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Role of 5-HT_{2A} and 5-$HT_{2B/2C}$ Receptors in the Behavioral Interactions Between Serotonin and Catecholamine Reuptake Inhibitors

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Dysfunction of monoamine neurotransmission seems to contribute to such pathopsychological states as depression, schizophrenia, and drug abuse. The present study examined the effects of the selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitor (SSRI) and antidepressant fluvoxamine on locomotor activity in rats following administration of the catecholamine reuptake inhibitor mazindol. Mazindol (1 mg/kg) did not alter locomotor activity; whereas, fluvoxamine (20 mg/kg) given alone induced a brief period of hypomotility. Hyperactivity was elicited in a dose-related manner when fluvoxamine (5–20 mg/kg) was combined with mazindol (1 mg/kg). The hyperactivity elicited by fluvoxamine (20 mg/kg) plus mazindol (1 mg/kg) was significantly attenuated by the 5-HT_{2A} receptor antagonist M100907 (2 mg/kg) and potentiated by the 5-$HT_{2B/2C}$ receptor antagonist SB 206553 (2 mg/kg). Neither antagonist significantly altered basal activity. The hyperactivity evoked by the combination of fluvoxamine and mazindol seems to be mediated in part by 5-HT_{2A} receptors; whereas, 5-$HT_{2B/2C}$ receptors may serve to limit this effect. Thus, the balance of activation between 5-HT_{2A} and 5-$HT_{2B/2C}$ receptors seems to contribute to the expression of locomotor hyperactivity evoked via combination of a 5-HT and a catecholamine reuptake inhibitor. A disruption in this balance may contribute to the expression of affective disorders, schizophrenia, and drug abuse. ***[Neuropsychopharmacology 24:319–329, 2001]***

KEY WORDS: *Dopamine; Locomotor activity; Serotonin; 5-HT_{2A} Receptors; 5-HT_{2C} Receptors*

Several lines of evidence suggest that dopamine (DA) and serotonin (5-hydroxy tryptamine; 5-HT) interact in the central nervous system and that dysfunction of these neurotransmitters may contribute to such pathopsychological states as depression, schizophrenia, and drug abuse (Kahn and Davidson 1993; Kosten et al. 1998). The 5-HT-containing cell bodies of the dorsal raphe nucleus project to DA cell bodies of the ventral tegmental area (VTA) and substantia nigra (SN), and to their terminal fields in the prefrontal cortex (PFC), nucleus accumbens (NAc), and striatum (Hervé et al. 1987; Steinbush et al. 1981; Van der Kooy and Hattori 1980). The precise nature of the interaction between 5-HT and DA has been difficult to elucidate, with both inhibitory and excitatory roles for 5-HT identified with respect to the neural activity of DA neurons and the release of DA. For example, electrophysiological studies in vivo suggest an inhibitory influence of 5-HT on DA cell bodies of the VTA and SN (Prisco et al. 1994; Kelland et al. 1990), and 5-HT inhibits DA release from striatal slices (Ennis et al. 1981; Westfall and Tittermary 1982). On the other hand, in vivo microdialysis studies consistently demonstrate that local infusion of 5-HT increases DA efflux in the PFC, NAc, and striatum (Benloucif et al.

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Received 22 May 2000; revised 1 September 2000; accepted 12 September 2000.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/01/$–see front matter
PII S0893-133X(00)00206-2

1993; Gobert and Millan 1999; Iyer and Bradberry 1996; Parsons and Justice 1993), possibly through stimulation of one or more of the 14 5-HT receptor subtypes characterized to date (Barnes and Sharp 1999).

The behavioral importance of 5-HT and DA interactions has been difficult to define. Much of this research has focused on modifications of behaviors evoked by enhanced DA neurotransmission consequent to psychostimulant administration. In early studies, reductions and enhancements of the locomotor stimulatory effects of cocaine (Scheel-Kruger et al. 1976) and amphetamine (Geyer et al. 1976) were reported following increased 5-HT synthesis or depletion of 5-HT, respectively. These and similar findings generated the hypothesis that 5-HT plays an inhibitory role in DA-mediated behavior. On the other hand, systemic administration of selective serotonin reuptake inhibitors (SSRIs) potentiated hyperactivity induced by amphetamine or cocaine in rats (Herges and Taylor 1998; Sills et al. 1999a, 1999b), but not in mice (Arnt et al. 1984; Maj et al. 1984; Reith et al. 1991). The ability of SSRIs to increase 5-HT efflux is well-documented (e.g., Guan and McBride 1988; Li et al. 1996), thus, these data imply a more sophisticated role for 5-HT in the control of DA-mediated behaviors than simple inhibition.

The mechanisms that underlie the potentiation of DA-mediated hyperactivity induced by SSRIs have been little studied. A possible pharmacokinetic interaction at the level of metabolic enzymes has been suggested to contribute to the potentiation of amphetamine-induced hyperactivity by SSRIs (Sills et al. 1999a, 1999b). However, a role for specific 5-HT receptors is suggested by the observation that the facilitation of cocaine-induced hyperactivity by fluoxetine was attenuated by the 5-HT_{1A} receptor antagonist WAY 100635 and the nonselective 5-HT_2 receptor antagonist ketanserin (Herges and Taylor 1998). The goal of the present study was to analyze further the 5-HT_2 receptor subtypes involved in SSRI-evoked potentiation of DA-elicited behaviors. To conduct these studies, we chose to utilize the catecholamine reuptake inhibitor mazindol, rather than the catecholamine releaser amphetamine (Heikkila et al. 1975) or cocaine that blocks the transporters for DA (K_i = 277 nM), 5-HT (K_i = 217 nM) and norepinep;hrine (NE; K_i = 144 nM) in rat brain synaptosomes (Koe 1976; Hyttel 1982). Mazindol binds with greater affinity to transporters for DA (K_i = 16 nM), 5-HT (K_i = 25 nM), and NE (K_i = 0.42 nM; Hyttel 1982) in rat brain synaptosomes, but has been characterized in vitro and in vivo as a potent inhibitor of DA and NE reuptake with less potency for 5-HT reuptake (Heikkila et al. 1977; Sugrue et al. 1977). The behavioral effects of mazindol have been attributed to catecholamine reuptake inhibition, especially that of DA, as DA receptor antagonists have been shown to attenuate both mazindol-induced anorexia and locomotor hyperactivity (Geveard and Takahashi 1999; Kruk and Zarrindast 1976).

The present study was designed to assess the hyperactivity evoked by combinations of the SSRI fluvoxamine with mazindol and the involvement of 5-HT_2 receptors in the interactive behavioral effects of these two drugs. The 5-HT_{2A} and 5-HT_{2C} receptors were hypothesized to be particularly important because of the moderate to dense localization of both transcript and protein for these receptors in SN and VTA as well as DA terminal regions of rat forebrain (Abramowski et al. 1995; Lopez-Gimenez et al. 1997; Pompeiano et al. 1994). Antagonism of 5-HT_{2A} (De Deurwaerdere and Spampinato 1999; Lucas and Spampinato 2000) and 5-HT_{2C} receptors (De Deurwaerdere and Spampinato 1999; Di Giovanni et al. 1999; Di Matteo et al. 1998; Lucas and Spampinato 2000) has been shown to influence DA function in areas of brain (e.g., NAc and striatum) thought to be important in motor activation, motivation, and reward. In the present study, locomotor activity was assessed following administration of the SSRI fluvoxamine in combination with a low dose of mazindol in rats. The selective 5-HT_{2A} receptor antagonist M100907 (Sorensen et al. 1993) and the 5-$HT_{2B/2C}$ receptor antagonist SB 206553 (Kennett et al. 1996) were employed to analyze the roles of these receptors in hyperactivity evoked by fluvoxamine plus mazindol. The doses of these 5-HT_2 receptor antagonists were chosen based on their documented efficacy following systemic administration (McCreary and Cunningham 1999; McMahon and Cunningham submitted; Moser et al. 1996).

METHODS

Animals

The subjects were 36 experimentally naive male Sprague–Dawley rats (Harlan, Houston, TX) weighing between 300–350 g at the beginning of the study. The rats were housed in pairs in a colony room that was maintained at a constant temperature (21–23°C) and humidity (40–50%); lighting was maintained on a 12-h light–dark cycle (07:00–19:00 h). 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 cm × 40 cm × 40 cm) was housed within sound-attenuating enclosures 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. Activity recorded in the inner 16 × 16 cm of the open field was counted as central activity, while the field bounded by the outer 16-cm band registered peripheral activity. Separate counts of peripheral and central activity were made by 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. Video cameras positioned above the chambers permitted continuous observation of behavior without disruption.

Behavioral Procedures

All rats were maintained in the colony room for a minimum of 1 week before behavioral testing for acclimation to daily handling procedures. At the time tests were to be conducted (between 09:00–12:00 h), all rats were habituated to the testing environment for 2 h per 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 administration of drugs. In Experiment 1, the group of rats ($n = 13$) received an injection of either saline (1 ml/kg, IP) or fluvoxamine (5, 10, or 20 mg/kg, IP), followed 30 min later by an injection of either saline (1 ml/kg, IP) or mazindol (1 mg/kg, IP). In Experiment 2, the group of rats ($n = 10$) received an injection of either vehicle (1 ml/kg of 1% Tween 80, IP) or M100907 (2 mg/kg, IP), followed 10 min later by an injection of either saline (1 ml/kg, IP) or fluvoxamine (20 mg/kg, IP), followed 30 min later by an injection of either saline (1 ml/kg, IP) or mazindol (1 mg/kg, IP). In Experiment 3, the group of rats ($n = 13$) received an injection of either vehicle (1 ml/kg of 45% β-cyclodextrin, IP) or SB 206553 (2 mg/kg, IP), followed 10 min later by an injection of either saline (1 ml/kg, IP) or fluvoxamine (20 mg/kg, IP), followed 30 min later by an injection of either saline (1 ml/kg, IP) or mazindol (1 mg/kg, IP). Rats within a given group received each of the experimental treatments assigned to that group for a total of eight tests that were randomized using a Latin square design. Doses of M100907 (McMahon and Cunningham in press) and SB 206553 (McCreary and Cunningham 1999) were chosen based upon our previous experience with these drugs. Measurement of locomotor activity counts began immediately following the mazindol or saline injection and was divided into 5-min bins for a total of 90 min for each of the groups. For each rat, the order of drug tests for the antagonists and fluvoxamine were counterbalanced, and the mazindol injections were given every other test. Test sessions were conducted every 2 to 3 days. Thus, mazindol injections occurred no more than once per week.

Data Analysis

Data were analyzed as horizontal (peripheral plus central) activity counts totaled for the 90-min test session or in 18 separate 5-min time bins following IP injection of mazindol or its saline control. Because group comparisons were specifically defined before the start of the experiment, these planned comparisons were conducted in lieu of an over-all F test in a multifactorial analysis of variance (ANOVA); this statistical analysis has been supported in a number of statistical texts (e.g., Keppel 1973). Thus, each experiment was subjected to a one-way ANOVA for repeated measures with levels of the treatment factor corresponding to the eight drug combinations administered to that group. Planned, pair-wise comparisons of the treatment means were made with Student-Newman-Keuls procedure (SAS for Windows, Version 6.12). Treatment × time interactions were analyzed using a two-way ANOVA for repeated measures. All statistical analyses were conducted with an experiment-wise error rate of $\alpha = 0.05$.

Drugs

Doses of all drugs refer to the weight of the salt. Cocaine hydrochloride (NIDA) and fluvoxamine maleate (Solvay, Marietta, GA) were prepared in 0.9% NaCl. Mazindol (Sandoz, Hanover, NJ) was prepared in 0.9% NaCl with mild acidification. SB 206553 [5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole); SmithKline Beecham, Frythe, Welwyn, UK] was prepared in 45% 2-hydroxypropyl-β-cyclodextrin (Sigma/RBI, Natick, MA). M100907 [R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol; Hoechst Marion Roussel, Cincinnati, OH] was prepared in a solution of 1% Tween 80 (Sigma, St. Louis, MO) in sterile distilled water. All drugs were injected IP in a volume of 1 ml/kg.

RESULTS

Effects of the SSRI Fluvoxamine Alone or in Combination with the Catecholamine Uptake Inhibitor Mazindol

In Experiment 1, the effects of saline or fluvoxamine (5, 10 or 20 mg/kg) in combination with saline or mazindol (1 mg/kg) were assessed. A dose of 1 mg/kg of mazindol was chosen for these analyses, because this dose was subthreshold for elicitation of hyperactivity, unlike 2 and 5 mg/kg, which evoked significant hyperactivity (data not shown). A main effect of treatment on total

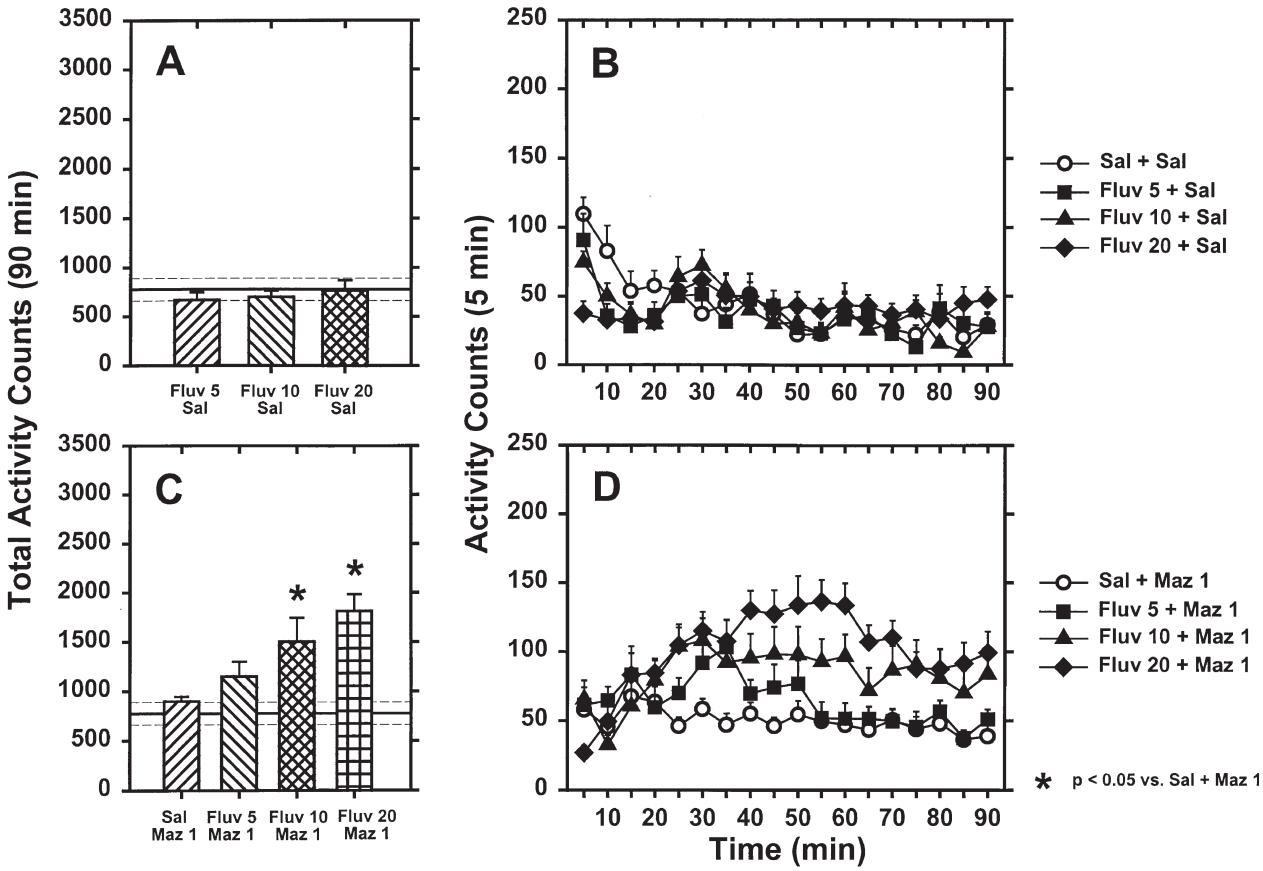

Figure 1. Locomotor activity after the SSRI fluvoxamine alone or in combination with the catecholamine uptake inhibitor mazindol. Left panel (**A** and **C**): Mean total horizontal activity (counts/90 min) (± S.E.M.) is depicted following IP injections of either saline (Sal) or fluvoxamine (Fluv; 5, 10, or 20 mg/kg) followed by saline or mazindol (Maz; 1 mg/kg). Mean total horizontal activity in saline-saline controls is expressed by the solid line with ± S.E.M. represented by the dashed lines. *, activity levels that were significantly different from saline-mazindol levels based on a Student-Newman-Keuls procedure ($p <$ .05). Right panel (**B** and **D**): Time course of horizontal activity (counts/5 min; ± S.E.M.) is depicted for the same tests indicated in **A** and **C**, respectively.

horizontal activity counts was observed [$F(7,96) = 9.69$, $p < .001$]. For visual simplicity, these data are graphed separately in Figure 1 top (**A** and **B**) and bottom (**C** and **D**). No significant differences in total horizontal activity were observed after 5, 10 or 20 mg/kg of fluvoxamine (Figure 1**A** and **B**) or mazindol (1mg/kg; Figure 1**C** and **D**) compared to saline controls ($p > .05$). In contrast, significant increases in total horizontal activity were observed after combination of fluvoxamine (10 or 20 mg/kg) and mazindol (1 mg/kg; Figure 1**C** and **D**) compared to mazindol alone ($p < .05$). A significant treatment × time interaction was observed for horizontal activity [$F(126,1386) = 3.41$, $p < .001$]. Fluvoxamine alone decreased horizontal activity at the beginning of the test session (<30 min; Figure 1**B**). Fluvoxamine at all doses in combination with mazindol produced significant hyperactivity compared to injection of mazindol alone beginning ~25 min after mazindol injection. The hyperactivity induced by mazindol in combination with 10 or 20 mg/kg of fluvoxamine was of longer duration than that observed following 5 mg/kg of fluvoxamine plus mazindol (Figure 1**D**).

Effects of the 5-HT_{2A} Receptor Antagonist M100907 on Locomotor Activity Evoked by Mazindol Alone or in Combination with Fluvoxamine

In Experiment 2, the effects of vehicle or M100907 (2 mg/kg) on horizontal activity evoked by saline or fluvoxamine (20 mg/kg) in combination with saline or mazindol (1 mg/kg) were assessed. A main effect of treatment on total horizontal activity counts was observed [$F(7,72) = 10.48$, $p < .001$]. For visual simplicity, these data are graphed separately in Figure 2 top (**A** and **B**) and bottom (**C** and **D**). Basal horizontal activity observed in vehicle-saline-saline controls was not significantly altered by fluvoxamine, M100907, or co-admin-

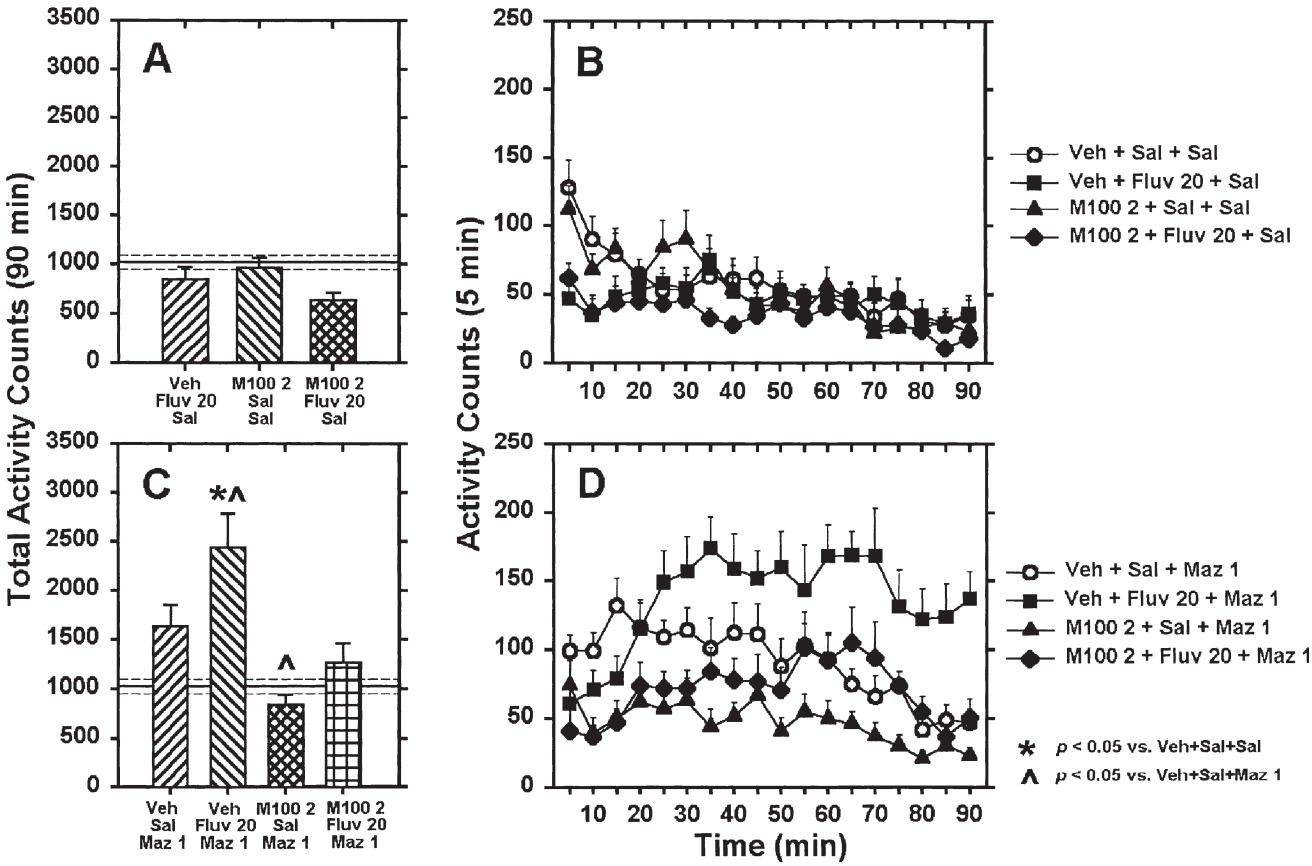

Figure 2. Effects of the 5-HT$_{2A}$ receptor antagonist M100907 on locomotor activity evoked by mazindol alone or in combination with fluvoxamine. Left panel (**A** and **C**): Mean total horizontal activity (counts/90 min) (± S.E.M.) is depicted following IP injections of either vehicle (Veh) or M100907 (M100; 2 mg/kg) followed by saline (Sal) or fluvoxamine (Fluv; 20 mg/kg) followed by saline or mazindol (Maz; 1 mg/kg). Mean total horizontal activity in vehicle-saline-saline controls is expressed by the solid line with ± S.E.M. represented by the dashed lines. *, activity levels that were significantly different from vehicle-saline-saline control levels. ^, activity levels that were significantly different from vehicle-saline-mazindol levels based on a Student-Newman-Keuls procedure ($p < .05$). Right panel (**B** and **D**): Time course of horizontal activity (counts/5 min; ± S.E.M.) is depicted for the same tests indicated in **A** and **C**, respectively.

istration of M100907 plus fluvoxamine ($p > .05$; Figure 2**A** and **B**). As noted in Experiment 1, total horizontal activity was significantly increased by the combination of fluvoxamine and mazindol compared to that observed after mazindol alone ($p < .05$; Figure 2**C** and **D**). In contrast, horizontal activity after M100907 in combination with fluvoxamine and mazindol was not significantly different from basal activity or that observed after mazindol alone ($p > .05$; Figure 2**C**). M100907 blocked the hyperactivity seen with fluvoxamine plus mazindol for the duration of the session (Figure 2**D**). Horizontal activity after M100907 in combination with mazindol alone was significantly different from that observed after mazindol alone ($p < .05$) but not from vehicle-saline-saline controls ($p > .05$; Fig. 2**C**). A significant treatment × time interaction was observed for horizontal activity in 5-min time bins [$F(119,1071) = 2.98$, $p < .001$]. As noted in Experiment 1, fluvoxamine depressed activity during the first 30 min of the session (Figure 2**B**).

Effects of the 5-HT$_{2B/2C}$ Receptor Antagonist SB 206553 on Locomotor Activity Evoked by Mazindol Alone or in Combination with Fluvoxamine

In Experiment 3, the effects of vehicle or SB 206553 (2 mg/kg) on horizontal activity evoked by saline or fluvoxamine (20 mg/kg) in combination with saline or mazindol (1 mg/kg) were assessed. A main effect of treatment on total horizontal activity counts was observed [$F(7,96) = 16.93$, $p < .001$]. For visual simplicity, these data are graphed separately in Figure 3 top (**A** and **B**) and bottom (**C** and **D**). In this experiment, a significant decrease in activity was observed after fluvoxamine compared to vehicle-saline-saline controls ($p < .05$; Figure 3**A** and **B**). In contrast, horizontal activity af-

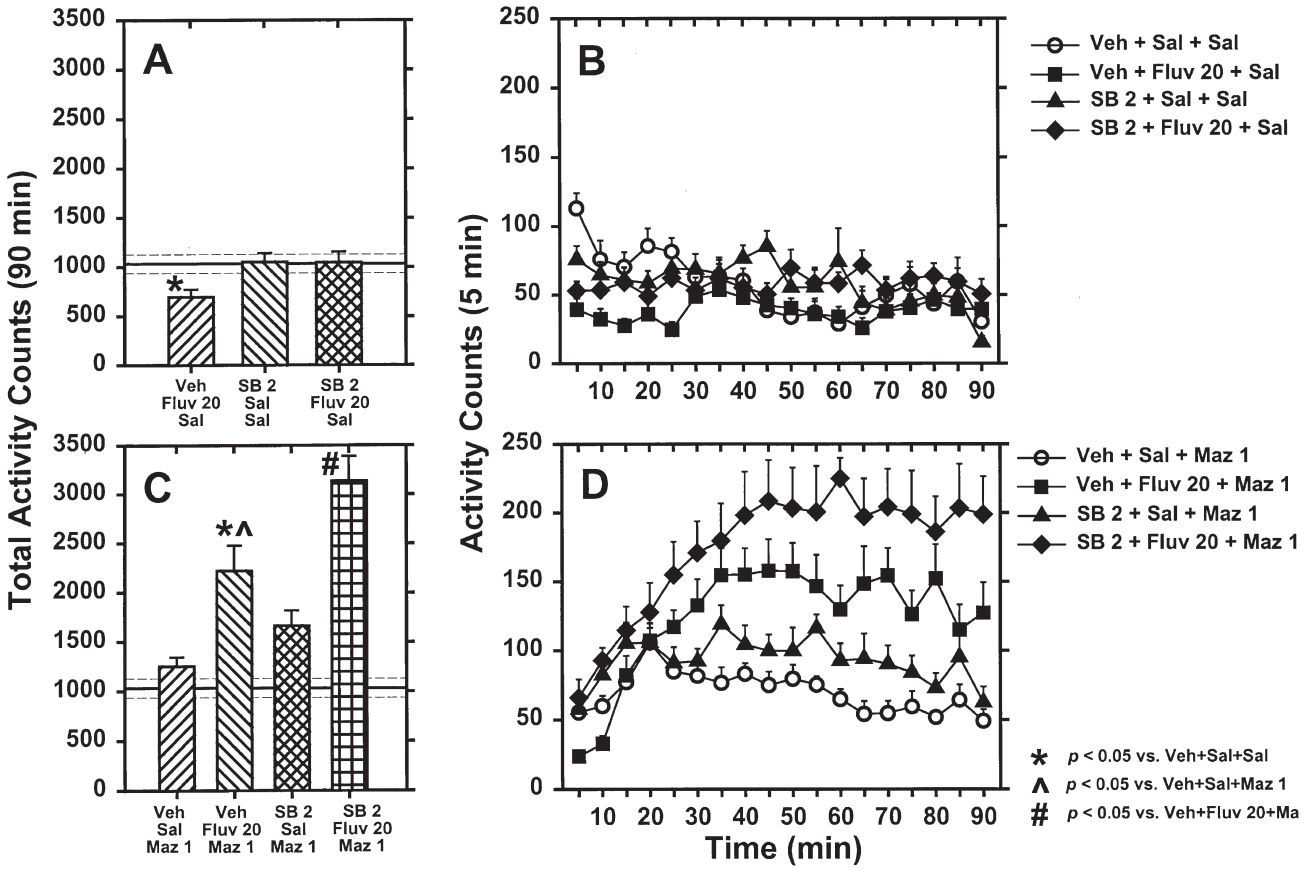

Figure 3. Effects of the 5-$HT_{2B/2C}$ antagonist SB 206553 on locomotor activity evoked by mazindol alone or in combination with fluvoxamine. See legend to Figure 2 for explanation of the figure. #, activity levels that were significantly different from vehicle-fluvoxamine-mazindol levels based on a Student-Newman-Keuls procedure ($p < .05$).

ter SB 206553 in combination with saline or fluvoxamine was not significantly different from that observed in vehicle-saline-saline controls ($p > .05$; Figure 3**A** and **B**). As noted in Experiments 1 and 2, total horizontal activity was significantly increased by the combination of fluvoxamine and mazindol compared to that observed after mazindol alone ($p < .05$; Figure 3**C** and **D**). Pretreatment with SB 206553 (2 mg/kg) significantly potentiated hyperactivity evoked by injections of fluvoxamine plus mazindol ($p < .05$; Fig. 3**C** and **D**). A significant treatment × time interaction was observed for horizontal activity in 5-min time bins [$F(119,1428) = 5.33$, $p < .001$]. Fluvoxamine-induced hypoactivity was evident during the first 30 min of the test session and was reversed by SB 206553 (Figure 3**B**). In addition, the peak and duration of hyperactivity observed after the fluvoxamine-mazindol combination was extended by pretreatment with SB 206553 (Figure 3**D**).

DISCUSSION

Based on previous evidence that 5-HT can influence DA function (see Introduction), the present study examined locomotor activity in rats after combination of the SSRI fluvoxamine and the catecholamine reuptake inhibitor mazindol. Mazindol alone at doses of 2 or 5 mg/kg induced a dose-related increase in locomotor activity that was characterized by a peak hyperactivity at 10 to 15 min postinjection and a duration of at least 90 min (maximum duration of the current test sessions; data not shown). Hyperactivity evoked by mazindol has been ascribed to inhibition of DA reuptake and is blocked by DA D_1- and D_2-like antagonists (Gevaerd and Takahashi 1999; Ross 1979). On the other hand, fluvoxamine tended to depress activity, particularly at the highest dose tested (20 mg/kg). This hypoactivity occurred 30 to 60 min after fluvoxamine injection and is most probably related to the flat body posture observed at that time point (data not shown), in the absence of other signs of the 5-HT syndrome (Grahame-Smith 1971). However, this hypomotility was short-lived, and the total horizontal activity for the duration of the session (90 min) was not significantly affected in two out of the three tests with 20 mg/kg of fluvoxamine. Minimal effects of fluvoxamine on locomotor activity have also been reported in mice (Maj et al. 1983; Reith et al. 1991).

Because fluvoxamine is an SSRI that possesses no appreciable affinity for NE or DA transporters or monoamine receptors (Wong et al. 1983; Hyttel 1994), reductions in locomotor activity evoked by fluvoxamine may be caused by elevated levels of endogenous 5-HT acting at specific 5-HT receptors (Benloucif et al. 1993; Gobert and Millan 1999; Iyer and Bradberry 1996; Parsons and Justice 1993). The 5-$HT_{2B/2C}$ receptor antagonist SB 206553, but not the 5-HT_{2A} receptor antagonist M100907, reversed the initial suppression of activity evoked by fluvoxamine. Hypoactivity resulting from activation of 5-HT_{2C} receptors has been well characterized by others (e.g., Curzon and Kennett 1990) and the present results suggest that indirect activation of 5-HT_{2C} receptors following reuptake inhibition may account for the transient induction of hypoactivity produced by fluvoxamine.

Fluvoxamine (5–20 mg/kg) dose-dependently evoked hyperactivity when given in combination with a dose of mazindol (1 mg/kg). This dose of mazindol was subthreshold for the elicitation of observable locomotor activation, but is a dose that has been reported to increase extracellular DA concentrations in rat striatum (Ng et al. 1992). These doses of fluvoxamine were reported to increase extracellular levels of 5-HT in frontal cortex without altering extracellular levels of DA or NE (Jordan et al. 1994). Based on these neurochemical data, one possible mechanism by which hyperactivity is elicited by fluvoxamine in combination with mazindol is via a 5-HT receptor-mediated enhancement of catecholaminergic neurotransmission. Such a mechanism would involve inhibition of 5-HT reuptake by fluvoxamine and indirect stimulation of specific 5-HT receptor subtypes.

The selective 5-HT_{2A} receptor antagonist M100907, at a dose previously shown to block the in vivo effects associated with 5-HT_{2A} receptor stimulation (Kehne et al. 1996b; Sorensen et al. 1993), reversed the hyperactivity induced by co-administration of fluvoxamine plus mazindol. M100907 is one of the few ligands available that has been shown to cross the blood–brain barrier and to discriminate between 5-HT_{2A} and 5-HT_{2C} receptors. In fact, M100907 possesses a 100-fold greater affinity for 5-HT_{2A} receptors (K_i = 0.85 nM) over 5-HT_{2C} (K_i = 88 nM) and α_1-adrenergic receptors (K_i = 128 nM), and negligible affinity for most other receptors, including DA D_1- and D_2-like receptors (>500 nM; Kehne et al. 1996a). The selectivity of M100907 is further suggested by the observation that doses of M100907 up to 30 times higher than those used here did not antagonize the behavioral effects of 5-HT_{2C}, D_2-like and α_1-adrenergic agonists (Dekeyne et al. 1999; Kehne et al. 1996a). Thus, the efficacy of M100907 to block hyperactivity is most likely attributable to a selective antagonism of 5-HT_{2A} receptors in vivo.

The level of tonic regulation of DA neurotransmission provided by 5-HT_{2A} receptors is somewhat controversial. Systemic injection of 5-HT_{2A} receptor antagonists did not potently alter basal behavior (present results; Kehne et al. 1996a, 1996b; McMahon and Cunningham in press; Sorensen et al. 1993; but see Gleason and Shannon 1998), basal cellular activity of DA somata (Sorensen et al. 1993) or striatal (Schmidt et al. 1992), accumbal (De Deurwaerdere and Spampinato 1999) or cortical DA efflux (Gobert and Millan 1999), suggesting that 5-HT_{2A} receptors provide little tonic control over DA function. The failure of the 5-$HT_{2A/2B/2C}$ receptor agonist 1-(2,5-dimethoxy-4-iodo)-2-aminopropane (DOI) to evoke striatal DA efflux in the presence of the 5-$HT_{2B/2C}$ receptor antagonist SB 206553 suggests further that selective 5-HT_{2A} receptor activation is not sufficient to provoke DA efflux in this DA terminal field (Lucas and Spampinato, 2000). However, under conditions of DA stimulation, 5-HT_{2A} receptors do seem to modulate DA outflow positively. For example, antagonism of 5-HT_{2A} receptors has been shown to attenuate striatal DA efflux stimulated by systemic administration of (±)-3,4-methylenedioxymethamphetamine (MDMA; Schmidt et al. 1992), blockade of D_2-like autoreceptors with haloperidol (Lucas and Spampinato 2000), and electrical stimulation of the dorsal raphe nucleus (De Deurwaerdere and Spampinato 1999). Systemic administration of M100907 also attenuated the suppression of DA cell firing evoked by amphetamine (Sorensen et al. 1993) and locomotor hyperactivity induced by amphetamine (Moser et al. 1996; Sorensen et al. 1993), cocaine (McMahon and Cunningham in press) or MDMA (Kehne et al. 1996b). Thus, the combination of fluvoxamine and mazindol mimics the activation of DA function under which 5-HT_{2A} receptors become functional, conditions under which M100907 is an effective antagonist of the resulting hyperactivity.

The mechanisms, triggers, and sites of action for 5-HT_{2A} receptors to control stimulated DA function have not yet been thoroughly clarified, although the mechanism may involve blockade of 5-HT_{2A} receptors that putatively control DA synthesis under conditions of stimulated DA neurotransmission (Lucas and Spampinato 2000; Schmidt et al. 1992). Despite the evidence to suggest that 5-HT_{2A} receptors exercise little tonic control over DA function under normal conditions (above), basal 5-HT concentrations may provide sufficient tone on 5-HT_{2A} receptors such that, under conditions of stimulated DA neurotransmission, antagonism of 5-HT_{2A} receptors triggers functional mechanisms that compensate for the overactivation of DA neurons. Such a mechanism might help to explain observations that M100907 can block the in vivo consequences of drugs thought to result predominantly in enhanced DA efflux, such as amphetamine (Moser et al. 1996; Sorensen et al. 1993), the DA reuptake inhibitor GBR 12909 (Carlsson 1995) and mazindol given alone (present results; Figure 2). On the other hand, further

increases in interstitial levels of 5-HT, such as following cocaine (McMahon and Cunningham in press), the 5-HT- and DA-releaser MDMA (Kehne et al. 1996b; Schmidt et al. 1992) or a combination of fluvoxamine plus mazindol (present results), may be required to uncover the control of DA function by 5-HT_{2A} receptors. In this case, one must postulate that drugs such as amphetamine, GBR 12909 and mazindol might alter interstitial levels of 5-HT, possibly below the limits of detectability for microdialysis experiments, that could contribute to the ability of 5-HT_{2A} receptors to control DA function. To complicate matters further, in addition to 5-HT-evoked increases in DA efflux (Benloucif et al. 1993; Gobert and Millan 1999; Iyer and Bradberry 1996; Parsons and Justice 1993), DA has also been shown to increase 5-HT release (Matsumoto et al. 1996), and these effects seem to be mediated, at least in part, by stimulation of specific 5-HT and DA receptors, respectively.

In contrast to the 5-HT_{2A} receptor antagonist M100907, pretreatment with the 5-$HT_{2B/2C}$ receptor antagonist SB 206553 (2 mg/kg) potentiated the hyperactivity elicited by fluvoxamine plus mazindol. Doses of SB 206553 in this range have previously been shown to effectively inhibit hypomotility induced by the 5-HT_2 receptor agonist m-chlorophenylpiperazine (Heisler and Tecott 2000) and the stimulus effects of the 5-HT_{2C} receptor agonist RO 60-0175 (Dekeyne et al. 1999), with little evidence of behavioral disruption when given alone (present results; Dekeyne et al. 1999; Kennett et al. 1996). The potentiation produced by SB 206553 may have involved the removal of a tonic, inhibitory influence of 5-HT_{2C} receptors on DA neurotransmission. In support of this hypothesis, SB 206553 was found to increase the firing rate of DA neurons in the VTA (Di Giovanni et al. 1999) and striatal DA efflux (De Deurwaerdere and Spampinato 1999; Di Matteo et al. 1998). Activation of 5-HT_{2C} receptors consequent to 5-HT reuptake inhibition induced by fluvoxamine might have reduced DA function stimulated by mazindol, thereby self-limiting the locomotor activation produced by the combination of fluvoxamine plus mazindol. As noted above, hypoactivity resulting from activation of 5-HT_{2C} receptors has been well characterized by others (Curzon and Kennett 1990). The present study suggests that the locomotor response to increased synaptic 5-HT in the face of modest catecholamine efflux depends upon the balance of 5-HT_{2A} and 5-HT_{2C} receptor activation. Thus, 5-HT_{2A} and 5-HT_{2C} receptors may serve opposing roles in the control of behaviors associated with increased catecholamine neurotransmission. Although we have focused the discussion on 5-HT-DA interactions, 5-HT interactions with NE (e.g., Saito et al. 1996) may be equally important, particularly given the affinity of mazindol for NE transporters (Hyttel 1982), and should not be overlooked.

A second mechanism that may account in part for the hyperactivity induced by the combination of fluvoxamine and mazindol involves potential pharmacokinetic interactions between these monoamine reuptake inhibitors. If acute administration of fluvoxamine impedes the metabolism of mazindol via inhibition of metabolic enzymes, increased brain concentrations of mazindol could contribute to the observed hyperactivity. A similar mechanism was proposed for the facilitation of amphetamine-induced hyperactivity and DA efflux in NAc by systemic fluoxetine; in that study, increased amphetamine levels were observed in the NAc of fluoxetine-treated rats (Sills et al. 1999a). Fluvoxamine has been shown to inhibit cytochrome P450 isozymes that catalyze the oxidative metabolism of certain drugs and could, therefore, increase brain levels of drugs metabolized by these isozymes (Hiemke and Hartter 2000). The ability of cytochrome P450 isozymes to contribute to the transformation of mazindol into imidazole-3-one, its primary metabolite in the rat, is currently unknown (Dugger et al. 1979). In vivo microdialysis is one strategy that could be used to determine the extent to which the observed synergism between fluoxetine and mazindol might be related to fluvoxamine-evoked increases in brain concentrations of mazindol (Sills et al. 1999a). However, we have recently shown that microinjections of fluvoxamine into the shell of the NAc result in an immediate enhancement of hyperactivity evoked by systemic cocaine (McMahon et al. 2000), a finding difficult to reconcile with a mechanism dependent upon inhibition of metabolism. Future studies of this nature as well as the establishment of a full dose response curve for mazindol in the presence of multiple doses of fluvoxamine will help to clarify the contribution of metabolic processes to this effect.

In conclusion, these results suggest that endogenous 5-HT regulates behavioral states ensuing from stimulated catecholamine neurotransmission, possibly by exerting excitatory and inhibitory tone on behavior through actions at 5-HT_{2A} and 5-HT_{2C} receptors, respectively. The present study further suggests that the balance of activation between 5-HT_{2A} and 5-HT_{2C} receptors may contribute to pathopsychological states associated with dysfunction of monoamine neurotransmission. Thus, 5-HT_{2A} and 5-HT_{2C} receptors might prove to be important targets for the successful development of pharmacotherapies for affective disorders, schizophrenia, and drug abuse.

ACKNOWLEDGMENTS

This research was supported by the National Institute on Drug Abuse Grants DA05708, DA06511 (to K.A.C.) and DA 05879 (to L.R.M.). The authors thank Mr. Michael G. Bankson for his thoughtful comments on this manuscript.

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