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Role of 5-HT_{1A} receptors in the ability of mixed 5-HT_{1A} receptor agonist/dopamine D_2 receptor antagonists to inhibit methylphenidate-induced behaviors in rats

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Abstract

Behavioral effects produced by the indirect-acting dopamine receptor agonist, methylphenidate (40 mg/kg i.p.) were examined in rats after administration of the 5-HT_{1A} receptor agonists (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and flesinoxan, the mixed 5-HT_{1A} receptor agonist/dopamine D_2 receptor antagonists buspirone and 1-[-4-fluorobenzoylamino)ethyl]-ethyl]-4-(7-methoxy-naphthyl)piperazine (S 14506), the neuroleptics haloperidol and clozapine, and the σ receptor ligand/partial 5-HT_{1A} receptor agonist α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol (BMY 14802). All of the compounds produced dose-related decreases in methylphenidate-induced stereotyped gnawing, and, as gnawing was inhibited, other methylphenidate-induced responses (i.e. sniffing, rearing and locomotion) appeared. Higher doses of haloperidol and buspirone, but none of the remaining compounds, inhibited these other responses, so that the behavior of the methyphenidate-treated animals became similar to that of normal controls. Pretreatment with the 5-HT_{1A} receptor antagonist *N*-[2-4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)-cyclohexanecarboxamide (WAY-100635; 0.63 mg/kg s.c.) blocked the ability of 8-OH-DPAT, S 14506 and flesinoxan to inhibit methylphenidate-induced gnawing, demonstrating the involvement of 5-HT_{1A} receptors in their ability to inhibit methylphenidate-induced behaviors. In contrast, pretreatment with WAY-100635 did not alter the ability of haloperidol, clozapine, buspirone, or BMY 14802 to inhibit methylphenidate-induced gnawing, or in the case of haloperidol and buspirone, to normalize behavior. The results indicate that mixed compounds with 5-HT_{1A} receptor agonist and dopamine receptor antagonist properties can be differentiated on the basis of the ability of WAY-100635 to reverse their effects on methylphenidate-induced behaviors.

Keywords: Haloperidol; Clozapine; BMY 14802; BMS-181100; Methylphenidate; Flesinoxan; Buspirone; 8-OH-DPAT ((±)-8-hydroxy-2-(di-*n*-propylamino)tetralin); S 14506; Gnawing; 5-HT_{1A} receptor agonist

1. Introduction

Widespread recognition that the atypical neuroleptic clozapine has significant 5-HT_2 receptor blocking properties has focused interest in the role of serotonin (5-hydroxytryptamine, 5-HT) in the therapeutic actions of a new generation of antipsychotics that may have a lower incidence of extrapyramidal side-effects (Meltzer et al., 1989; Skarsfeldt, 1995). Risperidone is one of the first of this new class of neuroleptics to reach clinical use, and was initially presented as a compound with therapeutic effects at doses with limited extrapyramidal side-effects (cf. Gerlach and Peacock, 1995). However, recent evidence that risperidone, which has a relatively high (approximately 20-fold) separation between in vitro affinities for 5-$HT_{2A/C}$ and dopamine D_2 receptors, is not entirely devoid of extrapyramidal side-effects (Kapur et al., 1995), has added impetus to the search for other atypical compounds, such as olanzapine (Moore et al., 1992, 1994), sertindole (Perregaard et al., 1992; Skarsfeldt and Perregaard, 1990) and ziprasidone (Seeger et al., 1995), with multiple dopamine, serotonin and norepinephrine receptor antagonist properties (cf. Gerlach and Peacock, 1995). However, the neuropharmacological basis for the hypothesis that a particular combination of different receptor affinities explains the superior clinical profile of atypical neuroleptics remains to be firmly established (Jackson et al., 1993), and further studies of 5-HT receptor involve-

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ment in the effects of neuroleptics in preclinical models are clearly needed.

Preclinical findings that 5-HT_{1A} receptor agonists reverse neuroleptic-induced catalepsy in rats and monkeys (Casey, 1993; McMillen et al., 1988; Neal-Beliveau et al., 1993; Wadenberg et al., 1994) suggest that compounds with both 5-HT_{1A} receptor agonist and dopamine receptor antagonist properties may have a lower incidence of extrapyramidal side-effects. This hypothesis was inadvertently tested some years ago with buspirone, a compound that resembled classical neuroleptics in preclinical tests (Wu et al., 1972), but was subsequently shown to have anxiolytic activity resulting from its 5-HT_{1A} receptor agonist properties (New, 1990). While apparently not efficacious in treating schizophrenia (Sathananthan et al., 1975), buspirone may be able to augment some effects of neuroleptics (Goff et al., 1991; Pantelis and Barnes, 1993). Buspirone is a partial 5-HT_{1A} receptor agonist (Yocca, 1990) and after oral administration is metabolized extensively on its first pass to 1-pyrimidylpiperazine, a compound with substantial α_2-adrenoreceptor blocking properties (Tollefson et al., 1991). Clearly, there is a need for more selective compounds with intrinsic activity at 5-HT_{1A} receptors higher than that of buspirone in order to adequately explore the possibility that the combination of 5-HT_{1A} receptor agonist/dopamine receptor antagonist properties will offer a superior clinical profile.

Recently, several different laboratories (Lowe et al., 1991; Perrone et al., 1994; Protais et al., 1994; Reitz et al., 1995) have reported the discovery of compounds that have high affinities for both dopamine and 5-HT_{1A} receptors and demonstrable activity in preclinical models of antipsychotic activity. However, the relatively selective 5-HT_{1A} receptor agonist (+)-8-hydroxy-2-(di-*n*-propyl-amino)tetralin (8-OH-DPAT) has several effects – inhibition of conditioned avoidance responding (Ahlenius, 1989; Wadenberg and Ahlenius, 1988) or apomorphine-induced behaviors (Liebman et al., 1989; Protais et al., 1994) – that are also produced by DA antagonists that lack affinity for 5-HT_{1A} receptors. Furthermore, 5-HT_{1A} receptor agonists, including 8-OH-DPAT and flesinoxan, have apparent inhibitory actions on dopamine neurotransmission (Ahlenius et al., 1991; Ichikawa et al., 1995; Johnson et al., 1993). Thus, it is conceivable that 5-HT_{1A} receptor agonists devoid of dopamine receptor antagonist properties are capable of mimicking antidopaminergic effects in preclinical models. Although such effects may nonetheless be predictive of antipsychotic potential, it is important to determine the extent to which the effects of mixed compounds are mediated by their 5-HT_{1A} receptor agonist properties.

In this study, the ability of buspirone and 1-[-4-fluorobenzoylamino)ethyl]-ethyl]-4-(7-methoxynaphthyl)-piperazine (S 14506), compounds that also have relatively high affinity for both 5-HT_{1A} and dopamine D_2 receptors (Maskall et al., 1995; Peroutka, 1985; Protais et al., 1994), to inhibit methylphenidate-induced gnawing and other behaviors (Koek and Colpaert, 1993) was compared with effects of relatively selective 5-HT_{1A} receptor agonists and of the antipsychotics haloperidol and clozapine. In order to determine the role of 5-HT_{1A} receptor agonist activity in the observed inhibition of methylphenidate-induced gnawing, the effects of these compounds were also examined after pretreatment with the 5-HT_{1A} receptor antagonist (*N*-[2-4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635) (Forster et al., 1995). The results demonstrate that buspirone, a compound that has prominent dopamine blocking properties in vivo, can be distinguished from other 5-HT_{1A} receptor agonists, such as S 14506 and flesinoxan.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Ico: OFA SD (I.O.P.S. Caw) Iffa Credo, France), weighing from 160 to 200 g at the time of the studies, were housed individually in plastic hanging cages (28 cm × 21 cm × 18 cm) with metal grid floors (RC Iffa Credo). Water, filtered at 0.22 μm, was freely available from an automatic dispenser. The animal storage and manipulation rooms were air-conditioned (temperature 22 ± 1°C; hygrometric degree 55 ± 5%), with lighting on from 07:00 to 19:00 h. All rats were cared for in accordance with the principles of laboratory animal care (Guide for the Care and Use of Laboratory Animals; US Department of Agriculture, Public Health Service, National Institutes of Health Publication No. 85-23, Revised 1985) and the protocol (No. 015) was carried out in accordance with local ethical committee guidelines for animal research. Animals were held in quarantine for 4–8 days, with free access to standard laboratory food (A04, 4AR, Epinay-sur-Orge, France) and filtered water (0.22 μ). Animals were food-restricted for 24 h before being used in the experiments.

2.2. Procedure

The observational method used, as described previously (Koek and Colpaert, 1993), was an adaptation of the method of Fray et al. (1980), combined with a time-sampling procedure (Waddington, 1986). Observations were made during a 10-min period starting 30 min after the injection of methylphenidate or saline using subgroups of four animals. Each min, each of the four rats was successively observed during a 10-s period (i.e. one animal every 15 s) for the presence or absence of locomotion, rearing, sniffing, gnawing and licking, and for the occurrence of the other directly observable phenomena listed in Table 1. This cycle was repeated 10 times during a 10-min period; thus, the incidence of a particular behavior could vary from 0 to 10 for the entire observation period. At the end

Table 1
Definitions of directly observable behavioral and other categories in rats (adapted from Fray et al., 1980; Irwin, 1968; Koek and Colpaert, 1993)

Category	Description
Locomotion [a]	Locomotion with all four legs moving
Rearing [a]	Standing on hindlegs, body fully extended
Sniffing [a]	Sniffing
Licking [a]	Licking the cage
Gnawing [a]	Gnawing the cage or body
Flat-body posture	The entire ventral surface of the animal being in contact with the cage floor
Lying	Lying without the entire ventral surface of the animal being in contact with the cage floor
Tremor	High-frequency, low-amplitude muscle contractions
Myoclonic convulsions	Isolated, jerky limb movements
Clonic convulsions	Repetitive jerks or twitches of the limbs
Tonic convulsions	Tonic extension of the hindlimbs, usually preceded by a flexion

[a] The behavior was considered present if the animal showed uninterrupted signs for at least 3 s.

of the observation period, the animals were tested for catalepsy (the animal maintained for at least 30 s, either (1) an abnormal cross-limbed position, imposed by the observer, in which the hindpaws were extended forward and placed on the forepaws, which were extended backwards, or (2) both forepaws on a cylindrical metal bar 1.25 cm in diameter, 10 cm elevation), akinesia (absence of movement after handling), and loss of righting (the animal remained in position for at least 10 s when placed on its back).

2.3. Analysis of data

Drug effects on methylphenidate-induced behaviors were evaluated for significance in two ways: (1) a posteriori comparisons made using Newman-Keuls procedure, with the estimated within-group error obtained from an analysis of variance of all groups tested with a particular compound irrespective of pretreatment, and $P < 0.05$ as the lower limit of statistical significance; and (2) based upon the incidence of each particular behavior observed in control animals treated with saline or with 40 mg/kg methylphenidate, dose-response functions were determined from the percentage of rats showing normalization or inhibition of gnawing (see Section 3.). ED_{50} values and their associated confidence limits and potency ratios were calculated with the Litchfield and Wilcoxon probit analysis procedure (Tallarida and Murray, 1987) implemented using a procedure that was written using the Research Programming Language (RPL) of RS/1 (Bolt, Beranek and Newman, Cambridge, MA). When fewer than two intermediate effects were observed, 0 and/or 100% effects were transformed by means of Berkson's adjustment (Hubert, 1984) to permit the use of the Litchfield and Wilcoxon procedure. The mimimally effective dose to produce other directly observable phenomena was defined, as described previously (Koek and Colpaert, 1993), as the lowest of two consecutive doses producing such phenomena; if any of the effects occured only at the highest dose tested, this dose was considered to be the minimally effective dose. Drugs were tested in 5 animals per dose, with the exception of 8-OH-DPAT and flesinoxan that were tested in 7 animals/dose because preliminary studies suggested the possibility that these latter compounds may produce only intermediate effects on gnawing.

2.4. Drugs

The drugs used were: haloperidol (Sigma, Chesnes, France), clozapine (Sandoz, Basle, Switzerland), BMY 14802 HCl (BMS-181100; α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol hydrochloride; Bristol Myers Squibb, Wallingford, CT, USA), methylphenidate HCl (Ciba-Geigy, Basle, Switzerland), 8-OH-DPAT HBr ((±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide), buspirone HCl (all from Research Biochemicals International, Natick, MA, USA), WAY-100635 dihydrochloride (*N*-[2-4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide dihydrochloride) and S 14506 (1-[-4-fluorobenzoylamino)ethyl]-ethyl]-4-(7-methoxy-naphthyl)piperazine) (both were synthesized by J. Maurel, Pierre Fabre Medicament, France). All compounds were prepared and administered in distilled water, with the exception of S 14506, which was prepared as a suspension in aqueous Tween 80 (2 drops/10 ml distilled water); an injection volume of 1 ml/100 g was used throughout and doses refer to the weight of the free base. WAY-100635 (0.63 mg/kg) or saline (1 ml/100 g) were administered s.c. 60 min before the observation session (−60 min), test compounds s.c. or i.p. at −45 min and methylphenidate i.p. at −30 min.

3. Results

3.1. Methylphenidate antagonism: controls

A total of 140 animals received a control treatment (i.e. saline or distilled water plus Tween) via either the s.c. or i.p. route, 30 min prior to the injection of methylphenidate

(40.0 mg/kg i.p.). Gnawing was particularly prominent after methylphenidate administration (9.7 ± 0.02; mean ± S.E.M.); sniffing, rearing and locomotion, although increased at doses from 0.63 to 10 (Koek and Colpaert, 1993) did not occur following administration of the 40 mg/kg dose. Gnawing scores of 9 or 10 were observed in more than 95% of the animals treated with the 40 mg/kg dose of methylphenidate; thus, a pretreatment was considered to have decreased the incidence of gnawing in an individual animal when a score less than 9 was observed. In control animals not receiving methylphenidate (n = 104), gnawing, locomotion, rearing, sniffing and licking each appeared with an average incidence of less than one, and the scores obtained in less than 5% of the animals were as follows: gnawing (1), locomotion (5), sniffing (9), rearing (7) and licking (1). Test compounds were therefore considered to have normalized methylphenidate-induced behaviors in individual animals if the following composite criterion was met: gnawing < 2, locomotion < 6, sniffing < 10, rearing < 8 and licking < 2. Licking, which was not observed in methylphenidate-treated rats, was not significantly affected by any of the compounds examined in this study and therefore the results are not shown.

WAY-100635 administered alone (0.63–10 mg/kg) did not significantly decrease the incidence of methylphenidate-induced gnawing or alter any other behavior (data not shown), and therefore did not normalize behavior; consequently, the 0.63 mg/kg dose was used as a pretreatment.

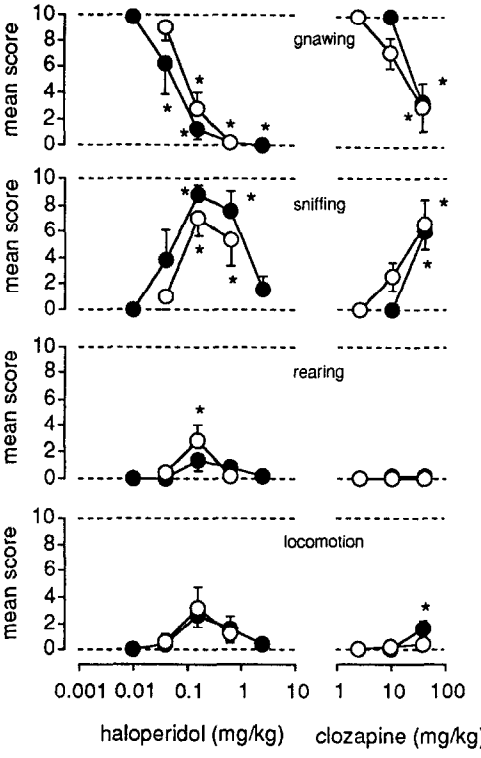

Fig. 1. Effects of the neuroleptics haloperidol and clozapine on methylphenidate-induced behaviors in the rat. Values represent the mean ± S.E.M. score of 5 animals treated with methylphenidate (40 mg/kg i.p.). Open symbols: saline pretreatment; closed symbols: WAY-100635 (0.63 mg/kg) pretreatment. See Section 2 for details of the behavioral scoring procedure. * $P < 0.05$ vs. lowest dose.

3.2. Methylphenidate antagonism

3.2.1. Effects of neuroleptics

Fig. 1 shows the effects of the neuroleptics haloperidol and clozapine on methylphenidate-induced behaviors. Haloperidol administered in combination with saline dose-dependently inhibited gnawing (Fig. 1, open symbols), and this effect was associated with the appearance of sniffing, rearing and locomotion. At the highest dose (0.63 mg/kg), levels of sniffing, rearing and locomotion were similar to those observed in saline-treated control animals. The analysis of the percentage of animals exhibiting gnawing scores lower than methylphenidate-treated control animals yielded an ED_{50} of 0.07 mg/kg (95% confidence limits 0.027-0.18 mg/kg, Table 2), and, according to the composite criterion, normalization of behavior occurred at approximately 4-fold higher doses (ED_{50} = 0.36 mg/kg, 0.14–0.93 mg/kg). Although pretreatment with WAY-100635 (0.63

Table 2
Ability of neuroleptics and compounds with 5-HT_{1A} receptor agonist properties to inhibit methylphenidate (40 mg/kg, i.p.)-induced gnawing and to normalize other mehylphenidate-induced behaviors in rats

Pretreatment	Saline				WAY-100635 (0.63 mg/kg)				Gnawing ratio WAY-100635 : saline
	Gnawing		Normalization of other behaviors		Gnawing		Normalization of other behaviors		
	ED_{50}	95% C.L.	ED_{50}	95% C.L.	ED_{50}	95% C.L.	ED_{50}	95% C.L.	
Haloperidol	0.070	0.027–0.18	0.36	0.14–0.93	0.057	0.019–0.17	0.36	0.090–1.5	1
Clozapine	11	2.3–54	> 40		20	N.D.	> 40		2
S 14506	0.087	0.021–0.36	> 10		1.9 [a]	0.56–6.4	> 40		22 [a]
Buspirone	1.1	0.43–2.9	5.7	2.2–15	1.2	0.63–2.4	5.7	2.2–15	1
Flesinoxan	0.98	0.27–3.6	> 40		49 [a]	18–129	> 160		50 [a]
8-OH-DPAT	0.37	0.07–1.91	> 10		7.9 [a]	2.9–21	> 10		21 [a]
BMY 14802	9.1	1.9–46	> 40		20	N.D.	> 40		2

[a] $P < 0.05$ vs. saline pretreatment. N.D., confidence limits could not be determined.

mg/kg) did not alter the ability of haloperidol to inhibit gnawing or normalize behavior, the dose-response functions for these behaviors were shifted slightly to the left (Fig. 1, closed symbols).

The neuroleptic clozapine significantly and dose-dependently inhibited methylphenidate-induced gnawing (Fig. 1; ED_{50} = 11 mg/kg, Table 2), and significantly altered sniffing scores, but did not affect rearing or locomotion. The analysis using the composite criterion showed that sniffing, rearing and locomotion were reduced to control levels in not more than 40% of the animals treated with the 40 mg/kg dose of clozapine, and neither this global measure nor the inhibition of gnawing were altered by pretreatment with WAY-100635.

3.2.2. *Effects of 5-HT_{1A} receptor agonists / mixed compounds*

As shown in Fig. 2 (open symbols), all of the compounds with 5-HT_{1A} receptor agonist properties examined in this study produced dose-related decreases in methylphenidate-induced gnawing, but differentially affected other behaviors induced by methylphenidate; further, pretreatment with WAY-100635 differentially altered their effects on methylphenidate-induced behaviors.

S 14506 produced significant decreases in methylphenidate-induced gnawing and increases in locomotion, sniffing and rearing. S 14506 dose-dependently inhibited methylphenidate-induced gnawing (ED_{50} = 0.087 mg/kg, 0.021–0.36 mg/kg), and inhibition of gnawing was associated with the appearance of sniffing, rearing and locomotion; however, unlike haloperidol, higher doses of S 14506 did not return behavior to levels observed in saline-treated control animals. Pretreatment with WAY-100635 attenuated the effects of S 14506 on methylphenidate-induced gnawing, sniffing, rearing and locomotion, as demonstrated by the increase of the lowest doses that produced significant effects on these behaviors; WAY-100635 treatment did not alter the inability of S 14506 to normalize behavior.

Buspirone produced significant decreases in methylphenidate-induced gnawing and increases in sniffing and locomotion. In contrast to the effects observed after administration of the other 5-HT_{1A} receptor agonists, buspirone normalized behavior in a dose-related manner (ED_{50} = 5.7 mg/kg). Pretreatment with WAY-100635 did not alter any of the effects of buspirone on the individual behaviors or affect the ability of buspirone to normalize behavior (Table 2).

Flesinoxan produced significant decreases in methylphenidate-induced gnawing and increases in sniffing, rearing and locomotion. Flesinoxan dose-dependently inhibited methylphenidate-induced gnawing (ED_{50} = 0.98

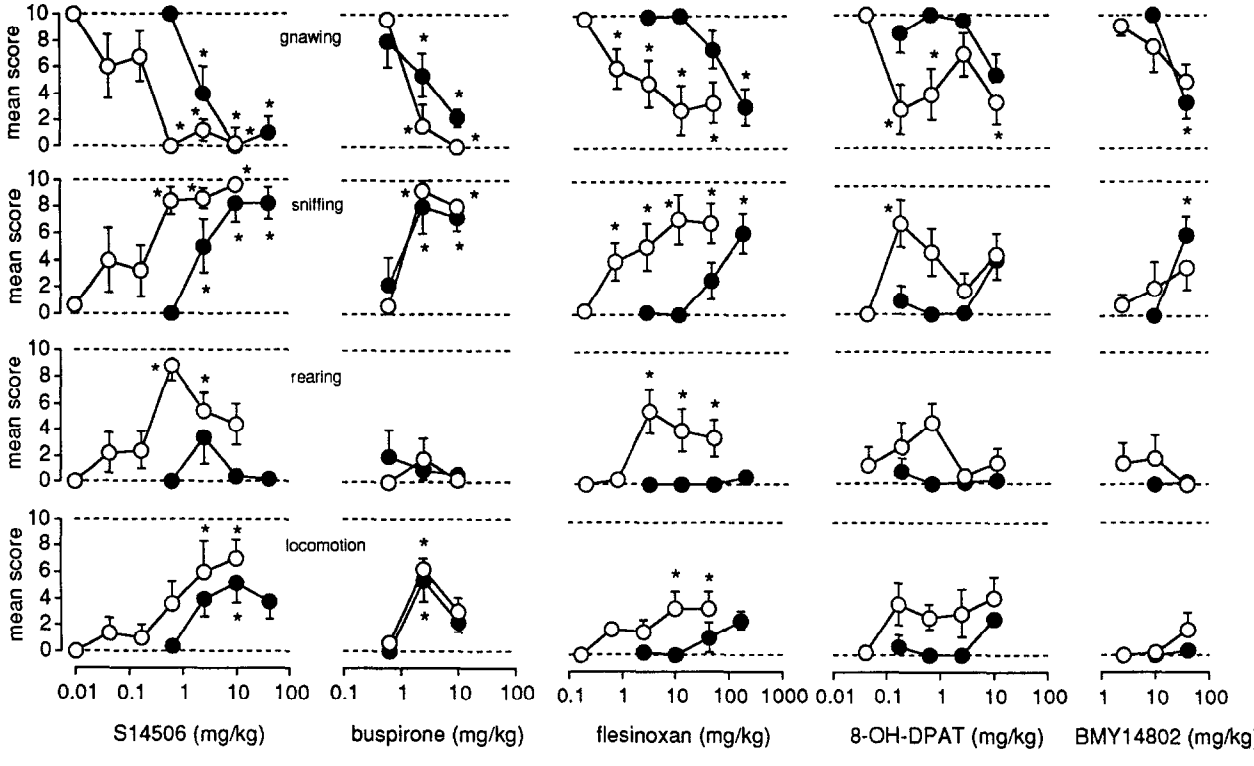

Fig. 2. Effects of 5-HT_{1A} receptor agonists and mixed 5-HT_{1A} receptor agonist/DA receptor antagonists, on methylphenidate-induced behaviors in the rat. All animals were treated with methylphenidate (40 mg/kg i.p.). Values represent the mean ± S.E.M. score (n = 5 rats/dose with the exception of 8-OH-DPAT and flesinoxan: n = 7/dose). Open symbols: saline pretreatment. Closed symbols: pretreatment with WAY-100635 (0.63 mg/kg). See Section 2 for further details of the behavioral scoring procedure. * $P < 0.05$ vs. lowest dose.

mg/kg, 0.27–3.6 mg/kg) and, at low doses this was associated with the successive appearance of sniffing, rearing and locomotion; however, behavior did not return to levels observed in saline-treated control animals after administration of higher doses of flesinoxan. Pretreatment with WAY-100635 attenuated the effects of flesinoxan on methylphenidate-induced gnawing, sniffing, locomotion and rearing as demonstrated by the increase of the lowest dose that produced significant effects on these behaviors.

8-OH-DPAT produced significant decreases in methylphenidate-induced gnawing and increases in sniffing. However, 8-OH-DPAT inhibited gnawing over a shallow, apparently biphasic dose-response function (ED_{50} = 0.37 mg/kg, 0.07–1.91 mg/kg), and, similar to that found with other relatively selective 5-HT_{1A} receptor agonists, 8-OH-DPAT did not normalize behavior. Pretreatment with WAY-100635 attenuated the effects of 8-OH-DPAT on methylphenidate-induced gnawing and sniffing, demonstrated by the absence of doses producing significant effects on these behaviors.

Although BMY 14802 inhibited gnawing and increased sniffing significant differences were observed only in animals pretreated with WAY-100635; BMY 14802 alone or in combination with WAY-100635 did not normalize behavior.

The rank order of potencies of the 5-HT_{1A} receptor agonists/mixed compounds to inhibit gnawing was S 14506 > 8-OH-DPAT > flesinoxan > buspirone > BMY 14802 (Table 2). Pretreatment with WAY-100635 (0.63 mg/kg) significantly shifted the inhibition of gnawing dose-response functions of 8-OH-DPAT, flesinoxan, and S 14506 to the right, increasing their ED_{50} values approximately 20–50-fold, but did not significantly affect the ability of buspirone or BMY 14802 to inhibit gnawing.

3.3. Other grossly observable behaviors

As shown in Table 3, all of the compounds examined in this study produced other observable behavioral effects, although these generally occurred at doses equal to or higher than those which inhibited gnawing, or in the case of haloperidol, normalized behavior. The antipsychotic clozapine produced flat-body posture and ataxia at 10 mg/kg and a high incidence of flat-body posture after administration of 40 mg/kg. A low incidence of catalepsy (i.e. seen in 1 of 5 animals) was found, after methylphenidate administration, in animals treated with the highest dose of haloperidol (0.63). All of the compounds with 5-HT_{1A} receptor agonist properties produced a high incidence of flat body posture (100%), generally at doses close to those which inhibited gnawing. In addition, 8-OH-DPAT, S 14506 and BMY 14802 at higher doses produced tremor, and 8-OH-DPAT produced convulsions, when combined with methylphenidate, although the incidence did not reach more than 60%. Lethality was observed in 1 of 7 animals treated with an intermediate dose of 8-OH-DPAT (2.5 mg/kg) in combination with saline, but was not observed with any other drugs tested in this study. In general, pretreatment with WAY-100635 raised the minimally effective dose that produced other behavioral effects for all of the 5-HT_{1A} receptor agonists. For example, the minimally effective dose of 8-OH-DPAT to produce other behavioral effects after saline pretreatment was 0.16 mg/kg, whereas after WAY-100635 pretreat-

Table 3
Other grossly observable effects produced by neuroleptics, 5-HT_{1A} receptor agonists and mixed 5-HT_{1A} receptor agonists/DA receptor antagonists in rats treated with methylphenidate (40 mg/kg i.p.)

	Dose range (mg/kg)	MED [a]	Maximal percentage incidence				
			Flat-body posture	Ataxia	Tremor	Convulsions	Catalepsy
Saline pretreatment							
Haloperidol	0.04–0.63	0.63					20
Clozapine	2.5–40	10	60 (40) [b]	20			
S 14506	0.01–10	0.01	100 (0.63)		60 (2.5)		
Buspirone	0.63–10	2.5	100 (10)				
Flesinoxan	0.16–40	2.5	100 (10)				
8-OH-DPAT	0.04–10	0.16	100 (2.5)	14 (10)	43 (2.5)	43 (2.5)	
BMY 14802	2.5–40	2.5	100 (40)		40 (40)		
WAY-100635 pretreatment							
Haloperidol	0.01–2.5	> 2.5					
Clozapine	10–40	40	20	40			
S 14506	0.63–40	0.63	100 (10)		20 (10)		
Buspirone	0.63–10	10	20				
Flesinoxan	2.5–160	160	43				
8-OH-DPAT	0.16–10	2.5	71 (10)			14 (2.5)	
BMY 14802	10–40	> 40					

[a] MED, minimally effective dose.
[b] Effective dose is shown in parentheses when higher than the MED.

ment, other behavioral effects were not observed until the administration of 2.5 mg/kg.

4. Discussion

The most important findings of this study are that the ability of the mixed 5-HT_{1A} receptor agonist/dopamine D_2 receptor antagonist buspirone to inhibit methylphenidate-induced gnawing and other behaviors cannot be blocked by administration of the silent 5-HT_{1A} receptor antagonist, WAY-100635, therefore demonstrating that its 5-HT_{1A} receptor agonist properties are not involved in its ability to inhibit gnawing and to normalize the behavior of methylphenidate-treated rats. In contrast, WAY-100635 produced parallel shifts to the right of the dose-response functions of the 5-HT_{1A} receptor agonists, 8-OH-DPAT, flesinoxan and S 14506 to inhibit methylphenidate-induced gnawing, indicating that their effects on this behavior are mediated via actions at 5-HT_{1A} receptors. Additionally, the effects of the antipsychotics haloperidol and clozapine were not altered by pretreatment with WAY-100635, further demonstrating the pharmacological specificity of the inhibition of methylphenidate-induced gnawing by 5-HT_{1A} receptor agonists and suggesting that blockade of tonic activity at 5-HT_{1A} receptors does not modulate the antidopaminergic effects of haloperidol and of clozapine examined here.

The indirect dopamine receptor agonist methylphenidate reliably produces gnawing, licking, sniffing, rearing and locomotion, characteristic of effects produced in rats by psychomotor stimulants of the amphetamine class (Lewander, 1977; Moore, 1978). Although its dose dependency was not examined here, methylphenidate increases sniffing, rearing and locomotion along inverted U-shaped dose-response curves (Koek and Colpaert, 1993). In the present study, haloperidol inhibited stereotyped gnawing in a dose-dependent manner, and at higher doses also inhibited other methylphenidate-induced behaviors, consistent with results previously obtained using this paradigm (Koek and Colpaert, 1993). Similarly in agreement with previous results, clozapine dose-dependently inhibited gnawing, but did not normalize other methylphenidate-induced behaviors (Koek and Colpaert, 1993). Interestingly, pretreatment with WAY-100635 did not alter the ability of clozapine to inhibit methylphenidate-induced gnawing, indicating that its reported partial 5-HT_{1A} receptor agonist properties (Newman-Tancredi et al., 1996) do not play a role in its ability to inhibit effects of methylphenidate. Moreover, pretreatment with WAY-100635 neither altered the ability of haloperidol nor the inability of clozapine (or other compounds, see below) to normalize behavior, suggesting that blockade of tonic activity at 5-HT_{1A} receptors does not alter the effects of these antidopaminergic compounds. On the other hand, WAY-100635 did produce a trend toward enhancement of the effects of haloperidol on gnawing and sniffing, suggesting that 5-HT_{1A} receptors may modulate some, but not all of the antidopaminergic effects of haloperidol. It should be emphasized, however, that the apparent lack of a role of 5-HT_{1A} receptor interactions in the effects of haloperidol and clozapine does not rule out the possibility that their effects can be modified by 5-HT_{1A} receptor agonists. A more detailed characterization of neuroleptic/5-HT_{1A} receptor interactions in this test, and in other preclinical models, such as conditioned avoidance responding, is currently being performed.

The differential effects of WAY-100635 in combination with selective (i.e. 8-OH-DPAT and flesinoxan) and non-selective 5-HT_{1A} receptor agonists (S 14506 and buspirone) reveals that these drugs do not inhibit methylphenidate-induced gnawing in the same manner. For example, the results obtained with flesinoxan, i.e. a parallel shift to the right of the inhibition of gnawing dose-response function, are consistent with its relatively selective affinity for 5-HT_{1A} receptors. In contrast, the lack of antagonism of the effects of buspirone on methylphenidate-induced gnawing is in agreement with findings that this compound has relatively high dopamine D_2 receptor affinity and demonstrable in vivo dopamine receptor antagonist properties (Maskall et al., 1995; Peroutka, 1985). Buspirone was also an exception among this group of compounds in that it normalized other methylphenidate-induced behaviors, a finding in agreement with previous results obtained under identical conditions (Koek and Colpaert, 1993). Although flesinoxan and S 14506 are more selective 5-HT_{1A} receptor agonists than buspirone, they have high affinity for dopamine receptors (Protais et al., 1994; Ybema et al., 1994) and might be expected to exhibit dopamine receptor antagonist properties in vivo. Nonetheless, their effects on methylphenidate-induced gnawing and other behaviors (i.e. sniffing and rearing) observed in this study were reversible by WAY-100635, demonstrating the existence of prominent 5-HT_{1A} receptor actions in vivo.

The 5-HT_{1A} receptor agonist S 14506 (Colpaert et al., 1992) has recently been described as a potential antipsychotic, based upon findings that it blocks apomorphine-induced climbing, and inhibits in vivo binding of [^{3}H]raclopride (Protais et al., 1994). However, in the present study, its effects in another behavioral model were shifted to the right in a surmountable manner by WAY-100635, clearly implicating the involvement of 5-HT_{1A} receptors in its presumed antidopaminergic effects. Thus, in spite of its high affinity for dopamine receptors, the present results indicate that 5-HT_{1A} receptor agonist properties of S 14506 mediate the reversal of methylphenidate-induced gnawing, and demonstrate the utility of WAY-100635 in dissociating the 5-HT_{1A} receptor agonist component from other activities of a reportedly mixed compound. It should be noted, however, that the present results do not exclude the possibility that S 14506 may exhibit in vivo dopamine receptor antagonist effects under different

conditions. Given that in vitro binding results indicate that there is only a 5-fold separation between 5-HT_{1A} and dopamine D_2 receptor affinities (Protais et al., 1994), it is conceivable that dopamine receptor antagonist properties of S 14506 would be 'unmasked' in the presence of WAY-100635 and would result in a normalization of methylphenidate-induced behaviors like that observed with haloperidol. S 14506, however, failed to completely inhibit all methylphenidate-induced behaviors, and, with the exception of locomotion, its effects were reversed by WAY-100635, and therefore appear to involve actions at 5-HT_{1A} receptors.

Although the apparent in vivo antidopaminergic effects of S 14506 may be largely attributed to its 5-HT_{1A} receptor agonist properties, examination of the maximal mean decreases in gnawing scores (Fig. 2) suggests that S 14506 and buspirone were both more effective than either 8-OH-DPAT or flesinoxan in inhibiting gnawing. That is, 8-OH-DPAT and flesinoxan, even when relatively high doses were administered, produced less than maximal decreases in methylphenidate-induced gnawing, whereas buspirone and S 14506 completely inhibited gnawing. These results suggest that, like buspirone, S 14506 may be more efficacious in inhibiting gnawing than the more selective 5-HT_{1A} receptor agonists. A role for in vivo dopamine antagonist properties in these effects of S 14506 appears unlikely, however, because they were reversed completely by WAY-100635.

Because inhibition of gnawing and the appearance of other behaviors were obtained with S 14506, flesinoxan, and, to some extent, 8-OH-DPAT, the results indicate that 5-HT_{1A} receptor agonists are consistently able to modify stimulant-induced behaviors. These findings, which, in general correspond to those of Protais et al. (1994), may be explained by results from a variety of neurochemical studies showing that 5-HT_{1A} receptors directly modulate dopamine neurotransmission (Ahlenius et al., 1989; Chen and Reith, 1995; Ichikawa et al., 1995). Of particular relevance for the present findings, it has been demonstrated that the high efficacy enantiomer of 8-OH-DPAT, *R*(+)-8-OH-DPAT inhibits amphetamine-induced dopamine release in a manner reversible by WAY-100635 (Ichikawa et al., 1995). Thus, the ability of compounds, such as S 14506 and flesinoxan, to inhibit gnawing and allow other methylphenidate-induced behaviors to appear is consistent with a direct modulatory role of 5-HT_{1A} receptors on the indirect receptor agonist effects of methylphenidate. Conversely, because even very high doses of WAY-100635 administered alone did not affect methylphenidate-induced behaviors, tonic activity at 5-HT_{1A} receptors probably does not modulate the stimulant-induced behaviors examined here.

The results obtained with the buspirone analog, BMY 14802 are interesting in light of its partial 5-HT_{1A} receptor agonist properties in vivo (Rabin and Winter, 1993; Sanger and Schoemaker, 1992) and putative antipsychotic properties demonstrated in preclinical models (cf. Debonnel and de Montigny, 1996). The effects on methylphenidate-induced gnawing in this study were clearly not affected by pretreatment with WAY-100635, demonstrating that 5-HT_{1A} receptors are not involved, and, because BMY 14802 lacks appreciable affinity for dopamine receptors, these results suggest that other mechanisms are responsible for the decrease in gnawing. Findings that BMY 14802 and other σ receptor ligands, e.g. rimcazole, interact with the dopaminergic system (Debonnel and de Montigny, 1996) and prevent some of the chronic effects of stimulants (Terleckyj and Sonsalla, 1994) suggest a possible mechanism, although the exact manner in which σ ligands act centrally still remains to be elucidated. Nonetheless, despite its inhibitory effects on methylphenidate-induced gnawing, BMY 14802 did not normalize behaviors and there are indications that it lacks efficacy in the treatment of schizophrenia (Gewirtz et al., 1994).

In summary, the results of this study demonstrate that the effects of the mixed 5-HT_{1A} receptor agonist/D_2 receptor antagonist buspirone are not altered by pretreatment with the 5-HT_{1A} receptor antagonist WAY-100635, similar to that found with the antipsychotics haloperidol and clozapine. Therefore, its effects on methylphenidate-induced gnawing cannot be attributed to the involvement 5-HT_{1A} receptors. In contrast, it was demonstrated that flesinoxan, 8-OH-DPAT and S 14506 reverse methylphenidate-induced gnawing via direct involvement of the 5-HT_{1A} receptor. It remains to be determined whether other recently discovered compounds with mixed 5-HT_{1A} receptor agonist/dopamine receptor antagonist properties (e.g. Reitz et al., 1995) can be distinguished from 5-HT_{1A} agonists in this preclinical model.

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