assessed after pretreatment with intra-NAc shell saline or SDZ 205,557 (0.5, 1, or 5 μg/0.2 μl/side) and systemic treatment with saline or cocaine (10 mg/kg; Figs. 6 and 7). A significant main effect of drug was observed for total horizontal activity during the first ($F_{7,49} = 11.00, p < .001$) and second 30-min intervals ($F_{7,49} = 7.30, p < .001$). For the first 30-min interval (Fig. 6A), systemic cocaine significantly increased horizontal activity after intra-NAc shell saline ($p < .05$), or 0.5 μg of SDZ 205,557 as well as intra-NAc shell pretreatment with 1 or 5 μg of SDZ 205,557 significantly attenuated cocaine-induced horizontal activity ($p < .05$). For the second 30-min interval (Fig. 6B), cocaine-induced horizontal activity was significantly elevated in rats infused with saline or 0.5 or 5 μg of SDZ 205,557 into the NAc shell ($p < .05$). Intra-NAc shell pretreatment with doses of SDZ 205,557 alone did not significantly alter basal horizontal activity ($p > .05$).

A significant main effect of drug was observed for total vertical activity during the first ($F_{7,49} = 2.47, p < .05$) and second ($F_{7,49} = 4.04, p < .01$) 30-min intervals. For the first 30-min interval (Fig. 7A), Student-Newman-Keuls procedure

**Fig. 4.** Total horizontal activity after intra-NAc shell pretreatment with the 5-HT<sub>4</sub> partial agonist RS 67333 (RS). (See legend to Fig. 1 for explanation of the figure.)

**Fig. 5.** Total vertical activity after intra-NAc shell pretreatment with the 5-HT<sub>4</sub> partial agonist RS 67333. (See legend to Fig. 1 for explanation of the figure.)

**Fig. 6.** Total horizontal activity after intra-NAc shell pretreatment with the 5-HT<sub>4</sub> antagonist SDZ 205,557. (See legend to Fig. 1 for explanation of the figure.)

**Fig. 7.** Total vertical activity after intra-NAc shell pretreatment with the 5-HT<sub>4</sub> antagonist SDZ 205,557. (See legend to Fig. 1 for explanation of the figure.)
revealed no significant differences in vertical activity among treatment combinations. For the second 30-min interval (Fig. 7B), systemic cocaine significantly elevated vertical activity regardless of the intra-NAc shell pretreatment (p < .05). Intra-NAc shell pretreatment with doses of SDZ 205,557 alone did not significantly alter basal vertical activity (p > .05).

### Discussion

The spontaneous activity of rats well habituated to the test environment was unaffected by the 5-HT<sub>4</sub> partial agonist RS 67333 or the 5-HT<sub>4</sub> antagonist SDZ 205,557 administered systemically or directly into the NAc shell. These findings are consistent with previous reports that 5-HT<sub>4</sub> antagonists have little effect on basal behaviors. For example, performance in the elevated plus-maze (Silvestre et al., 1996; Kennett et al., 1997) and social interaction test in rats (Kennett et al., 1997) or the light/dark test in mice (Cheng et al., 1994; Costall and Naylor, 1997) was unaffected by 5-HT<sub>4</sub> antagonists, including SDZ 205,557, although another 5-HT<sub>4</sub> antagonist, RS 67532, was reported to significantly reduce basal locomotion in rats (Fontana et al., 1997). In general, these observations suggest that 5-HT<sub>4</sub> receptors may not be tonically activated under basal conditions. To a certain extent, this hypothesis is upheld by neurochemical studies of DA and 5-HT efflux. For example, basal DA efflux measured in vivo in rat substantia nigra (Thorpe et al., 1998), NAc (Taylor and Routledge, 1996), and striatum (De Deurwaerdere et al., 1997) was unaffected by 5-HT<sub>4</sub> antagonists, although DA efflux decreased in rat striatum after local application of the 5-HT<sub>4</sub> antagonist GR 113808 (Steward et al., 1996). Interestingly, the influence of GR 113808 on basal DA release significantly declined when assessed in striatal slices, suggesting that the modulatory control of 5-HT<sub>4</sub> receptors on DA release may be largely dependent on the activity of 5-HT terminals (Steward et al., 1996). In contrast to DA release, a facilitatory endogenous tone on 5-HT<sub>4</sub> receptors, which control 5-HT efflux, has been noted. The local or systemic administration of the 5-HT<sub>4</sub> antagonist GR 125,487D lowered 5-HT efflux in the hippocampus of freely moving rats (Ge and Barnes, 1996), whereas intranigral perfusion with the 5-HT<sub>4</sub> antagonist RS 39604 lowered 5-HT efflux in this region (Thorre et al., 1998). Perhaps the tonic nature of 5-HT<sub>4</sub> control of DA efflux is dependent on the level of 5-HT innervation to particular brain nuclei and the relative local density of 5-HT<sub>4</sub> receptors. This speculation notwithstanding, some of the confusion with regard to 5-HT<sub>4</sub> actions may be related to the use of different antagonists with different profiles of action, such as different affinities and efficacies at 5-HT<sub>4</sub> receptors.

Both automated activity measurements and observational analyses indicated that systemic administration of SDZ 205,557 effectively attenuated cocaine-evoked horizontal and vertical activities in the same dose range in which this 5-HT<sub>4</sub> antagonist was ineffective in altering spontaneous activity in habituated rats. In two previous studies, the 5-HT<sub>4</sub> antagonist GR 125487D was reported to suppress basal and cocaine-evoked activity nonselectively (Ohuoha et al., 1998), whereas the 5-HT<sub>4</sub> antagonist SB 204070 did not alter hyperactivity evoked by systemic administration of amphetamine, nicotine, or morphine (Reavill et al., 1998). Thus, the present findings are the first to indicate that the systemic administration of a 5-HT<sub>4</sub> antagonist effectively blocked hyperactivity evoked by a psychostimulant in the absence of changes in basal behavior.

The systemic doses of SDZ 205,557 that were effective in the present study are equivalent to the doses used in other behavioral paradigms. For example, systemic doses of SDZ 205,557 (≤1 mg/kg) were reported to significantly reverse the anxiolytic profile of diazepam (Costall and Naylor, 1997) or the combined injection of 5-HT<sub>2C/2A</sub> antagonist ritanserin and 5-hydroxytryptophan in the mouse light/dark test (Cheng et al., 1994). Although the biological half-life of SDZ 205,557 was estimated to be 23 min in the micropig due to rapid hydrolysis of the ester moiety present in this compound (Eglen et al., 1993), species differences may exist given that this and other studies (Cheng et al., 1994; Costall and Naylor, 1997) have demonstrated that low doses of SDZ 205,557 can influence behavior in rodents for much longer periods. For example, systemic administration of SDZ 205,557 (administered 45 min before the injection of cocaine and subsequent locomotor testing) significantly reduced cocaine-induced hyperactivity during the first 30 min of testing, indicating that this compound can influence behavior for at least 1 h. Systemic administration of RS 67333 did not alter cocaine-evoked activity, although this partial 5-HT<sub>4</sub> agonist would be expected to act as a 5-HT<sub>4</sub> antagonist under the conditions of increased 5-HT tone (Ruffolo, 1982) such as that after 5-HT reuptake inhibition evoked by cocaine. In a dose range similar to that used here, RS 67333 has been reported to act as a 5-HT<sub>4</sub> agonist to reverse deficits in spatial learning and memory induced by the central actions of atropine in rats (Fontana et al., 1997), suggesting that the failure of RS 67333 to affect basal or cocaine-stimulated locomotor activity was not related to a failure of this compound to penetrate the blood-brain barrier.

In contrast to the lack of efficacy of systemic RS 67333, intra-NAc shell microinjection of RS 67333 significantly attenuated cocaine-induced horizontal activity during the first
and second 30 min of testing, without significantly altering cocaine-induced vertical activity. Similarly, intra-NAc shell microinjection of SDZ 205,557 significantly suppressed cocaine-induced horizontal activity, although only during the first 30 min of testing. The longer duration of action of RS 67333 is consistent with the proposal that this drug is more resistant to metabolism than SDZ 205,557 (Fontana et al., 1997). These data are the first to suggest that the locomotor stimulatory effects of cocaine may involve actions at central 5-HT4 receptors and to implicate 5-HT4 receptors in the control of output from the NAc under stimulated conditions. Systemic administration of cocaine increases the efflux of 5-HT in the NAc (Reith et al., 1997), and given that 5-HT4 receptors are densely localized to the shell of the NAc, subsequent activation of 5-HT4 receptors, which could stimulate DA efflux in the NAc, may contribute to the behavioral effects of cocaine.

The ability of intra-NAc shell microinjection of RS 67333 and SDZ 205,557 to attenuate cocaine-evoked hyperactivity is presumably related to actions at 5-HT4 receptors. Both RS 67333 ($K_i = 2\text{nM};$ Eglen et al., 1995) and SDZ 205,557 ($K_i = 10.6\text{nM};$ Schiavi et al., 1994) exhibit high affinity for 5-HT4 receptors, but RS 67333 is an agonist with low intrinsic efficacy (Eglen et al., 1995). Perhaps RS 67333 acts as an antagonist upon microinjection into the shell of the NAc because of a low receptor reserve in this region and the propensity of partial agonists to act as antagonists under these conditions (Rufolo, 1982). However, RS 67333 does possess affinity for $\sigma_1$ and $\sigma_2$ receptors (Eglen et al., 1993), and the NAc contains $\sigma$ receptors (Bouchard and Quirion, 1997). Because $\sigma$ receptors have been noted to modulate DA release in limbic and cortical sites of guinea pig brain (Weatherspoon et al., 1996), the possibility that RS 67333 is acting at $\sigma$ receptors in NAC to affect the inhibition of cocaine-induced hyperactivity observed in the present study cannot presently be ruled out. A similar concern exists with regard to the selectivity of actions of SDZ 205,557 because this compound also possesses affinity for 5-HT3 receptors ($K_i = 341\text{nM};$ Schiavi et al., 1994). The local application of 5-HT3 receptor agonists into the NAc has been reported to increase DA efflux, an effect that is blocked by 5-HT3 antagonists (Chen et al., 1991). Cocaine-induced hyperactivity in rodents is reportedly attenuated by systemic pretreatment with 5-HT3 antagonists (e.g., Reith, 1990; Svingsø and Hitzemann, 1992). Because many of the 5-HT3 ligands previously studied possess actions at 5-HT4 receptors (Eglen et al., 1995), actions at both 5-HT3 and 5-HT4 receptors may contribute to the observed antagonistic effects of SDZ 205,557 on hyperactivity evoked by systemic cocaine administration.

In summary, both the 5-HT4 partial agonist RS 67333 and the 5-HT4 antagonist SDZ 205,557 successfully attenuated the stimulatory properties of cocaine when microinjected into the NAc shell, whereas systemic administration of SDZ 205,557 blocked cocaine-activated behaviors. These data suggest the importance of NAc shell 5-HT4 receptors in the behavioral effects of cocaine, and based on the proposal that stimulation of 5-HT4 receptors activates DA release, one possible explanation of the present findings is that both RS 67333 and SDZ 205,557 blocked the actions of 5-HT at 5-HT4 receptors, thereby lowering DA efflux in the shell of the NAc. However, definitive tests of this hypothesis will require combined neurochemical and behavioral analyses. Elucidation of the extent to which 5-HT4 receptors regulate behavioral processes dependent on mesolimbic DA pathways will help to clarify whether the 5-HT4 receptor might prove to be a novel target for the development of medications useful in the treatment of both drug dependence and psychiatric disorders. Furthermore, pharmacotherapy with 5-HT4 ligands may be associated with an acceptable side effect profile given that the observed antagonism of cocaine-induced hyperactivity by RS 67333 and SDZ 205,557 occurred at doses that did not appear to alter spontaneous behavior.

References

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