Contribution of Serotonin (5-Hydroxytryptamine; 5-HT) 5-HT₂ Receptor Subtypes to the Hyperlocomotor Effects of Cocaine: Acute and Chronic Pharmacological Analyses

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Received March 24, 2004; accepted May 6, 2004

ABSTRACT

The role of serotonin (5-hydroxytryptamine; 5-HT) 5-HT₂ receptor subtypes (5-HT₂AR, 5-HT₂BR, and 5-HT₂CR) in acute cocaine-evoked hyperactivity was compared with their contribution to the development and expression of locomotor sensitization upon repeated, intermittent treatment with cocaine (10 mg/kg/day for 5 days) in male Wistar rats. Cocaine-evoked hyperactivity was significantly enhanced by pretreatment with the preferential 5-HT₂AR antagonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and the 5-HT₂CR antagonist SDZ SER-082 [(+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methylindolo(1,7-BC)(2,6) naphthyridine fumarate]. The 5-HT₂AR antagonist SR 46349B [1(2,5-dimethoxy-4-iodophenyl)ethyl]oxy]-iminomethyl)-1(2-fluorophenyl)-3-(4-hydroxyphenyl)-2(E)-propene] and the preferential 5-HT₂CR agonist MK 212 [6-chloro-2-(1-piperazinyl)pyrazine HCl] (2 mg/kg) significantly attenuated acute cocaine-evoked hyperactivity; however, a lower dose of MK 212 (0.3 mg/kg) enhanced cocaine-evoked hyperactivity. The 5-HT₂CR agonist BW 723C86 (1-[5-(2-thienylmethoxy)-1H-3-indolyl]propan-2-amine HCl) and the 5-HT₂AR antagonist SB 204741 [N-(1-methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl) urea] had no effect on cocaine-evoked hyperactivity. Repeated treatment with cocaine alone resulted in a 2-fold increase in hyperactivity upon challenge with cocaine 5 days after termination of the cocaine regimen (sensitization). The 5-HT₂AR antagonist SR 46349B also blocked cocaine-evoked hyperactivity following repeated cocaine treatment, whereas the other 5-HT₂R ligands were ineffective. When any of the 5-HT₂R ligands was coadministered with cocaine during the treatment regimen (10 mg/kg/day for 5 days), the development of sensitization was unchanged as measured by the level of cocaine-evoked hyperactivity upon challenge 5 days after termination of the treatment. The present study implies that 5-HT₂AR and 5-HT₂CR exert opposing influence upon hyperactivity evoked by acute administration of cocaine; this balance is altered following repeated cocaine administration.

Cocaine enhances dopamine (DA), serotonin (5-hydroxytryptamine; 5-HT), and norepinephrine neurotransmission through inhibition of their respective reuptake inhibitors (Koe, 1976). Enhancement of DA, particularly within the DA mesoaccumbens (“reward”) pathway, is important in the locomotor-stimulant, reinforcing, and discriminative stimulus effects of cocaine (Pettit et al., 1984; Delfs et al., 1990; Callahan et al., 1997). However, the 5-HT system has also been shown to play a vital role in the modulation of DA mesoaccumbens pathways (Schmidt et al., 1992; De Deurwaerdere and Spampinato, 1999; Di Matteo et al., 1999; Gobert et al., 2000) and has been implicated in the mediation of cocaine-evoked behaviors, including cocaine-induced hyperactivity (McCreary and Cunningham, 1999; McMahon and Cunningham, 2001; McMahon et al., 2001; Filip and Cunningham, 2002, 2003; Fletcher et al., 2002, 2004; Bubar et al., 2003).

The 5-HT₂A receptor (5-HT₂AR) and the 5-HT₂CR appear to have opposing influences on DA neurotransmission and psy-

ABBREVIATIONS: DA, dopamine; 5-HT, 5-hydroxytryptamine, serotonin; 5-HT₂AR, serotonin₂ receptor; SR 46349B, 1(2-[dimethylaminooxyiminomethyl]-1(2-fluorophenyl)-3-(4-hydroxyphenyl)-2(E)-propene; MK 212, 6-chloro-2-(1-piperazinyl)pyrazine HCl; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; SDZ SER-082, [(+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methylindolo(1,7-BC)(2,6) naphthyridine fumarate; BW 723C86, 1-[5-(2-thienylmethoxy)-1H-3-indolyl]propan-2-amine HCl; SB 204741, N-(1-methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl) urea; ANOVA, analysis of variance; M100907, R-(+)-(2,3-dimethyl-4-phenyl-5-piperidinyl)-3-pyrrolidinylcarboxamidemethanol; SB 242084, 6-chloro-5-methyl-1-[2-(2-methylpyridyl)-3-oxoylpyrid-5-yl] carbamoyl]indoline; SB 206553, N-3-pyrrolidinyl-5-dihydro-5-methylbenzo[1,2-b:4,5-b']dipyrrrole-1(2H)-carboxamide hydrochloride; GABA, γ-aminobutyric acid; NAc, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area.
chostimulant-evoked behaviors. Microdialysis assays suggest that the 5-HT2AR can enhance DA neurotransmission under "stimulated" conditions such as after amphetamine administration (Schmidt et al., 1992) or dorsal raphe nucleus stimulation (De Deurwaerdere and Spampinato, 1999), whereas the 5-HT2AR appears to exert inhibitory control over brain DA pathways (De Deurwaerdere and Spampinato, 1999; Di Matteo et al., 1999; Gobert et al., 2000). In keeping with a potentiative role for the 5-HT2AR over DA mesoaccumbens circuits, 5-HT2AR antagonists have been shown to block the hyperlocomotor (Filip et al., 2001; McMahon and Cunningham, 2001) and discriminative stimulus effects (McMahon and Cunningham, 2001; but see Callahan and Cunningham, 1995; Meert and Janssen, 1992), as well as relapse to self-administration evoked by cocaine (Fletcher et al., 2002). Conversely, systemic administration of brain-penetrant 5-HT2CR antagonists has been shown to potentiate these same behavioral effects of cocaine (McCready and Cunningham, 1999; Fletcher et al., 2002). These data suggest that the 5-HT2R family may be functional and oppositional regulators of the neural substrates that control responsiveness to cocaine.

Modifications in serotonin function may be involved in the processes that underlie "behavioral sensitization" (Cunningham et al., 1992; Filip et al., 2001; Przegalinski et al., 2001). Behavioral sensitization is the enhancement of locomotor hyperactivity and stereotypies demonstrated upon challenge with cocaine during withdrawal from repeated, intermittent cocaine administration (for review, see Vanderschuren and Kalivas, 2000). This behavioral model has been used extensively to analyze the neural modifications associated with chronic cocaine exposure and withdrawal (White and Kalivas, 1998).

The present series of experiments was conducted to compare the ability of selective agonists and antagonists for specific 5-HT2R subtypes to modulate locomotor activity evoked by acute cocaine administration versus their ability to modulate the acquisition or expression of locomotor sensitization to cocaine. A repeated cocaine treatment regimen of 10 mg/kg/day for 5 days has previously been shown to induce locomotor sensitization when expression is measured 5 days after the last treatment injection (Filip et al., 2001; Przegalinski et al., 2001). To examine the ability of the 5-HT2R ligands to alter acquisition of cocaine sensitization, the ligands were administered before each daily injection of cocaine during the repeated treatment regimen, whereas the ability of the 5-HT2R ligands to alter expression of sensitization was determined via administration of the ligands before challenge with cocaine 5 days after the termination of repeated cocaine treatment. We hypothesized that the selective 5-HT2AR antagonist SR 46349B and the preferential 5-HT2AR agonist MK 212 would limit acute cocaine (10 mg/kg)-evoked hyperactivity and the development and/or expression of sensitization to cocaine, whereas the preferential 5-HT2AR agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-amino propane (DOI) and the selective 5-HT2CR antagonist SDZ SER-082 were expected to enhance these cocaine-evoked behaviors. Although DOI has moderate affinity for all three 5-HT2R subtypes (see Table 1), the effects of DOI on acute cocaine-evoked hyperactivity are thought to be primarily mediated by the 5-HT2AR, since the behavioral effects of DOI (e.g., wet dog shakes) are preferentially blocked by 5-HT2AR, but not 5-HT2BR/2CR, antagonists (Kennett, 1993; Schreiber et al., 1995). Since 5-HT2AR expression in the brain is low (Duxon et al., 1997), the 5-HT2AR agonist BW 723C86 and the 5-HT2CR antagonist SB 204741 were predicted to have little or no effect on acute cocaine-evoked hyperactivity or cocaine sensitization.

### Materials and Methods

#### Animals

Male Wistar rats (n = 832; Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland) weighing 250 to 270 g at the beginning of the experiment were used. The rats were housed eight per cage in standard plastic rodent cages (57 cm × 35 cm × 20 cm) 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 7:00 AM) and had continuous access to tap water and rodent chow except during experimental sessions. All experiments were conducted during the light phase of the light/dark cycle (between 9:00 Am and 3:00 PM) and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with approval from the Bioethics Commission as compliant with the Polish Law (21 August 1997).

#### Drugs

The following drugs, their full chemical names (when relevant), the supplier, and the route of injection, respectively, were as follows: BW 723C86 [1-[5-(2-thienylmethyl)-1H-3-indolyl]propan-2-amine HCl; Tocris Cookson, Bristol, UK; i.p.], cocaine HCl (Merck, Darmstadt, Germany; i.p.), DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-amino propane HCl; Sigma-Aldrich, St. Louis, MO; i.p.], MK 212 [6-chloro-2-(1-piperazinyl)pyrazine HCl; Tocris Cookson; i.p.], SB 204741 [N-(1-methyl-5-indolyl)-N’-(3-methyl-5-isothiazolyl) urea; Tocris Cookson; i.p.], SDZ SER-082 [(+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methylnindolo(1,7-BC)(2,6) napthyridine fumarate; Tocris Cookson; i.p.], and SR 46349B [(1Z)-2-(dimethylamino)ethoxyiminio]-1(2-fluorophenyl)-3-(4-hydroxyphenyl)-2(E)-propene; Sanofi-Sythelabo, Paris, France; s.c.]. To achieve dissolution, DOI, MK 212, and SDZ SER-082 were dissolved in saline (0.9% NaCl), BW 723C86

<table>
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<th>Table 1</th>
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<tr>
<td>Affinity (K&lt;sub&gt;i&lt;/sub&gt;, nM) of ligands for 5-HT&lt;sub&gt;2&lt;/sub&gt;R subtypes</td>
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<th>5-HT&lt;sub&gt;2C&lt;/sub&gt;R</th>
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<td>125</td>
</tr>
<tr>
<td>DOI</td>
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<td>39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MK 212</td>
<td>15,848</td>
<td>1258</td>
<td>630</td>
</tr>
<tr>
<td>SB 204741</td>
<td>5011</td>
<td>15</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>SR 46349B</td>
<td>5.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;100</td>
<td>120&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>SDZ SER-082</td>
<td>630&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58</td>
<td>15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> EC<sub>50</sub> (nM).
<sup>b</sup> IC<sub>50</sub> (nM).
<sup>c</sup> K<sub>i</sub> (nM).
and SB 204741 were suspended in aqueous 1% Tween solution, and SR 46349B was dissolved in two to three drops of ethanol and diluted as required in distilled water. All drugs were injected in a volume of 1 ml/kg. The doses of drugs were chosen based on their functional selectivity at a particular 5-HT₁ᵣ (Cunningham et al., 1986; Rinaldi-Carmona et al., 1992; Schreiber et al., 1995; Kennett et al., 1997; Gobert et al., 2000); the affinity profiles for each of the 5-HT₂ᵣ ligands are presented in Table 1.

Apparatus

Locomotor activity was monitored and quantified in clear Plexiglas chambers (43 cm × 43 cm × 25 cm) housed inside Opto-Varimex activity monitors surrounded with a 15 × 15 array of photocell beams located 3 cm from the floor surface (Columbus Instruments, Columbus, OH). Interruptions of these photocells resulted in horizontal activity defined as distance traveled (expressed in centimeters). Records of horizontal activity were made by the control software (Columbus Instruments) for subsequent statistical evaluation.

Procedures

Effects of 5-HT₂ᵣ Ligands on Acute Cocaine-Evoked Locomotor Activity. Rats were habituated to the test environment for 2 h/day on each of the 2 days before the start of the experiment, and on each test day for 1 h before the start of the test session. Animals were tested only one time, and separate groups of animals (n = 8/group) were pretreated with either the 5-HT₂ᵣ agonist DOI (0.1–1 mg/kg), 5-HT₂ᵣ antagonist SR 46349B (0.25–1 mg/kg), 5-HT₂ᵣ agonist BW 723C86 (3–10 mg/kg), 5-HT₂ᵣ antagonist SB 204741 (1–3 mg/kg), 5-HT₂ᵣ agonist MK 212 (0.1–2 mg/kg), 5-HT₂ᵣ antagonist SDZ SER-082 (0.25–1 mg/kg), or the appropriate vehicle 30 min (DOI, BW 723C86, MK 212, SB 204741), the rats received an i.p. treatment injection of either saline (1 ml/kg) or cocaine (10 mg/kg) and were returned to the test environment, and their locomotor activity was recorded for 60 min. Each rat underwent only one test session.

Effects of 5-HT₂ᵣ Ligands on the Acquisition of Behavioral Sensitization to Cocaine. On each day for 5 consecutive days, rats (n = 8/group) were removed from their home cage, weighed, and injected with either DOI (0.1–1 mg/kg), SR 46349B (0.25–1 mg/kg), BW 723C86 (3–10 mg/kg), SB 204741 (1–3 mg/kg), MK 212 (0.1–2 mg/kg), or SDZ SER-082 (0.25–1 mg/kg) and returned to their home cage; 30 to 40 min later, rats received an injection of cocaine (10 mg/kg) and were immediately returned to their home cage. Control rats (n = 8/group) were injected with the appropriate vehicle (1 ml/kg; see Drugs, above) before an injection of saline (1 ml/kg) or cocaine (10 mg/kg). Measurements of locomotor activity began immediately after the second (saline or cocaine) injection and lasted 60 min.

Results

Hyperactivity Induced by Acute Cocaine Administration

Effects of the 5-HT₂ᵣ Agonist DOI on Cocaine-Induced Hyperactivity. A main effect of pretreatment (F₁,₅₆ = 4.74, p < 0.01), treatment (F₀,₇ = 42.12, p < 0.001), and a pretreatment × treatment interaction (F₃,₅₆ = 2.90, p < 0.05) was observed for total horizontal activity summed across the 1-h session. DOI (0.1–1 mg/kg) administered before a systemic saline injection did not alter basal locomotor activity (p > 0.05). Pretreatment with DOI dose dependently increased the horizontal activity induced by cocaine (10 mg/kg), reaching significance at the highest dose (1 mg/kg) of DOI tested (p < 0.05; Fig. 1A).

Effects of the 5-HT₂ᵣ Antagonist SR 46349B on Cocaine-Induced Hyperactivity. A main effect of pretreatment (F₃,₅₆ = 6.99, p < 0.001), treatment (F₀,₇ = 29.49, p < 0.001), and a pretreatment × treatment interaction (F₃,₅₆ = 2.95, p < 0.05) was observed for total horizontal activity summed across the 1-h session. Pretreatment with SR 46349B (0.25–1 mg/kg) dose dependently attenuated cocaine-induced horizontal activity (p < 0.05); activity levels observed following pretreatment with 1 mg/kg SR 46349B were significantly decreased (p < 0.001; Fig. 1B) to levels that were not significantly different from vehicle + saline controls (p > 0.05). SR 46349B (0.25–1 mg/kg) tested alone did not significantly alter basal locomotor activity (p > 0.05).
Effects of the 5-HT2CR Antagonist SDZ SER-082 on Cocaine-Induced Hyperactivity. A main effect of treatment ($F_{1.5} = 40.31, p < 0.001$), but not pretreatment ($F_{2.42} = 1.67, p > 0.05$) or a pretreatment × treatment interaction ($F_{2.42} = 1.58, p > 0.05$), was observed for total horizontal activity summed across the 1-h session. Neither of the doses of BW 723C86 (3 and 10 mg/kg) significantly altered either basal or cocaine-induced horizontal activity ($p > 0.05$; Fig. 2A).

Effects of the 5-HT2BR Antagonist SB 204741 on Cocaine-Induced Hyperactivity. A main effect of treatment ($F_{1.5} = 42.00, p < 0.001$), but not pretreatment ($F_{2.42} = 0.04, p > 0.05$) or a pretreatment × treatment interaction ($F_{2.42} = 0.33, p > 0.05$), was observed for total horizontal activity summed across the 1-h session. Neither of the doses of SB 204741 (1 and 3 mg/kg) significantly altered basal or cocaine-induced horizontal activity ($p > 0.05$; Fig. 2B).

Effects of 5-HT2CR Agonist MK 212 on Cocaine-Induced Hyperactivity. A main effect of pretreatment ($F_{4.70} = 5.08, p < 0.01$), treatment ($F_{1.45} = 44.39, p < 0.001$), and a pretreatment × treatment interaction ($F_{4.70} = 4.28, p < 0.01$) was observed for total horizontal activity summed across the 1-h session. Pretreatment with 0.3 mg/kg MK 212 enhanced cocaine-induced horizontal activity ($p < 0.05$), whereas 2 mg/kg MK 212 significantly reduced cocaine-induced increases in locomotor activity ($p < 0.05$) to levels that were not significantly different from vehicle + saline controls ($p > 0.05$; Fig. 3A). However, 2 mg/kg MK 212 significantly reduced basal locomotor activity ($p < 0.05$; Fig. 3A).

Effects of the 5-HT2CR Antagonist SDZ SER-082 on Cocaine-Induced Hyperactivity. A main effect of pretreatment ($F_{3,56} = 15.97, p < 0.01$), treatment ($F_{1.7} = 64.25, p < 0.001$), and a pretreatment × treatment interaction ($F_{3,56} = 17.32, p < 0.001$) was observed for total horizontal activity summed across the 1-h session. Pretreatment with SDZ SER-082 increased the horizontal activity induced by cocaine (10 mg/kg) in a dose-dependent manner; a significant enhancement was observed after 1 mg/kg SDZ SER-082 ($p < 0.001$; Fig. 3B). SDZ SER-082 (0.25–1 mg/kg) did not alter basal locomotor activity ($p > 0.05$).
Cocaine Sensitization

Rats repeatedly treated with vehicle (VEH) + saline (SAL; 1 ml/kg; open bars) or cocaine (COC; 10 mg/kg; hatched bars) once a day for 5 days. Five days after the last treatment, rats were challenged with the acute injection of cocaine (10 mg/kg) + saline (SAL; 1 ml/kg; open bars) or cocaine (COC; 10 mg/kg; hatched bars) once a day for 5 days. Data points represent the mean horizontal distance traveled (± S.E.M.) over the 1-h recording period from eight rats. *p < 0.05 versus repeated SAL-COC challenge group; #p < 0.05 versus repeated COC-COC challenge group.

Effects of 5-HT₂R Ligands on the Development of Cocaine Sensitization

Rats received five daily pretreatments with vehicle (VEH; 1 ml/kg; open bars) or cocaine (COC; 10 mg/kg; hatched bars) once a day for 5 days. Five days after the last repeated treatment, rats were challenged with the 5-HT₂AR agonist DOI (0, 0.1, 0.3, or 1.0 mg/kg) + cocaine (10 mg/kg) (A), or the 5-HT₂AR antagonist SR 46349B (0, 0.25, 0.5, or 1 mg/kg) + cocaine (10 mg/kg) (B). Data points represent the mean horizontal distance traveled (± S.E.M.) over the 1-h recording period from eight rats. *p < 0.05 versus repeated SAL-COC challenge group.

Table 2

Effects of the 5-HT₂R ligands on the development of cocaine sensitization

Rats were treated repeatedly with vehicle (VEH; saline (SAL), vehicle + cocaine (COC; 10 mg/kg), or a 5-HT₂R ligand + cocaine (10 mg/kg) for 5 days. Five days after termination of the repeated regimen, rats were challenged with cocaine (10 mg/kg) and locomotor activity was measured. Data points represent the mean horizontal distance traveled (cm ± S.E.M.; 8 rats/group) in response to the cocaine challenge.

<table>
<thead>
<tr>
<th>Repeated Treatment</th>
<th>Distance Traveled</th>
<th>Repeated Treatment</th>
<th>Distance Traveled</th>
</tr>
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<tr>
<td>VEH/SAL</td>
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<td>3967 ± 499*</td>
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<td>2855 ± 971</td>
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<td>SR 46349B 1.0/COC</td>
<td>3732 ± 995*</td>
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<tr>
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<td>MK 212 2.0/COC</td>
<td>3386 ± 729*</td>
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*p < 0.05 vs. VEH/SAL.
mental groups pretreated with DOI (0.1–1 mg/kg) did not significantly alter hyperactivity expressed upon challenge with cocaine (10 mg/kg) 5 days after termination of the repeated cocaine regimen; pretreatment with 0.5 and 1.0 mg/kg SR 46349B significantly suppressed cocaine-evoked hyperactivity (p < 0.05; Fig. 4B).

Effects of the 5-HT_{2A}R Agonist BW 723C86 on Expression of Cocaine Sensitization. A main effect of treatment was observed for total horizontal activity summed across the 60-min session (F_{4,35} = 12.44, p < 0.001). BW 723C86 had no effect on the expression of sensitization since pretreatment with BW 723C86 (3 or 10 mg/kg) did not significantly alter hyperactivity induced by challenge with cocaine (10 mg/kg) 5 days after termination of the repeated cocaine regimen (p > 0.05; Fig. 5A).

Effects of the 5-HT_{2C}R Agonist MK 212 on Expression of Cocaine Sensitization. A main effect of treatment was observed for total horizontal activity summed across the 60-min session (F_{4,40} = 12.64, p < 0.001). None of the doses of SB 204741 significantly altered hyperactivity expressed upon challenge with cocaine (10 mg/kg) 5 days after termination of the repeated cocaine regimen (p > 0.05; Fig. 5B).

Effects of the 5-HT_{2C}R Agonist SDZ SER-082 on Expression of Cocaine Sensitization. A main effect of treatment was observed for total horizontal activity summed across the 60-min session (F_{4,34} = 5.42, p < 0.01). This dose of MK 212 also suppressed basal locomotor activity (see Fig. 3A).

**Discussion**

The present studies were conducted to compare the ability of 5-HT_{2C}R agonists and antagonists to alter acute cocaine-evoked hyperactivity with the ability of these same ligands to alter the acquisition and/or expression of cocaine sensitization. The results suggest that the 5-HT_{2C}R plays a stimulatory role in cocaine hyperactivity induced by either acute cocaine administration or challenge with cocaine 5 days after...
termination of the sensitization regimen, while having no influence upon the acquisition of locomotor sensitization. Conversely, the 5-HT$_2$cR appears to have an inhibitory role in cocaine hyperactivity induced by acute cocaine administration, while having little influence upon the acquisition of sensitization or cocaine-evoked hyperactivity following the sensitizing cocaine regimen. The 5-HT$_2$aR has no overt role in elicitation of cocaine-evoked hyperactivity or cocaine sensitization.

The present observations following administration of the 5-HT$_2$aR antagonist SR 46349B support and extend previous findings that the selective 5-HT$_2$aR antagonist M100907 (McMahon and Cunningham, 2001; Fletcher et al., 2002) and the nonselective 5-HT$_2$R antagonist ketanserin (Fili et al., 2001; McMahon and Cunningham, 2001) attenuated cocaine-evoked hyperactivity at doses of the antagonists that did not alter basal activity levels. SR 46349B (present study) and ketanserin (Fili et al., 2001) also attenuated hyperactivity induced by challenge with cocaine 5 days after termination of the sensitizing cocaine regimen. In keeping with these results, we are the first to report that the preferential 5-HT$_2$aR agonist DOI enhanced the locomotor-activating effects of cocaine administered acutely, at doses of DOI that did not alter basal locomotor activity. After a sensitizing regimen of cocaine, however, DOI is no longer capable of further enhancing hyperactivity seen upon cocaine challenge 5 days after termination of the repeated cocaine regimen. In addition, cotreatment with neither DOI nor SR 46349B during the repeated cocaine regimen effectively altered the course of sensitization, suggesting that the role for 5-HT$_2$aR in acquisition of cocaine sensitization is minimal. This may be attributed to the rapid desensitization and down-regulation of 5-HT$_2$aR that can occur following repeated administration of either 5-HT$_2$aR antagonists or antagonists (for review, see Gray and Roth, 2001) and, potentially, cocaine (e.g., Darmani et al., 1997; but see Baumann and Rothman, 1998). Thus, although the 5-HT$_2$aR appears to be integral for the enhancement of hyperactivity induced by acute administration of cocaine, the functional role of the 5-HT$_2$aR appears to be altered with repeated cocaine administration.

Loss of the enhancement of cocaine-induced hyperactivity by the preferential 5-HT$_2$aR agonist DOI was observed in rats exposed to repeated cocaine administration. An alteration in the expression of the 5-HT$_2$aR or its downstream components after repeated cocaine administration may account for this observation. Repeated cocaine administration has been shown to result in short-term supersensitivity of 5-HT$_2$aR during withdrawal (Baumann and Rothman, 1998) that appears to be associated with an increase in membrane-associated $G_{q11}$ protein expression, rather than a specific increase in 5-HT$_2$aR ($B_{max}$) expression (Carrasco et al., 2003). Since cocaine-induced 5-HT efflux is enhanced in cocaine-sensitized animals (Parsons and Justice, 1993), it is possible that the 5-HT$_2$aR is in a state of maximal stimulation following cocaine challenge in the sensitized animals. Under these circumstances, DOI may be unable to provide any additional stimulation of 5-HT$_2$aR over levels of activation induced by endogenous 5-HT, which accumulates in the synapse after cocaine administration alone (Parsons and Justice, 1993). Conversely, the high-affinity 5-HT$_2$aR antagonist SR 46349B may effectively compete with 5-HT for 5-HT$_2$aR binding sites (Rinaldi-Carmona et al., 1992), and thus, SR 46349B blockade of 5-HT$_2$aR would continue to exert its inhibitory effect upon hyperactivity evoked by cocaine challenge, as was observed in the present study. This hypothesis is further supported by the present observation that, in animals treated with the repeated cocaine regimen, the levels of hyperactivity induced by challenge with cocaine alone were greater than those elicited by acute administration of DOI + cocaine in animals that had not been previously exposed to cocaine. Thus, the present results suggest that adaptation of the 5-HT$_2$aR or its downstream signaling components may occur following repeated cocaine administration and that these modifications may contribute to the inability of the 5-HT$_2$aR agonist to modulate the sensitized response to cocaine.

Alternatively, it is important to note that DOI is not a selective 5-HT$_2$aR agonist (see Table 1), and thus may also act at other 5-HT$_2$R subtypes. Considering the opposing actions of the 5-HT$_2$aR and 5-HT$_2$cR on cocaine-evoked hyperactivity (present results; McMahon and Cunningham, 2001; Fletcher et al., 2002), simultaneous stimulation of 5-HT$_2$aR and 5-HT$_2$cR by DOI may have contributed to the lack of effect of DOI on cocaine-evoked hyperactivity following the sensitization regimen.

Administration of the selective 5-HT$_2$cR antagonists SDZ SER-082 (present study) or SB 242084 (Fletcher et al., 2002) as well as the 5-HT$_2$b/2cR antagonist SB 206553 (McCreary and Cunningham, 1999) enhanced cocaine-evoked hyperactivity, suggesting that the 5-HT$_2$cR exerts an inhibitory influence on acute cocaine-evoked hyperactivity. Interestingly, a biphasic effect of the preferential 5-HT$_2$cR agonist MK 212 on cocaine-evoked hyperactivity was observed; enhancement and suppression were elicited by a low (0.3 mg/kg) and high dose of MK 212 (2 mg/kg), respectively. The high dose of MK 212 (2 mg/kg) was also shown to significantly suppress basal locomotor activation. A reciprocal biphasic effect was previously demonstrated following administration of the 5-HT$_2$b/2cR antagonist SB 206553, with lower doses suppressing and higher doses enhancing cocaine-evoked hyperactivity (McCreary and Cunningham, 1999); however, a significant biphasic effect was not observed following administration of the selective 5-HT$_2$cR antagonist SDZ SER-082 in the present study. The biphasic nature of the response may be due to the lack of complete selectivity of either MK 212 or SB 206553 for the 5-HT$_2$cR and, in particular, the lack of selectivity over the 5-HT$_2$aR. However, the inability of the selective 5-HT$_2$bR agonists and antagonists to alter cocaine-evoked hyperactivity as observed in the present and other studies (Fletcher et al., 2002) suggests that this distinction is unlikely attributable to the 5-HT$_2$bR.

A potential explanation for the biphasic effects of 5-HT$_2$aR agonists and antagonists on cocaine-induced hyperactivity is the differential influence of various populations of 5-HT$_2$Rs within the DA mesolimbic pathways. In the ventral tegmental area (VTA), the origin of the DA mesolimbic pathways, 5-HT$_2$Rs appear to be located on both DA and $\gamma$-aminobutyric acid (GABA) neurons (Eberle-Wang et al., 1997; Bubar and Cunningham, 2003). Systemic administration of 5-HT$_2$cR antagonists has been shown to either decrease (Blackburn et al., 2002) or increase (Di Matteo et al., 1999) the firing rate of spontaneously active VTA DA neurons, suggesting that different populations of 5-HT$_2$cRs within the
VTA may differentially activate VTA DA neurons (Blackburn et al., 2002). In addition, 5-HT2C-Rs are also located in the nucleus accumbens (NAc) and prefrontal cortex (PFC), the terminal regions of the DA mesolimbic pathways. Microinjection of 5-HT2C-R agonists into the NAc (Filip and Cunningham, 2002) or PFC (Filip and Cunningham, 2003) enhanced or reduced, respectively, cocaine-evoked hyperactivity, with reciprocal effects observed following antagonist administration (Filip and Cunningham, 2002, 2003). These data suggest that 5-HT2C-Rs in the NAc and PFC exert opposing influence upon cocaine-evoked hyperactivity. Thus, the ability of systemically administered 5-HT2C-R agonists and antagonists to alter cocaine-evoked hyperactivity in a biphasic manner may be attributed to the oppositional influence of 5-HT2C-R populations within and/or between brain regions associated with the DA mesolimbic pathways.

In contrast to the profound effects of 5-HT2C-R ligands on cocaine-evoked hyperactivity following acute cocaine administration, neither MK 212 nor SDZ SER-082 effectively altered the acquisition of sensitization. The 5-HT2C-R antagonist SDZ SER-082 also had no effect on cocaine-evoked hyperactivity 5 days after the repeated cocaine sensitization regimen, whereas only the highest dose of the 5-HT2C-R agonist MK 212 (2 mg/kg), which suppressed basal activity alone, was effective in suppressing cocaine-evoked hyperactivity. The lack of effects of the 5-HT2C-R ligands on cocaine-evoked hyperactivity following the cocaine sensitization regimen suggests that repeated cocaine administration results in alterations in the contribution of 5-HT2C-R to cocaine-evoked hyperactivity.

The loss of effect of both the 5-HT2C-R agonist and antagonist following the cocaine sensitization regimen suggests that the function of these receptors or their downstream signaling components is altered significantly following repeated exposure to cocaine. Unfortunately, to our knowledge, alterations in the sensitivity or expression of the 5-HT2C-R or its key signaling components subsequent to repeated cocaine administration have not yet been examined. Recent evidence from our laboratory, however, suggests that 5-HT2C-R sensitivity is decreased during withdrawal from a sensitizing regimen of the 5-HT/Da releaser (+)-3,4-methylenedioxymethamphetamine (Bubar et al., 2002); thus a similar consequence may occur following repeated cocaine administration. Since the 5-HT2C-R appears to predominantly exert an inhibitory influence upon DA mesocumbens pathway activity (De Deurwaerder and Spampinato, 1999; Di Matteo et al., 1999; Gobert et al., 2000) and cocaine-evoked hyperactivity (present study; Fletcher et al., 2002), down-regulation of the 5-HT2C-R following repeated cocaine administration would likely result in a decrease in 5-HT2C-R-mediated inhibition. This loss of 5-HT2C-R inhibition would be expected to enhance activation of the DA mesocumbens pathway and cocaine-evoked hyperactivity. This hypothesis is consistent with enhanced cocaine-evoked DA release (Vanderschuren and Kalivas, 2000) and sensitization of hyperactivity, as well as the loss of influence of the 5-HT2C-R ligands observed following repeated cocaine administration (present results).

In conclusion, the present results confirm previous evidence that the 5-HT2AR and 5-HT2CR exert opposing modulatory actions on hyperactivity evoked by acute cocaine administration, whereas 5-HT2BRs do not appear to be involved in elicitation of cocaine-evoked hyperactivity. In addition, we provide evidence that repeated cocaine administration may result in adaptations that contribute to the expression of sensitization and that alter the ability of 5-HT2AR and 5-HT2CR ligands to modulate cocaine-evoked hyperactivity.

Acknowledgments
We gratefully acknowledge the technical assistance of Ewa Nowak and Sanofi-Synthelabo for their generous gift of SR 46349B.

References


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