Dopamine Uptake Inhibitor-Induced Rotation in 6-Hydroxydopamine-Lesioned Rats Involves Both D1 and D2 Receptors but Is Modulated through 5-Hydroxytryptamine and Noradrenaline Receptors

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Received September 16, 2004; accepted November 12, 2004

ABSTRACT

Dopamine uptake inhibitors may provide a means of sustaining endogenous and exogenous striatal dopamine levels in Parkinson’s disease, but most are not selective and also inhibit the noradrenaline and 5-hydroxytryptamine (5-HT) transporters. To determine the involvement of the individual monoamine transporters in the production of motor activity, the effect of the nonselective monoamine uptake inhibitor BTS 74 398 1-[1-(3,4-dichlorophenyl)cyclobutyl]-2-(3-diaminomethylaminopropylthio) ethanone monocitrate and the selective dopamine, GBR 12909 [1-(2-(bis-(4-fluorphenyl)-methyl)ethyl)-4-(3-phenylpropyl)piperazine] dihydrochloride, noradrenaline (nisoxetine), and 5-HT (fluvoxamine) reuptake inhibitors on circling in the unilateral 6-hydroxydopamine-lesioned rat was investigated. GBR 12909 induced ipsilateral circling, but fluvoxamine and nisoxetine were without effect. However, when administered with GBR 12909, fluvoxamine enhanced rotation, whereas nisoxetine had no effect. The results suggest that 5-HT, but not noradrenaline, reuptake inhibition facilitates dopamine-mediated motor activity. To test this hypothesis, BTS 74 398 was administered in combination with selective dopamine, 5-HT, and noradrenaline receptor antagonists. Both D1 and D2 receptor antagonists, SCH 23390 [R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine] and raclopride, inhibited BTS 74 398-induced circling. In contrast, the 5-HT1A and 5-HT1A/B antagonists, WAY 100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide maleate) and pindolol, and the 5-HT2A antagonist, ketanserin, had no effect. The nonspecific 5-HT1/2 antagonists, methysergide and metergoline, and the specific 5-HT2C antagonist, N-desmethylclozapine, enhanced BTS 74 398-induced circling, as did the α2-adrenoceptor antagonist idazoxan. Overall, the data suggest that inhibition of the 5-HT and noradrenaline transporters modulates dopamine uptake inhibitor-mediated motor activity. However, the mechanism of this interaction is complex, involving opposing effects of noradrenaline and 5-HT agonism and antagonism.

The interaction among noradrenaline, 5-HT, and dopamine in the control of motor behavior is complex. For example, depletion of striatal 5-HT increases spontaneous motor behavior in rats (Carter and Pycock, 1979), and manipulation of cerebral 5-HT levels modulates striatal dopamine content (De Deurwaerdere et al., 1996) and dopamine-mediated motor behaviors (Bubar et al., 2003). High and low levels of central 5-HT stimulation can attenuate and potentiate, respectively, dopamine-mediated stereotypes (Carter and Pycock, 1981). The effects of 5-HT are mediated through numerous receptor subtypes, many of which are present in the striatum and directly modulate dopamine levels and/or motor behavior induced by directly or indirectly acting dopa-

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SSRI, 5-HT reuptake inhibitor; BTS 74 398, 1-[1-(3,4-dichlorophenyl)cyclobutyl]-2-(3-diaminomethylaminopropylthio) ethanone monocitrate; GBR 12909, 1-(2-(bis-(4-fluorphenyl)-methyl)ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride; SCH 23390, R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide maleate; ANOVA, analysis of variance.
amine agonists (Bonhomme et al., 1995; Ward et al., 1995; De Deurwaerder et al., 1997). Stimulation of presynaptic 5-HT\textsubscript{1A} receptors decreases 5-HT release (Hjorth, 1996), leading to increased locomotor behavior and improvements in motor function in both 6-OHDA-lesioned rats and MPTP-treated monkeys, models of the nigrostriatal dopamine depletion observed in Parkinson’s disease (Dawson and Nguyen, 2000; Bibbiani et al., 2001). In contrast, 5-HT\textsubscript{2A} receptor antagonism attenuated dopamine-mediated locomotion in the 6-OHDA-lesioned rat, whereas it was enhanced by 5-HT\textsubscript{2C} receptor antagonists (Fox and Brotchie, 2000; McMahon and Cunningham, 2001b; Oh et al., 2002).

The contribution of noradrenaline to motor activity is equally confounding. Striatal noradrenergic depletion enhances locomotor activity (Schwarting and Carey, 1988), whereas lesions of the locus coeruleus decrease dopamine levels in both nucleus accumbens and striatum (Lategan et al., 1990), suggesting a modulatory effect of noradrenaline on nigrostriatal dopaminergic neurones. Importantly, \alpha\textsubscript{2}-adrenoceptor antagonists alter \textit{L}-dopa- and apomorphine-induced motor responses in the MPTP-treated marmoset and 6-OHDA-lesioned rat, respectively (Bezard et al., 1999; Chopin et al., 1999; Henry et al., 1999).

The dopamine reuptake inhibitors, mazindol and nomifensine, have shown some efficacy in ameliorating the symptoms of the motor disorder Parkinson’s disease (Park et al., 1981), caused primarily by the loss of dopaminergic neurones in the nigro-striatal pathway; however, additional losses of serotonergic and noradrenergic neurones may also contribute, and their contribution to the disease manifestation remains unknown (Hornykiewicz, 1975). Neither mazindol nor nomifensine are selective dopamine reuptake inhibitors, blocking noradrenaline and 5-HT reuptake to varying degrees (Cheetham et al., 1998), but the contribution this has on their antiparkinsonian actions is unknown. The reported effects of 5-HT reuptake inhibitors (SSRIs) on the motor symptoms of Parkinson’s disease are inconsistent (Linazasoro, 2000; Goy et al., 2003), supporting the concept of a highly complex relationship between dopaminergic systems and modulation of 5-HT levels in the control of motor activity. In contrast, the influence of noradrenaline reuptake inhibition on motor behavior in Parkinson’s disease appears totally unknown because until recently, there were no selective noradrenaline reuptake inhibitors available for clinical use.

Consistent with the effects of mazindol and nomifensine in patients, both selective and nonselective dopamine uptake inhibitors alleviate motor deficits in the MPTP-treated common marmoset (Hansard et al., 2002b, 2004). BTS 74 398 (1-[1-(3,4-dichlorophenyl)cyclobutyl]-2-(3-dimethylaminopropyl)thio)ethanone monocitrate) is a nonselective monoamine uptake inhibitor with high affinity for noradrenaline, dopamine, and 5-HT reuptake transporters. An investigation into the possible contributions of 5-HT and noradrenaline uptake-inhibiting components in reducing parkinsonian symptoms in this model produced confounding data (Hansard et al., 2002a). Coadministration of an SSRI, fluvoxamine, with GBR 12909 [1-(2-(bis-(4-fluorophenyl)-methyl)ethyl)-4-(3-phenylpropyl)piperazine] dihydrochloride], a selective dopamine uptake inhibitor, reversed all motor improvements produced by GBR 12909 alone; thus, the origin of the potent effect of BTS 74 398 in this appears unclear. To clarify the mechanism through which BTS 74 398 exerts its effects, this study utilizes the 6-OHDA-lesioned rat to investigate the relative roles of dopamine, noradrenaline, and 5-HT in the motor actions of monoamine reuptake inhibitors. Specifically, this study utilizes uptake inhibitors selective for the dopamine, serotonin, and noradrenaline transporters, GBR 12909, fluvoxamine, and nisoxetine, respectively. The effects of single and combined administration on rotational behavior are evaluated to approximate the effects of BTS 74 984. These initial experiments showed that 5-HT reuptake inhibition facilitated dopamine reuptake inhibitor-mediated circling. To further examine the relative influences of individual monoamine receptors, the effect of coadministering BTS 74 398 with the selective D\textsubscript{1} and D\textsubscript{2} dopamine receptor antagonists SCH 23390 \([R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine]\) and raclopride and, subsequently, the nonselective 5-HT antagonists, methysergide and merville; 5-HT\textsubscript{1} antagonists, WAY 100635 \([N\{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl\}-N\{2-pyridinyl-cyclohexanecarboxamide maleate\}\) and pindolol; and 5-HT\textsubscript{2} antagonists, N-desmethyloclazapine and ketanserin. Additionally, the influences of \alpha\textsubscript{2}-antagonist idazoxan on rotational responses were also assessed.

Materials and Methods

Animals. Male Wistar rats (220–250 g; Tucks Ltd., Essex, UK) were housed in pairs under 12-h light/dark cycle with an environment of 50% humidity and temperature of 21 °C. Rats were given standard Purina rat chow and water ad libitum. Powdered chow with water (mash) was supplied for animals following surgery until their weight was stabilized. All experiments were carried out in accordance with Animals (Scientific Procedures) Act 1996 Home Office regulations (Project License 90/4619).

Drugs. BTS 74 398 was provided by Shire Pharmaceuticals (Basingstoke, Hampshire, UK). Nisoxetine hydrochloride, SCH 23390, ketanserin, N-desmethylclozapine, methysergide, and WAY 100635 were obtained from Tocris Cookson Inc. (Bristol, UK) and raclopride from AstraZeneca Pharmaceuticals LP (Wilmington, DE). Fluvoxamine maleate was provided by Solvay Pharmaceuticals (Weesp, Holland). Metergoline, pindolol, apomorphine hydrochloride, 6-hydroxydopamine hydrochloride, and GBR 12909 were obtained from Sigma Chemical (Poole, Dorset, UK). GBR 12909 was dissolved in 50% ethanol before further dilution with saline to a maximum of 10% ethanol. N-desmethylclozapine was dissolved in 0.01 M acetic acid and brought to pH 7 with 0.1 M NaOH. Pindolol was dissolved in a minimal quantity of 0.1 M HCl and corrected to pH 7 with 0.1 M NaOH. Apomorphine hydrochloride was dissolved in 0.9% saline containing 0.05% ascorbic acid. All other drugs were dissolved in 0.9% saline and administered in a volume of 1 ml/kg. Concentrations were calculated from the free-base weight of the compounds.

Surgery. Rats were anesthetized with halothane (3–4% in 95% O\textsubscript{2}, 5% CO\textsubscript{2} carrier gas) in an induction chamber before being placed on a Kopf stereotaxic frame where anesthesia was maintained with 0.5 to 1.5% halothane. A small area of the skull was exposed and a hole drilled 0.8 mm in diameter using a hand drill (coordinates: AP, 2.2 mm; ML, −1.5 mm) (Paxinos and Watson, 1987). A 10-μl Hamilton syringe was lowered to 8 mm below the dura, and 6-hydroxydopamine (8 μg in 4 μl of 0.9% saline containing 0.05% ascorbic acid) was injected over 4 min at a constant rate. The needle remained in situ for a further 4 min before being removed, and the wound was cleaned and sutured. Fluoxetine hydrochloride (2.5 mg/kg) was administered postoperatively for pain relief, and 10 ml/kg 0.9% saline containing 5% glucose was given to ensure hydration. Three weeks after surgery, the circling response of 6-OHDA-lesioned rats to the administration of apomorphine hydrochloride (0.5 mg/kg s.c.) was
assessed. Rats were only used for further investigation if they showed more than six contralateral rotations per minute.

Dose-Response Relationships for Monoamine Reuptake Inhibitor-Induced Circling Behavior. 6-OHDA-lesioned rats \((n = 8)\) were acclimatized in automated rotometers for 30 min (MED Associates, St. Albans, VT), and a 30-min baseline recording of 45° movements in clockwise and anticlockwise directions was taken. Animals were then given fluvoxamine \((1, 3, 10, \text{ or } 30 \text{ mg/kg i.p.})\), nisoxetine \((3, 10, \text{ or } 30 \text{ mg/kg i.p.})\), GBR 12909 \((3, 10, 30, \text{ or } 60 \text{ mg/kg i.p.})\), or drug vehicle in a random order according to a modified Latin square. Circling behavior in both clockwise and anticlockwise directions was monitored for 5 h after drug administration. A minimum interval of 72 h was allowed between drug treatments.

Combined Administration of Selective Monoamine Reuptake Inhibitors. A dose of GBR 12909 \((30 \text{ mg/kg i.p.})\) that produced a submaximal rotational response was used in further studies. Fluvoxamine and nisoxetine did not evoke circling; therefore, the doses that did not cause noticeable side effects were used for subsequent study, namely nisoxetine \((10 \text{ mg/kg i.p.})\) and fluvoxamine \((3 \text{ mg/kg i.p.})\). Combinations of the drugs were administered to each rat in a random order according to a modified Latin square design. Rats were acclimatized for 30 min, a 30-min baseline was recorded, and drugs were then administered simultaneously. Circling behavior in both clockwise and anticlockwise directions was recorded continuously for a 5-h period. A minimum of 72 h was allowed between drug treatments.

Modification of the BTS 74 398-Induced Circling Response. 6-OHDA-lesioned rats \((n = 8–12)\) were acclimatized to the rotometers for 30 min, and baseline circling was recorded before drug administration. BTS 74 398 \((4.7 \text{ mg/kg i.p.})\) the ED\(_{50}\) for circling behavior identified in previous studies in this laboratory, dose-response curve reproduced in Fig. 1) was administered 30 min later, and rotational behavior in both directions was recorded for a further 5 h. SCH 23390 \((0.005, 0.01, 0.05, \text{ and } 0.1 \text{ mg/kg i.p.})\), raclopride \((0.1, 0.5, 1, \text{ and } 5 \text{ mg/kg i.p.})\), ketanserin, pindolol, methysergide, N-desmethylclozapine \((all 1 \text{ mg/kg i.p.})\), WAY 100635 \((0.5 \text{ mg/kg i.p.})\), metergoline \((5 \text{ mg/kg i.p.})\), and idazoxan \((1 \text{ mg/kg i.p.})\) or drug vehicle were administered prior to BTS 74 398. Doses used were obtained from the literature based on changes in induced motor activity (Fox and Brotchie, 1996; Chopin et al., 1999; Bibbiani et al., 2001; McMahon and Cunningham, 2001a; S. Cheetham, unpublished data). A modified Latin square was used to randomize the dosing order and to ensure all rats received all drugs at all concentrations. A minimum of 72 h was allowed between drug treatments.

Statistical Analysis. Total numbers of rotations in each direction over the 5-h recording periods were calculated, and data are shown as mean ± S.E.M. The total number of ipsilateral rotations induced by each selective monoamine reuptake inhibitor was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison post hoc test, comparing all doses with saline-induced response. In the combination of selective monoamine uptake inhibitors, the total number of ipsilateral rotations was analyzed by one-way ANOVA with Newman-Keuls multiple comparison test. Ipsilateral rotations evoked by BTS 74 398 in combination with the antagonists was analyzed by one-way ANOVA followed by Dunnett’s multiple comparison test comparing BTS 74 398-induced ipsilateral circling to BTS 74 398 plus antagonist responses.

Results

The Effect of Selective and Nonselective Reuptake Inhibitors on the Circling Behavior of 6-OHDA Rats. Administration of GBR 12909 produced dose-dependent, ipsilateral circling that lasted for 4 to 5 h at 10 and 30 mg/kg and over 5 h at 60 mg/kg (Fig. 2a). Increasing doses augmented both the magnitude and duration of the circling response (Fig. 2b). Mild stereotypy, mainly sniffing, was observed that increased in duration but not intensity with increasing doses. GBR 12909 \((30 \text{ mg/kg})\) produced a submaximal response in circling and so was used in subsequent studies.

Administration of nisoxetine \((3 \text{ mg/kg i.p.})\) evoked low levels of ipsilateral circling \((83 ± 24 \text{ ipsilateral turns/5 h at 3 mg/kg, data not shown})\) indistinguishable in nature and quantity from that observed in saline-treated rats \((65 ± 15 \text{ ipsilateral turns in 5 h})\). No further increases occurred at 10 or 30 mg/kg i.p. \((85 ± 20 \text{ ipsilateral turns; 134 ± 47.8, respectively, } p > 0.05)\). These responses were not significantly different from those produced by saline treatment. No stereotypic behaviors were observed, but at the highest dose of nisoxetine \((30 \text{ mg/kg i.p.})\), the animal’s posture was hunched and abnormal; as a consequence, nisoxetine at a dose of 10 mg/kg i.p. was chosen for subsequent study.

Administration of fluvoxamine evoked ipsilateral turning \((66 ± 16 \text{ ipsilateral turns at 1 mg/kg i.p.; 66 ± 25 at 3 mg/kg i.p., 40 ± 11 at 10 mg/kg i.p., and 40 ± 13 at 30 mg/kg, data not shown})\) that was not different from that produced by saline treatment \((65 ± 15 \text{ ipsilateral turns in 5 h; } p > 0.05)\). However, at the highest dose used, rats exhibited a flat-body posture and drogging of the hind paws. This was also present
to a milder degree at 10 mg/kg; as a consequence, a dose of 3 mg/kg was used for subsequent studies.

The Effect of Combinations of Selective Monoamine Reuptake Inhibitors on Ipsilateral Circling. The total number of rotations induced by GBR 12909 was not altered by the coadministration of nisoxetine, and no other behavioral changes were observed (Fig. 3a). However, there was an increase in total rotation produced by GBR 12909 coadministered with fluvoxamine (3 mg/kg; see Fig. 2a) compared with GBR 12909 alone. The time course of drug effect showed that there appeared to be an increase in both the peak intensity of circling and the duration of the response (Fig. 3b). Combined administration of GBR 12909 (30 mg/kg), nisoxetine (10 mg/kg), and fluvoxamine (3 mg/kg) significantly increased the total number of ipsilateral turns compared with GBR 12909 alone and GBR 12909 in combination with nisoxetine but not GBR 12909 in combination with fluvoxamine (Fig. 3, a and b).

The Effect of Antagonists on Baseline Circling Behavior. SCH 23390 (0.1 mg/kg i.p.), raclopride (5 mg/kg i.p.), metergoline (5 mg/kg i.p.), methysergide (1 mg/kg i.p.), WAY 100635 (0.5 mg/kg i.p.), pindolol (1 mg/kg i.p.), ketanserin (1 mg/kg i.p.), N-desmethyloleizapine (1 mg/kg i.p.), and idazoxan (1 mg/kg i.p.) did not produce a rotational response that was different from saline in the 30 min before BTS 74 398 administration (data not shown, but see time course in Figs. 4b and 5b; see Fig. 7).

The Effect of Dopamine Antagonists on BTS 74 398-Induced Ipsiversive Circling. BTS 74 398 (4.7 mg/kg i.p.) induced ipsilateral circling lasting for 4 to 5 h following drug administration (dose-response curve from unpublished data, reproduced as Fig. 1). Pretreatment with SCH 23390 (0.005 and 0.01 mg/kg i.p.) did not alter the rotational response.

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Fig. 2. The ability of the dopamine reuptake inhibitor GBR 12909 (3, 10, 30, and 60 mg/kg) to induce ipsilateral circling behavior in 6-OHDA-lesioned rats. Data are presented as: a, total number of ipsilateral turns in 5 h; and b, time course of the response (number of ipsilateral turns per 10 min over the 5-h time period, error bars are omitted for clarity) and are shown as mean ± S.E.M., total turns analyzed by one-way ANOVA with Newman-Keuls (*, p < 0.05; ***, p < 0.001 compared with saline).

Fig. 3. The ability of combinations of the monoamine reuptake inhibitors fluvoxamine (fluv; 3 mg/kg), nisoxetine (nis; 10 mg/kg), and GBR 12909 (GBR; 30 mg/kg) on the ipsilateral circling response of 6-OHDA-lesioned rats (n = 8). Data presented as: a, total number of ipsilateral turns in 5 h (one-way ANOVA with Newman-Keuls multiple comparison test, *, p < 0.05; ***, p < 0.001 compared with saline; †, p < 0.05; ‡, p < 0.01 compared with GBR alone; ††, p < 0.05 compared with GBR/nisoxetine); and b, time course of the response (number of ipsilateral turns per 10 min over the 5-h time period, error bars are omitted for clarity) and are shown as mean ± S.E.M.
produced by BTS 74 398. However, higher doses of SCH 23390 (0.05 and 0.1 mg/kg i.p.) completely inhibited BTS 74 398-induced circling at a level that did not differ from saline treatment (Fig. 5).

Raclopride (0.1–5 mg/kg i.p.) evoked a dose-dependent reduction in the total number of ipsilateral rotations induced by BTS 74 398 (Fig. 5a). At the highest dose administered, raclopride (5 mg/kg) completely inhibited circling, and the total number of turns over 5 h did not differ from that seen in saline-treated animals (Fig. 5).

**The Effect of 5-HT Antagonists on BTS 74 398-Induced Ipsilateral Circling.** Metergoline (5 mg/kg i.p.), methysergide (1 mg/kg i.p.), and N-desmethyllozapine (1 mg/kg i.p.) increased the total number of ipsilateral rotations induced by BTS 74 398 administration (Figs. 6 and 7, a and b).

The time course suggests that the peak intensity of circling was enhanced but that the duration of the BTS 74 398-induced response remained unchanged. WAY 100635 (0.5 mg/kg i.p.), pindolol (1 mg/kg i.p.), and ketanserin (1 mg/kg i.p.) did not alter the ipsilateral turning response evoked by BTS 74 398 (Figs. 6 and 7, a and b).

**The Effect of an α2-Antagonist on BTS 74 398-Induced Ipsilateral Circling.** Idazoxan (1 mg/kg i.p.) pretreatment increased ipsilateral rotation induced by BTS 74 398 compared with the effects of BTS 74 398 administered alone (Fig. 6). The time course suggests that the peak rotational rate was increased but that the duration of the response was not different from that produced by BTS 74 398 alone (Fig. 7c).
Discussion

The monoamine uptake inhibitor BTS 74 398 effectively reversed motor deficits in MPTP-treated primates (Hansard et al., 2004), but investigation into the contribution 5-HT and noradrenaline uptake inhibition made to this effect were confounding (Hansard et al., 2002a). BTS 74 398 was found to be highly potent, but a combination of GBR 12909 with 5-HT uptake inhibition negated all the benefits afforded by the dopamine uptake inhibitor alone. Neither had there been any investigation of the dopamine receptors involved in the response. Against this background, the relative involvement of dopamine, noradrenaline, and 5-HT in motor behavior was investigated in the 6-OHDA-lesioned rat.

The selective dopamine reuptake inhibitor GBR 12909 evoked ipsilateral circling in the 6-OHDA-lesioned rat, presumably by increasing dopamine levels in the intact striatum. As previously found, neither 5-HT nor noradrenaline reuptake inhibitors produced rotational behavior over that seen following vehicle administration (Kimmel and Holtzman, 1998). This suggests that the circling response evoked by nonselective monoamine reuptake blockers is predominantly mediated by dopamine reuptake inhibition. Nisoxetine had no effect on the circling response induced by GBR 12909, whereas fluvoxamine potentiated GBR 12909-induced circling both in the presence and absence of nisoxetine, indicating a modulatory role for 5-HT but not noradrenaline in the ipsiversive rotational behavior induced by nonselective monoamine reuptake inhibitors, such as BTS 74 398, in the 6-OHDA-lesioned rat. However, there are caveats in the interpretation of these data. The model used produces selective unilateral dopaminergic denervation without a reduction in serotoninergic transmission. Indeed, there is evidence of sprouting of serotoninergic fibers and increases in 5-HT content in the lesioned striatum (Zhou et al., 1991). Thus, alterations of dopamine-mediated circling by serotoninergic...
agents could be mediated either by enhancing dopamine transmission on the intact striatum or altering serotonergic transmission on the lesioned side to further unbalance striatal output. This seems unlikely given that neither fluvoxamine nor the 5-HT antagonists used had any effect alone on circling behavior and only produced an effect when dopamine levels were elevated by coadministration of dopaminergic agents. This implies that the effect of 5-HT is mediated by a modulation of dopaminergic transmission in the intact striatum. Further experiments lesioning basal ganglia output structures on the intact side of the brain will confirm the location of these effects.

The data presented here are consistent with the body of evidence that alterations in 5-HT transmission can modulate both dopamine release and dopamine-mediated motor behavior. In particular, SSRIs do not alter basal dopamine efflux but enhance dopamine levels when dopaminergic function is elevated (Sershen et al., 2000). Similarly, basal locomotion is not increased when SSRIs are given alone, but in conjunction with dopamine reuptake inhibitors, an increase is observed (McMahon and Cunningham, 2001b). Based on the effects of the selective uptake inhibitors, the actions of BTS 74 398 appear to be primarily due to an enhancement of dopaminergic transmission but also partially mediated by increased 5-HT activation. A primary role for the dopaminergic system is supported by the ability of the D1 and D2 agonists, SCH 23390 and raclopride, to completely inhibit circling behavior. The doses at which raclopride inhibited BTS 74 398-induced circling are within the range reported to induce mild catalepsy (Hillegaart and Ahlenius, 1987), which must be considered. The effect of SCH 23390 on spontaneous motor behavior in the doses used is unclear, but previous reports suggest that motor inhibition occurs during the first 40 min after drug administration (Meyer et al., 1992). In BTS 74 398-induced rotation lasting over 4 h, no recovery of the circling response was observed, and obvious behavioral changes were not observed after pretreatment with either raclopride or SCH 23390. This suggests that the reduction in motor response to BTS 74 398 is not the result of a general motor inhibition and that both D1 and D2 receptors are involved in mediating the circling response. This agrees with a previous study in intact rats in which both selective D1 and D2 receptors were observed in the basis of previous experiments showing either behavioral activation or inhibition of dopamine agonist-evoked changes in locomotion (Fox and Brotchie, 1996; Chopin et al., 1999; Bibbiani et al., 2001; McMahon and Cunningham, 2001a; S. Cheetham, unpublished data). Interestingly, the nonselective 5-HT agonists methysergide and metergoline, acting nonselectively on 5-HT1 and 5-HT2 receptors, potentiated the BTS 74 398-induced circling, an effect mirrored by the selective 5-HT2C antagonist N-desmethyclozapine. These data suggest that 5-HT2C receptors are normally inhibitory on motor activity, either directly or through control of dopamine, such that 5-HT2C antagonists increase locomotion. The influence of 5HT2C receptors on motor control has previously been demonstrated by augmentation of locomotion induced by the combined administration of mazindol and fluvoxamine by the 5-HT2C antagonist, SB 206553 (McMahon and Cunningham, 2001b). Furthermore, D1 and D2 agonist-induced circling behavior in 6-OHDA-lesioned rats is potentiated by 5-HT2C antagonism (Fox and Brotchie, 1996, 2000). The locomotor effects of cocaine are attenuated by 5-HT2A antagonism as is mazindol- and fluvoxamine-mediated hyperactivity (O’Neill and Shaw, 1999; McMahon and Cunningham, 2001a,b). Additionally, striatal preprotachykinin mRNA expression is modulated by 5-HT2A receptor activation, with synergistic interactions with D1 receptors in subregions of the striatum (Gresch and Walker, 1999; Basura and Walker, 2001). Given this evidence of interactions between 5-HT2A and dopamine receptors, the lack of effect of 5-HT2A antagonism in reducing the BTS 74 398-mediated response is surprising. Further exploration of this effect with more selective antagonists of 5-HT receptors may provide more conclusive data. BTS 74 398 increases dopamine overflow in the striatum (S.C. Cheetham, personal communication), but its effects on noradrenaline and 5-HT levels are unknown; therefore, the only conclusion that can be reached is that 5-HT reuptake inhibition is highly likely to play some modulatory role in the efficacy of BTS 74 398.

In contrast to the effects of SSRIs on GBR 12909-induced circling, the coadministration of nisoxetine with GBR 12909 had no effect on the circling response. Like nisoxetine, α2-adrenoceptor antagonists do not initiate rotational behavior in 6-OHDA-lesioned rats but dose-dependently augment circling produced by both directly and indirectly acting dopamine receptor agonists (Mavridis et al., 1991; Chopin et al., 1999). Pretreatment with the α2-adrenoceptor antagonist idazoxan potentiated the BTS 74 398-induced ipsilateral circling. However, there was no apparent effect of idazoxan on the duration of the BTS 74 398-induced rotational response, contrasting with the prolongation of L-dopa-mediated improvement in locomotion in MPTP-treated marmosets (Henry et al., 1999). These behavioral observations are supported by evidence that α2-adrenoceptor agonists directly inhibit striatal dopamine release, and although antagonism of α2-adrenoceptors by atipamezole did not affect dopaminergic overflow when administered alone, it can enhance L-dopa-evoked dopamine release (Yavich et al., 2003).

The ability of idazoxan to potentiate BTS 74 398-induced circling contrasts with the lack of effect of nisoxetine on GBR 12909-mediated rotational behavior. The dose of nisoxetine administered was the highest that could be given without inducing abnormal posturing; however, it is possible that the level of noradrenaline reuptake inhibition produced by nisoxetine was insufficient to influence the response to GBR 12909 or to reproduce the reuptake inhibition of the noradrenaline transporter induced by BTS 74 398. Additionally, idazoxan is a high-affinity ligand at imidazoline and 5-HT1A receptor sites as well as an α2-adrenoceptor antagonist (Newman-Tancredi et al., 1998). Although an action on imidazoline sites is unlikely to explain the potentiation of BTS 74 398-induced circling by idazoxan, 5-HT1A agonism could mediate this response, although the present study did not show any
effect of 5-HT1A antagonism on the circling induced by BTS 74 398.

In summary, the circling behavior in the 6-OHDA-lesioned rat induced by BTS 74 398 is primarily mediated by dopamine reuptake inhibition through both D1 and D2 receptors. The 5-HT reuptake inhibiting properties of BTS 74 398 may modulate the dopamine-mediated response through 5-HT2 receptors. Although these data suggest that noradrenaline reuptake inhibition does not contribute to BTS 74 398-induced circling, the finding of α2-adrenergic receptor antagonist-mediated potentiation suggests some involvement of the noradrenergic system.

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