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Antagonism at 5-HT_{2A} receptors potentiates the effect of haloperidol in a conditioned avoidance response task in rats

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Abstract

High affinity for serotonin-2A (5-HT_{2A}) over dopamine (DA) D_2 receptors is a leading hypothesis for clozapine's favorable therapeutic profile. Recent preclinical studies also indicate that a sufficient antipsychotic effect might be obtained by a combined high 5-HT_{2A}/low D_2 receptor blockade. Thus, addition of a 5-HT_{2A} receptor antagonist to an ineffective dose of a D_2 receptor antagonist produces a robust antipsychotic-like effect in the conditioned avoidance response (CAR) test. Electrophysiological and biochemical studies also show that 5-HT_{2A} receptor antagonists can confer an atypical (clozapine-like) profile on a D_2 receptor antagonist. Improved therapeutic efficacy by adjunctive 5-HT_{2A} receptor antagonist treatment to a traditional D_2 receptor blocking regimen has been suggested. However, the ability of 5-HT_{2A} receptor blockade to protect against, or ameliorate, parkinsonian symptoms still remains unclear. Using the CAR and the catalepsy (CAT) tests as indices for antipsychotic activity and extrapyramidal side effect (EPS) liability, respectively, the effects of the selective 5-HT_{2A} receptor antagonist MDL 100,907 in combination with the DA D_2 receptor antagonists haloperidol or raclopride were studied in rats. Haloperidol (0.025 or 0.1 mg/kg sc, −30 min) produced a dose-dependent suppression of CAR. Pretreatment with MDL 100,907 (0.5, 1.0, or 1.5 mg/kg sc; −60 min) enhanced and prolonged the haloperidol-induced suppression of CAR without escape failures. MDL 100,907 (1 mg/kg sc, −60 min) had no effect on CAT when coadministered with ineffective doses of raclopride. Raclopride (1 mg/kg sc, −30 min) alone produced a submaximal cataleptic response that was significantly enhanced by pretreatment with MDL 100,907. The present results confirm and extend previous results by showing that 5-HT_{2A} receptor blockade can enhance the antipsychotic-like effects of a very low dose of a commonly used traditional antipsychotic. 5-HT_{2A} receptor blockade does not, however, prevent EPS (CAT). The therapeutic advantage of this combination might, therefore, operate within a fairly narrow window.

Keywords: Serotonin-2A receptor; Dopamine D_2 receptor; Conditioned avoidance response behavior; Catalepsy; Atypical antipsychotic drugs; Rat

1. Introduction

In traditional receptor binding assays, the atypical antipsychotic clozapine appears to have fairly low affinity for the dopamine (DA) D_2 receptor (Seeman, 1992; see also Seeman et al., 1997). Yet, clozapine is more efficacious and also produces markedly fewer parkinsonian extrapyramidal side effects (EPS) than traditional neuroleptics. More recent findings indicate, however, that therapeutic doses of clozapine might in fact occupy higher levels of D_2 receptors than initially thought. In receptor binding assays, this is presumably masked by the fact that clozapine binds more "loosely" to the D_2 receptor than traditional neuroleptics, and thus gets more rapidly and readily displaced by commonly used radioligands or by release of endogenous DA (Seeman and Tallerico, 1998). In addition, clozapine has a multireceptor affinity profile that makes it difficult to determine which receptors, or combinations of receptor affinity ratios, might significantly further contribute to its superior efficacy. However, a high affinity ratio for the serotonin-2A (5-HT_{2A}) over the DA D_2 receptor is one leading hypothesis for clozapine's favorable therapeutic profile (Meltzer, 1989, 1995a,b).

Recent preclinical studies do indeed suggest that a sufficient antipsychotic effect might be obtained by a

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combined high 5-HT_{2A}/low DA D_2 receptor blockade. The conditioned avoidance response (CAR) test is a common animal screening test for potentially antipsychotic drugs (see e.g. Arnt, 1982; Wadenberg and Hicks, 1999). Adjunctive administration of 5-$HT_{2A/2C}$ (ritanserin) or selective 5-HT_{2A} (MDL 100,907) receptor antagonists to an ineffective dose of a selective DA D_2 receptor antagonist produces a robust antipsychotic-like suppression of CAR (Wadenberg et al., 1996, 1998a). Electrophysiological and biochemical findings have demonstrated an ability of ritanserin to confer an atypical (clozapine-like) profile on the DA D_2 receptor antagonist raclopride (Andersson et al., 1995; see also Svensson et al., 1993). Thus, for example, ritanserin potentiates the stimulatory effects of raclopride on neuronal activity and DA release selectively in rat mesocorticolimbic brain areas (Andersson et al., 1995). Similarly, systemic administration or local application of MDL 100,907 into the rat medial prefrontal cortex (mPFC) produces a marked increase in DA efflux in this area (Schmidt and Fadayel, 1995). Improved therapeutic efficacy, particularly regarding negative symptoms, by the adjunctive treatment with a 5-HT_{2A} receptor antagonist to a traditional DA D_2 receptor blocking agent has also been reported in clinical studies (Carman et al., 1995; Duinkerke et al., 1993; see Reyntjens et al., 1986).

While preclinical studies seem to suggest that adjunctive 5-HT_{2A} receptor blockade might allow for a sufficient antipsychotic effect in spite of a low (sub-pseudo-parkinsonian) D_2 receptor occupancy, the ability of 5-HT_{2A} receptor blockade to actually protect against, or ameliorate parkinsonism remains unclear. Using the rodent catalepsy (CAT) model of neuroleptic-induced parkinsonism (see e.g. Wadenberg, 1996), several studies have shown that CAT induced by DA D_2 receptor blockade cannot be reversed by pretreatment with 5-HT_{2A} receptor antagonists (Arnt et al., 1986; Elliott et al., 1990; Wadenberg, 1992; Wadenberg et al., 1996). Furthermore, several clinical studies found no amelioration of parkinsonism (see e.g. Duinkerke et al., 1993; Korsgaard and Friis, 1986) or of tardive dyskinesia (Meco et al., 1989) following adjunctive treatment with 5-HT_{2A} receptor antagonists. However, some investigators have reported an attenuation of neuroleptic-induced CAT following pretreatment with some 5-HT_2 receptor antagonists (Bligh-Glover et al., 1995; Bonhomme et al., 1997; Neal-Beliveau et al., 1993).

To increase clinical relevance, the present study used a well-established traditional antipsychotic haloperidol (Janssen, 1967) in the CAR test for antipsychotic activity. As a complement to our previously reported specific DA D_2/5-HT_{2A} receptor interaction in the CAR test, we used the selective DA D_2 receptor antagonist raclopride (Köhler et al., 1985) to investigate the effects of additional 5-HT_{2A} receptor antagonism on extrapyramidal motor functions (i.e. CAT). MDL 100,907 (Sörensen et al., 1993; see also Dudley et al., 1990) was used as a selective 5-HT_{2A} receptor antagonist.

2. Methods

2.1. Animals

Adult male Sprague–Dawley rats (280–300 g) were purchased from Sasco King (Omaha, NE), and housed in an AALAC-approved facility for a minimum of 3 days prior to CAR training or entering into CAT experiments. Animals observed for CAT were housed two per cage throughout the study. Food and water were available ad libitum. Animals in the CAR experiments were housed singly after training was initiated, and were kept on a calorie-restricted diet throughout the study. Water was available ad libitum to the CAR-trained rats. All animals were maintained on a reversed 12-h on/off light cycle (lights off at 0600), with a room temperature of $21 \pm 1^{\circ}C$. Relative humidity averaged 50% but varied with ambient conditions. All experiments were performed between the 4th and 10th hour of the dark cycle.

2.2. Drugs

Haloperidol (Sigma, St. Louis, MO, USA), raclopride tartrate (Astra, Södertälje, Sweden), and *R*-(+)-α (2,3-dimethoxyphenyl)-1-[2(4-fluorophenethyl)]-4-piperidinemethanol (MDL 100,907; Hoechst Marion Roussel, Cincinnati, OH, USA) were used. Haloperidol and MDL 100,907 were dissolved in a minimal amount of glacial acetic acid and made up to volume with isotonic glucose. Raclopride was dissolved in distilled water. MDL 100,907 (or vehicle) and haloperidol or raclopride were given 60, 30, and 60 min, respectively, before the first observation time (CAR and CAT). All drugs were given subcutaneously in a volume of 2 ml/kg body weight. Experiments involving the CAR test used a set of seven to eight animals for each dose of haloperidol tested. For further description, see the section on CAR behavior. Experiments involving the CAT test used eight animals per treatment group.

2.3. CAR behavior

Rats were trained and tested in a computer-assisted, two-way active avoidance (shuttlebox) apparatus equipped with a tilting grid floor with microswitch detection, and connected to a high-resistance power supply. The boxes were divided into two compartments of equal size by a partition with one opening. Upon presentation of the 80-dB white noise conditioned stimulus (CS), the animals had 10 s to move from one compartment of the shuttlebox into the other. If the rat remained in the same compartment for more than 10 s, the unconditioned stimulus (UCS) was presented as an intermittent electric shock in the grid floor until an escape was performed. If the animal did not respond within 60 s, including the first 10 s, the trial was terminated (escape failure). Intertrial intervals varied at random between 20 and 40

Table 1
Lack of effects of haloperidol (0.01 mg/kg) alone, and in combination with MDL 100,907 on CAR in rats

	Time after haloperidol (min)			
Drug (mg/kg)	Pre	30	90	240
Vehicle/vehicle	100 ± 3.8	100 ± 0.0	100 ± 2.5	95 ± 5.3
Vehicle/haloperidol (0.01)	100 ± 0.0	100 ± 2.5	100 ± 0.0	100 ± 5.5
MDL (0.1)/haloperidol	100 ± 2.5	95 ± 5.0	100 ± 5.0	100 ± 0.0
MDL (1.0)/haloperidol	100 ± 0.0	95 ± 5.5	100 ± 10.5	95 ± 12.0

MDL 100,907 and haloperidol were administered 60 and 30 min, respectively, before the first postinjection observation time. Shown are medians ± semi-interquartile range based on repeated observations of the same seven animals serving as their own controls in a changeover design (Li, 1964). Statistical evaluation was performed by means of the Friedman two-way ANOVA by ranks (Seigel and Castellan, 1988).

All Friedman values resulted in $P>.05$.

s. The following variables were recorded: *avoidance* (response to CS within 10 s); *escape* (response to CS + UCS); *escape failures* (failure to respond to CS and CS + UCS); and *intertrial crosses*. The animals were trained on consecutive days until they reached >90% conditioned avoidance. Experimental manipulations were always preceded by a pretest. All pretest and experimental sessions were run for 10 min resulting in an approximate number of 20–22 trials in any given session. The number of trials in which an avoidance response occurred was divided by the total number of trials per session to determine the percent avoidance response. For further details, see Wadenberg et al. (1998b).

The same animals were tested repeatedly according to a randomizing changeover design (Li, 1964) serving as their own controls.

2.4. Catalepsy

Animals were placed on an inclined (60°) grid. To establish a reliable baseline, the first 30 s were excluded from the actual rating time. The time the rat remained in the same position was thereafter measured for a maximum of 2.5 min. CAT was scored from 0–5 according to the time (minutes; square root transformation) the animal remained immobile: 0 = 0–0.08, 1 = 0.09–0.35, 2 = 0.36–0.80, 3 = 0.81–1.42, 4 = 1.43–2.24, 5 = ≥2.25, i.e. if the rat remained immobile for ≥2.25 min, a score of 5 was recorded (cf. Ahlenius and Hillegaart, 1986).

2.5. Statistics

Statistical evaluation was performed by means of the Friedman two-way ANOVA by ranks, followed by the Wilcoxon matched-pairs signed-ranks test for appropriate post-hoc comparisons (CAR), or by means of the Kruskal–Wallis one-way ANOVA followed by the Mann–Whitney *U* test (CAT) (Siegel and Castellan, 1988).

The study was approved by the Scott & White IACUC committee for animal research.

3. Results

In the CAR experiments, no escape failures were recorded at any time or under any treatment condition (i.e. a decrease in avoidance responding was always accompanied by a corresponding increase in escapes).

3.1. Effects of haloperidol alone and in combination with MDL 100,907 on a CAR behavior in rats

The DA D_2 receptor antagonist haloperidol (0.01 mg/kg sc) alone, or in combination with MDL 100,907 (0.1 or 1.0 mg/kg sc) had no effect on CAR (Table 1).

Haloperidol (0.025 or 0.1 mg/kg sc, −30 min) alone suppressed CAR (Table 2). Pretreatment with the selective 5-HT_{2A} receptor antagonist MDL 100,907 (0.5, 1.0, or 1.5 mg/kg sc, −60 min) enhanced the haloperidol (0.1 mg/kg)-induced suppression of CAR ($P<.05$). The effect of MDL 100,907 (1.5 mg/kg) was still present and statistically significant at 240 min after haloperidol (0.1 mg/kg) administration (Table 2). Pretreatment with MDL 100,907 (1 mg/kg sc, −60 min) enhanced haloperidol (0.025 mg/kg sc, −30 min)-induced suppression of CAR ($P<.01$). The effect lasted at least 4 h (Table 2).

A dose–response presentation of the effects on CAR of haloperidol (0.01, 0.025, or 0.1 mg/kg sc, −30 min) alone and following pretreatment with MDL 100,907 (1 mg/kg sc, −60 min) is shown in Fig. 1.

3.2. Effects of haloperidol alone and in combination with MDL 100,907 on the number of intertrial crosses

Haloperidol (0.01 mg/kg) had no effects on the number of intertrial crosses compared to vehicle-treated con-

Table 2
Effects of haloperidol (0.025 or 0.1 mg/kg) alone, and in combination with MDL 100,907 on CAR in rats

	Time after haloperidol (min)				
Drug (mg/kg)	Pre	30	90	240	480
Vehicle/vehicle	100 ± 3	100 ± 0	100 ± 2	95 ± 5	97 ± 5
Vehicle/haloperidol (0.025)	100 ± 5	44 ± 9**	6 ± 6**	95 ± 8	98 ± 2
Vehicle/haloperidol (0.1)	95 ± 5	29 ± 36**	6 ± 21**	81 ± 27*	94 ± 15
MDL (1.0)/halo (0.025)	100 ± 2	0 ± 8††	0 ± 0†	14 ± 14††	95 ± 19
MDL (0.5)/halo (0.1)	97 ± 8	0 ± 4†	0 ± 0†	28 ± 32	81 ± 26
MDL (1.0)/halo(0.1)	92 ± 6	0 ± 3†	0 ± 0†	53 ± 26	79 ± 7
MDL (1.5)/halo (0.1)	97 ± 3	0 ± 6†	0 ± 0†	8 ± 31†	95 ± 8

MDL 100,907 and haloperidol were administered 60 and 30 min, respectively, before the first postinjection observation time. Shown are medians ± semi-interquartile range based on repeated observations of the same eight animals serving as their own control in the changeover design (Li, 1964). Statistical evaluation was performed by means of the Friedman two-way ANOVA by ranks followed by the Wilcoxon matched-pairs signed-ranks test for appropriate post hoc comparisons (Seigel and Castellan, 1988).

* $P<.05$, compared to vehicle-treated controls.

** $P<.01$, compared to vehicle-treated controls.

† $P<.05$, compared to animals treated with haloperidol alone.

†† $P<.01$, compared to animals treated with haloperidol alone.

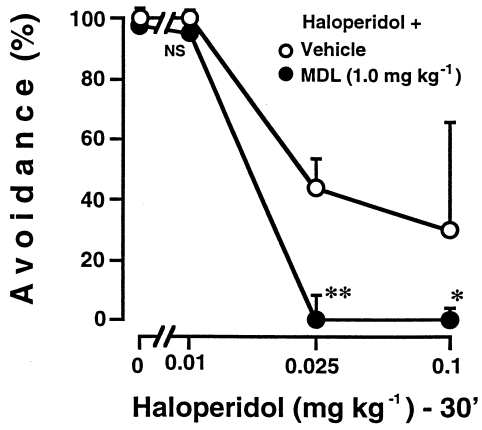

Fig. 1. Effects of haloperidol alone, and in combination with MDL 100,907 on CAR in rats — dose response. MDL 100,907 and haloperidol were administered 60 and 30 min, respectively, before the first observation time. Shown are medians ± semi-interquartile range based on repeated observations of the same seven or eight animals serving as their own controls in a changeover design (Li, 1964). Statistical evaluation was performed by means of the Friedman two-way ANOVA followed by the Wilcoxon matched-pairs signed-ranks test for appropriate post-hoc comparisons (Siegel and Castellan, 1988). $^{ns}P>.05$; $*P<.05$; $**P<.01$.

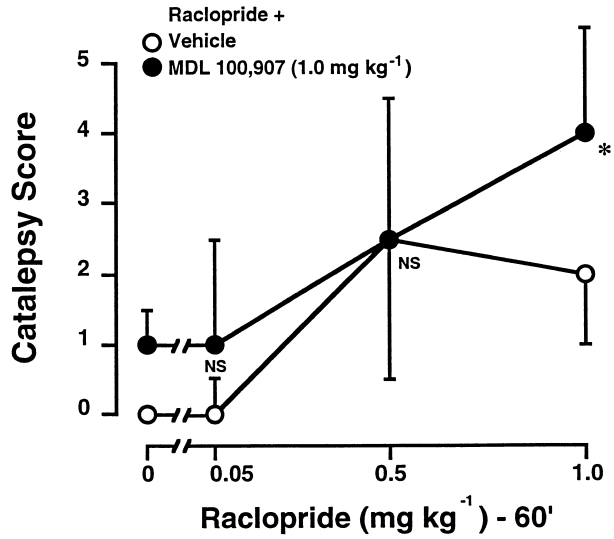

Fig. 2. Effects of raclopride alone, and in combination with MDL 100,907 on CAT in rats — dose response. MDL 100,907 and raclopride were administered 60 and 30 min, respectively, before the first postinjection observation time. Shown are medians ± semi-interquartile range based on repeated observations of eight animals per treatment group. Statistical evaluation was performed by means of the Kruskal–Wallis one-way ANOVA followed by the Mann–Whitney U test for comparisons between animals treated with raclopride alone and animals treated with the combination of MDL 100,907 and raclopride (Siegel and Castellan, 1988). $^{ns}P>.05$; $*P<.05$.

trols. Neither did pretreatment with MDL 100,907 (0.1 or 1.0 mg/kg) alter the number of intertrial crosses (data not shown).

Haloperidol (0.1 mg/kg) alone decreased the number of intertrial crosses significantly compared to controls only at the 240-min observation time ($P<.01$). A possible enhancement of the haloperidol-induced decrease in the number of intertrial crosses by pretreatment with MDL 100,907 (0.5–1.5 mg/kg) could not be detected because the number of intertrial crosses following treatment with haloperidol alone approached zero (Table 3). Haloperidol (0.025 mg/kg) alone did not affect the number of intertrial crosses. Combined treatment with MDL 100,907 (1 mg/kg) and haloperidol (0.025 mg/kg) produced a measurable reduction in the number of intertrial crosses as compared to the vehicle controls at the 240-min observation time ($P<.01$). (Table 3).

3.3. Effects of MDL 100,907 on raclopride-induced CAT in rats

The selective DA D_2 receptor antagonist raclopride (0.05 or 0.5 mg/kg sc, −30 min) alone did not significantly induce CAT compared to vehicle-treated controls. In the group treated with 0.5 mg/kg raclopride, this was due to high between-subject variability. Pretreatment with MDL 100,907 (1 mg/kg sc, −60 min) did not alter the effects of raclopride. Raclopride at 1 mg/kg sc (−30 min) produced a statistically significant CAT compared to vehicle-treated controls ($P<.01$). Pretreatment with MDL 100,907 (1 mg/

Table 3
Effects of haloperidol (0.025 or 0.1 mg/kg) alone, and in combination with MDL 100,907 on the number of intertrial crosses in rats

	Time after haloperidol (min)				
Drug (mg/kg)	Pre	30	90	240	480
Vehicle/vehicle	6.0 ± 3.3	2.5 ± 2.5	3.0 ± 3.5	6.5 ± 4.3	3.5 ± 3.0
Vehicle/haloperidol (0.025)	5.5 ± 5.3	1.0 ± 0.5	0.0 ± 0.0	4.0 ± 2.0	1.0 ± 3.8
Vehicle/haloperidol (0.1)	5.0 ± 3.5	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0**	1.0 ± 1.8
MDL (1.0)/halo (0.025)	7.0 ± 1.3	0.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.0††	1.0 ± 2.3
MDL (0.5)/halo (0.1)	6.0 ± 2.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.5	3.5 ± 1.8
MDL (1.0)/halo (0.1)	5.0 ± 3.8	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0	0.5 ± 0.8
MDL (1.5)/halo (0.1)	5.5 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.5	0.0 ± 1.8

MDL 100,907 and haloperidol were administered 60 and 30 min, respectively, before the first postinjection observation time. Shown are medians ± semi-interquartile range based on repeated observations of the same eight animals serving as their own controls in a changeover design (Li, 1964). Statistical evaluation was performed as in Table 2.

** $P<.01$, compared to vehicle-treated controls.

†† $P<.01$, compared to animals treated with haloperidol alone.

kg sc, −60 min) produced an enhancement of raclopride (1 mg/kg)-induced CAT compared to animals treated with raclopride alone ($P<.05$). MDL 100,907 (1 mg/kg) alone did not produce CAT.

Fig. 2 illustrates the dose–response effect of raclopride alone and in combination with MDL 100,907 on CAT.

4. Discussion

In the present study, pretreatment with the 5-HT_{2A} receptor antagonist MDL 100,907 significantly enhanced and prolonged haloperidol-induced suppression of CAR without producing escape failures at any time. In combination with ineffective doses of the DA D_2 receptor antagonist raclopride, MDL 100,907 did not influence CAT. However, in combination with a dose of raclopride that by itself produced a submaximal CAT in most animals, pretreatment with MDL 100,907 significantly enhanced the raclopride-induced CAT.

The present study demonstrates effects of MDL 100,907 on the suppression of CAR induced by the clinically commonly used antipsychotic haloperidol that are consistent with previous findings using raclopride (Wadenberg et al., 1996, 1998a), a compound not used in the clinic as an antipsychotic but often used in preclinical studies as a pharmacological research tool because of its selectivity for DA D_2 receptors. In previous studies in our laboratory (Wadenberg et al., 1996, 1998a), it was shown that a 5-$HT_{2A/2C}$ receptor antagonist (ritanserin) or a 5-HT_{2A} receptor antagonist (MDL 100,907) could enhance or induce, respectively, antipsychotic-like effects (i.e. selective suppression of CAR) in the presence of a threshold (0.1 mg/kg) or ineffective (0.05 mg/kg) dose of raclopride. However, another research group has reported failure of ritanserin to enhance the antipsychotic-like effects of raclopride or haloperidol in the CAR test (Prinssen et al., 1996). This could be because ritanserin is a less selective compound, and the dose used was fairly low.

Considering the fact that the present effect can be reliably reproduced using compounds from different chemical classes, it seems unlikely that the effect is due to pharmacokinetic interactions. A selective suppression of CAR has also been reported by cotreatment with the selective 5-HT_{2A} receptor antagonist amperozide and haloperidol in threshold doses (Egbe et al., 1990). In addition, it was recently found that local application of MDL 100,907 into the nucleus accumbens$_{shell}$ (NAS_{shell}) produced a similar selective suppression of CAR in the presence of a subthreshold dose (0.01 mg/kg) of systemically administered haloperidol (Hicks et al., 1999).

It also seems unlikely that the observed effects of MDL 100,907 (0.5–1.5 mg/kg) could be due to additional D_2 receptor blockade by MDL 100,907 itself. Compared to the less selective 5-HT_2 receptor antagonist ketanserin, MDL 100,907 is consistently reported to display high (sub-nanomolar) selectivity for 5-HT_{2A} receptor populations (mainly located in brain cortical areas), and extremely low nonspecific binding, in in vitro studies in humans and monkeys (López-Giménez et al., 1998), in rats (Johnson et al., 1996; López-Giménez et al., 1997), in in vivo studies in rats (Zhang and Bymaster, 1999), and in human positron emission tomography (PET) studies (Ito et al., 1998; Andrée et al., 1998). Furthermore, preclinical characterization of MDL 100,907 reports a K_i value for in vitro D_2 receptor binding of >2250 nM, and an ED_{50} of >30 mg/kg for reduction of apomorphine-induced climbing in rats (index of D_2 receptor antagonism) (Sörensen et al., 1993; Schmidt et al., 1995; Kehne et al., 1996). In a recent behavioral study, using 1 mg/kg of MDL 100,907, it was found that MDL 100,907 was unable to reverse apomorphine-induced disruption of prepulse inhibition (PPI) in rats, a primary dopaminergic animal model of sensorimotor gating deficits in schizophrenia (Geyer et al., 1999). In this context, it should also be noted that this same dose of MDL 100,907 restores disruption of PPI in rats induced by the 5-$HT_{2A/C}$ receptor agonist DOI or the NMDA receptor antagonist dizocilpine (Sipes and Geyer, 1995; Varty et al., 1999). Since clozapine (but not always traditional antipsychotics) displays similar properties, this has been taken as an indication of potentially atypical antipsychotic properties.

The CAR test is commonly used for the screening of potential antipsychotics. In this test, the characteristic feature of antipsychotic compounds is that, contrary to compounds without antipsychotic activity, they display a reasonable dose range within which they produce a selective suppression of CAR behavior but have no effects on escape behavior. This is in essence the criterion of the test (see e.g. Wadenberg and Hicks, 1999). Although some false positives have been reported, the test is considered to have high predictive validity (see Wadenberg and Hicks, 1999). Since antipsychotic drugs are presumed to exert their main therapeutic effect via an action at the DA mesocorticolimbic system (Carlsson, 1988; Owens and Risch, 1995), it seems reasonable to believe that this pathway is also involved in CAR behavior. The NAS_{shell} is an important substructure of the DA mesocorticolimbic system (Heimer et al., 1993). This substructure is presumed to be of importance in the pathophysiology of schizophrenia, and is also thought to be involved in behavior arising from emotion and motivation (Mogenson et al., 1980). Therefore, it is interesting to note that local application of the DA D_2 receptor antagonist (−)sulpiride into the NAS_{shell}, but not the dorsolateral neostriatum, suppresses CAR (Wadenberg et al., 1990). Furthermore, the atypical antipsychotic clozapine also produces a selective suppression of CAR following local application into the NAS_{shell} (see Wadenberg and Hicks, 1999). Therefore, it seems likely that the CAR test primarily involves DA mesocorticolimbic pathways not thought to be immediately involved in the mediation of extrapyramidal motor functions.

Finally, it should be noted that the effect of ritanserin on haloperidol-induced effects has also been studied in the paw test that is designed to test both antipsychotic activity and EPS liability in rats (see Ellenbroek and Cools, 1988). In this test, ritanserin was reported to reduce the effects of haloperidol (Ellenbroek et al., 1994). This finding is interesting but, unfortunately, difficult to fully interpret since the paw test is not commonly used in laboratories that do behavioral research.

The enhancement of haloperidol-induced suppression of CAR by pretreatment with MDL 100,907 was in part paralleled by a similarly enhanced decrease in the number of intertrial crosses. This is consistent with previous findings (Wadenberg et al., 1998a). These observations most likely reflect effects on exploratory locomotor activity mediated via mesolimbic DA pathways since locomotor activity has been shown to be dependent on an intact mesolimbic DA neural transmission (see e.g. Fink and Smith, 1980). It should also be noted that haloperidol-induced suppression of locomotor activity cannot be antagonized by pretreatment with the anticholinergic scopolamine (Ahlenius and Hillegaart, 1986). Although sedation would also decrease locomotor activity, this is most likely not the reason for the decrease in intertrial crosses since drugs that produce general sedation either have no effect at all in the CAR test, or do also produce escape failures (see Wadenberg and Hicks, 1999). There were no escape failures at any treatment or time in the present study

In the present study, pretreatment with MDL 100,907 significantly enhanced raclopride (1 mg/kg)-induced CAT. This indicates that selective 5-HT_{2A} receptor blockade is not able to protect against, and might in some situations worsen, EPS. In support for this notion, an increase in neuroleptic-induced CAT following pretreatment with the 5-$HT_{2A/2C}$ receptor antagonists ritanserin, mianserin, and ICI-170,809 has been reported (Elliott et al., 1990). Furthermore, it is interesting to note that while selective 5-HT_{2A} receptor blockade does not protect against EPS (CAT), selective 5-HT_{2C} receptor blockade has been reported to actually reverse haloperidol-induced CAT (Reavill et al., 1999). It has previously been shown that the 5-$HT_{2A/C}$ receptor agonist DOI reverses raclopride-induced CAT, and that the anticataleptic effects of DOI are reversed by pretreatment with the 5-$HT_{2A/C}$ receptor antagonist ritanserin (Wadenberg and Ahlenius, 1995). Considering the results reported by Reavill et al. (1999), the anticataleptic effect of DOI seems most probably to be mediated via the 5-HT_{2A} receptor. This seems to suggest that clozapine's favorable EPS profile may in part be due to its antagonism at 5-HT_{2C}, rather than 5-HT_{2A}, receptors.

Clinical reports of improvements in EPS following ritanserin as an adjunctive treatment are inconsistent. The reports often describe an amelioration of neuroleptic-induced Parkinsonian tremor (see e.g. Kapur, 1996). The CAT test, however, does not assess neuroleptic-induced tremor but more specifically address motor rigidity in general and the well-known difficulty in parkinsonism to initiate voluntary movements (see e.g. Wadenberg, 1996).

Preliminary data from a currently ongoing series of experiments to investigate the quantitative relationship between D_2 receptor occupancy and CAT in rats have shown that manifest CAT occurs only when striatal D_2 receptor occupancy is >80% (Wadenberg et al., 2000). This correlates extremely well with human PET data (Farde et al., 1992), and seems to be true for traditional as well as atypical antipsychotics. For raclopride and haloperidol, used in the present study, a striatal D_2 receptor occupancy >80% occurs at around 2 and 0.2 mg/kg, respectively (Wadenberg et al., 2000; in preparation). A complete selective suppression of CAR, even with traditional antipsychotics known to produce troublesome EPS, is usually obtained at doses lower than those needed to produce a robust CAT. The present and previous studies show that doses of D_2 receptor blocking agents needed for selective suppression of CAR can be further decreased in the presence of 5-HT_{2A} receptor blockade.

It has been suggested that while positive symptoms of schizophrenia might be due to an overactivity in the DA mesolimbic system (i.e. the NAS_{shell} area), the cognitive deficits associated with negative symptoms of schizophrenia might be due to a subsequent compensatory feedback mechanism that in turn decreases DA activity in the prefrontal cortex (PFC; see e.g. Angrist et al., 1980). On the other hand, increases in DA activity in the mPFC has been reported to produce a decrease in subcortical DA function (Schmidt and Fadayel, 1995). Clozapine, with high affinity for 5-HT_2 receptors, has been shown to preferentially increase DA efflux in the rat mPFC (Moghaddam and Bunney, 1990). Similarly, MDL 100,907 produces a marked increase in DA efflux in the rat mPFC both when injected systemically and following local application into the mPFC (Schmidt and Fadayel, 1995). It is therefore an intriguing thought that the behavioral effects of MDL 100,907, in the presence of a weak DA receptor blockade as observed in the present and previous studies, might indirectly be due to its influence on dopaminergic activity in the brain mPFC.

In conclusion, the 5-HT_{2A} receptor antagonist MDL 100,907 significantly enhanced and prolonged suppression of CAR induced by a submaximal dose of haloperidol without producing escape failures. In combination with ineffective doses of the DA D_2 receptor antagonist raclopride, MDL 100,907 had no effects on CAT. In combination with a dose of raclopride that by itself produced submaximal CAT, however, pretreatment with MDL 100,907 enhanced raclopride-induced CAT.

The present results suggest that, in the presence of 5-HT_{2A} receptor blockade, sufficient antipsychotic effect might be obtained with a lower dose (than usually needed) of the traditional antipsychotic haloperidol. The results in the CAT test indicate, however, that the therapeutic advantage of this combination might operate within a fairly narrow window, and point to the need for further careful

investigations on the interaction between MDL 100,907 (as it hopefully becomes available again) and haloperidol in this respect.

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