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Short communication

Effects of WAY 100635 on antipsychotic-induced catalepsy in 5-HT depleted animals: a role for tonic activation of 5-HT_{1A} receptors

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Abstract

We recently observed that the 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)-cyclohexanecarboxamide (WAY 100635) enhanced antipsychotic-induced catalepsy, which we hypothesized to be due to a blockade of tonic 5-HT_{1A} receptor activation. Here, we examined this hypothesis by studying the effects of WAY 100635 in animals that were depleted of 5-HT by repeated treatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine methyl ester. Depletion of 5-HT abolished the enhancement by WAY 100635 of catalepsy induced by low doses of the antipsychotics nemonapride and raclopride, in agreement with the hypothesis that WAY 100635 enhances catalepsy by blocking tonic 5-HT_{1A} receptor activation. Given the predictive validity of catalepsy, these findings indicate that 5-HT_{1A} receptor blockade may enhance the extrapyramidal side-effects of antipsychotics in humans.

Keywords: Neuroleptic; Antipsychotic; Catalepsy; Extrapyramidal side-effect; 5-HT (5-hydroxytryptamine, Serotonin) depletion; Dopamine D_2-like receptor; 5-HT_{1A} receptor; *p*-Chlorophenylalanine; Nemonapride; Raclopride; WAY 100635

1. Introduction

A growing number of studies show that stimulation of 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptors attenuates the extrapyramidal side-effects of antipsychotics. For example, 5-HT_{1A} receptor agonists attenuate antipsychotic-induced extrapyramidal side-effects in human (Yoshida et al., 1998) and non-human primates (Christoffersen and Meltzer, 1998), and antipsychotic-induced catalepsy in rats (for review, see Wadenberg (1996)). The increased interest in 5-HT_{1A} receptors in antipsychotic research is evidenced by reports of novel antidopaminergic compounds with affinity at 5-HT_{1A} receptors (for references, see Prinssen et al. (1999)). An example is nemonapride, a novel antipsychotic that is used in the clinic, and that, at 5-HT_{1A} receptors, has both high affinity (4.5 nM, versus 0.12 nM at dopamine D2 receptors; Assié et al., 1993) and high intrinsic activity (Assié et al., 1997). We recently found that the 5-HT_{1A} receptor agonist properties of nemonapride reduced its propensity to induce catalepsy at high doses (Prinssen et al., 1998), as expected. Unexpectedly, we also found that the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)-cyclohexanecarboxamide (WAY 100635; Forster et al., 1995) enhanced the cataleptic effects of low, dopamine D_2-like receptor-selective doses of nemonapride as well as those of the dopamine D_2-like receptor antagonists raclopride and haloperidol (Prinssen et al., 1998), and hypothesized that these effects were due to blockade of tonic 5-HT_{1A} receptor activation (Prinssen et al., 1998).

If tonic 5-HT_{1A} receptor activation indeed inhibits antipsychotic-induced catalepsy, this could have implications for the extrapyramidal side-effects of mixed dopamine/5-HT_{1A} receptor ligands, because it would suggest that antipsychotics with additional 5-HT_{1A} receptor antagonist properties may produce a high incidence of extrapyramidal side-effects. To examine the hypothesis that blockade of tonic 5-HT_{1A} receptor activation by WAY 100635 underlies its ability to enhance antipsychotic-induced catalepsy, we performed two experiments: (1) we tried to replicate the effects of WAY 100635 on catalepsy induced by low doses of the antipsychotics nemonapride (YM-09151-2; Usuda et al., 1981) and raclopride (Köhler et al., 1985) in animals treated repeatedly with saline (i.e., the control

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conditions for the 2nd experiment), and (2) we studied the effects of WAY 100635 on catalepsy induced by nemonapride and raclopride in animals treated repeatedly with the 5-HT synthesis inhibitor *p*-chlorophenylalanine methyl ester (which strongly decreases basal 5-HT concentrations in vivo, e.g., O'Connell et al., 1991). In the second experiment, we studied not only lower, but also higher doses of nemonapride, to examine whether, in 5-HT depleted animals, WAY 100635 would still be able to alter the effects of these higher, 5-HT_{1A} receptor activating, doses of nemonapride (like it did in intact animals, Prinssen et al., 1998), in the possible absence of effects of WAY 100635 on lower, dopamine receptor-selective, doses. In each experiment, a fixed dose of WAY 100635 was used (i.e., 0.63 mg/kg), based on our previous study (Prinssen et al., 1998).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Ico: OFA SD (IOPS Caw) Iffa Credo, Lyon, France), weighing 180 ± 20 g on arrival, were group-housed in polypropylene cages containing bedding material ($n = 6$ per cage, internal dimensions: 17 × 38 × 15 cm^3; $W \times L \times H$) in an environmentally controlled room (temperature, 21 ± 1°C, and relative humidity, 55 ± 5%) on a 12:12 h light:dark cycle (lights on at 0700 h). Food (standard rat chow; AO4, UAR, Epinay sur Orge, France) and filtered (0.22 μm) water were continuously available. A 5-day acclimatisation period was allowed before animals were used in experiments. Three days before the test, the animals were moved to an environmentally controlled laboratory but remained group-housed ($n = 2$–3 per cage as described above) during the repeated treatment phase. The acute experimental conditions were identical to those used in experiments on which the present study was based (Prinssen et al., 1998), i.e., the animals were individually housed in polycarbonate hanging cages with a grid floor (internal dimensions: 18 × 31 × 18 cm^3; $W \times L \times H$) 22 h before testing, where they had free access to water, but not food. Animals were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, USA, 1996) and the European Directive 86/609, and the protocol (No. 85/2) was carried out in compliance with French regulations and with local ethical committee guidelines for animal research.

2.2. Procedure

In the catalepsy procedure (cf. Prinssen et al., 1998, 1999), animals were first examined in the cross-legged position (CLP) test, and immediately thereafter, in the bar test. In the CLP test, the hindlimbs were placed over the ipsilateral forelimbs and the time was determined during which an animal remained in this position up to a maximum of 30 s. In the bar test, the forelimbs were placed on a horizontal, cylindrical metal bar (diameter, 1.25 cm; 10 cm above the table) and the time during which both forelimbs remained on the bar was determined up to a maximum of 30 s. Both tests were repeated three times (intertrial time: 3 min) and the mean of the three trials was used for analysis. Animals were put back in their home cage after each set of tests.

2.3. Analysis of data

Two dependent variables were used in this study, i.e., the duration of catalepsy in the CLP and the bar tests, which were analyzed separately. Interactions between WAY 100635 and the antipsychotics were analyzed with a two-way analysis of variance, with factors pretreatment and dose, followed by Dunnett's post-hoc tests for individual comparisons ($P < 0.05$). Because the experiments were designed to detect increases compared with saline-treated controls, one-tailed tests were used.

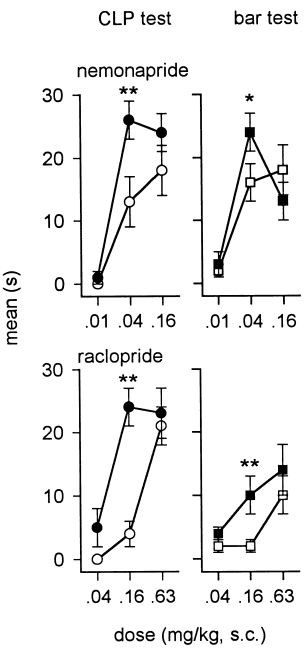

Fig. 1. The effects of the 5-HT_{1A} receptor antagonist WAY 100635 on catalepsy induced by the dopamine D_2-like receptor antagonist/5-HT_{1A} receptor agonist nemonapride and the dopamine D_2-like receptor antagonist raclopride in animals that were pretreated with saline (both 3 and 2 days before test). Upper panels: effects of nemonapride in combination with saline (open symbols) or WAY 100635 (0.63 mg/kg; closed symbols) in the CLP test and the bar test. Lower panels: effects of raclopride in combination with saline (open symbols) or WAY 100635 (0.63 mg/kg; closed symbols) in the CLP and bar tests. Shown are the mean values ± S.E.M. ($n = 7$ per group). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with animals treated with the corresponding dose of the antipsychotic, based on Dunnett's post-hoc tests with a one-tailed probability.

2.4. Drugs

The following drugs were used: DL-*p*-chlorophenylalanine methyl ester HCl (Sigma, St. Louis, MO, USA), raclopride tartrate (Research Biochemicals, Natick, MA, USA), nemonapride (YM-09151-2) and WAY 100635 diHCl (provided by J.L. Maurel, Centre de Recherche Pierre Fabre). All drugs were dissolved in distilled water, except nemonapride, which was dissolved in distilled water with a drop of lactic acid, after which the pH was adjusted to 5–7 with a 4% solution of sodium hydroxide. All drugs were administered in a volume of 10 ml/kg. Doses refer to the free base. Animals were administered *p*-chlorophenylalanine methyl ester (160 mg/kg, i.p.), or saline, both 3 and 2 days before tests. On the test day, WAY 100635 (0.63 mg/kg, s.c.) or saline was administered at 70 min, and one of several doses of an antipsychotic (s.c.) or saline at 55 min before the first set of catalepsy tests. Our earlier studies showed that 0.63 mg/kg WAY 100635, as well as a four-fold higher dose (2.5 mg/kg), does not induce catalepsy when administered alone (Prinssen et al., 1998, 1999).

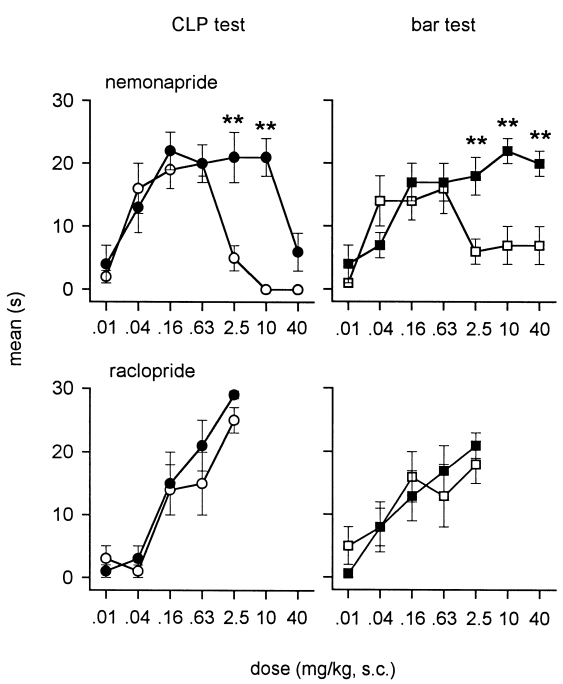

Fig. 2. The effects of the 5-HT_{1A} receptor antagonist WAY 100635 on catalepsy induced by nemonapride and raclopride in animals that were pretreated with the 5-HT synthesis inhibitor *p*-chlorophenylalanine methyl ester (160 mg/kg, both 3 and 2 days before test). Upper panels: effects of nemonapride in combination with saline (open symbols) or WAY 100635 (0.63 mg/kg; closed symbols) in the CLP test and the bar test. Lower panels: effects of raclopride in combination with saline (open symbols) or WAY 100635 (0.63 mg/kg; closed symbols) in the CLP and bar tests. Shown are the mean values ± S.E.M. ($n = 9$ per group for nemonapride, $n = 7$ per group for raclopride). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with animals treated with the corresponding dose of the antipsychotic, based on Dunnett's post-hoc tests with a one-tailed probability.

3. Results

In animals treated repeatedly with saline, pretreatment with WAY 100635 (0.63 mg/kg; Fig. 1, closed symbols) significantly enhanced the effects of low doses of nemonapride (i.e., 0.04 mg/kg) and raclopride (i.e., 0.16 mg/kg) in both tests.

In animals treated repeatedly with *p*-chlorophenylalanine, nemonapride induced catalepsy in the CLP and bar tests in a biphasic manner (Fig. 2, upper panels, open symbols). Pretreatment with WAY 100635 (0.63 mg/kg; closed symbols) prevented the decrease in catalepsy at higher doses of nemonapride, but did not alter significantly the effects of low doses of nemonapride (0.01–0.63 mg/kg). Raclopride dose-dependently induced catalepsy in both tests (Fig. 2, lower panels, open symbols). Pretreatment with WAY 100635 did not significantly alter the effects of raclopride (closed symbols).

4. Discussion

Recently, we observed that WAY 100635 significantly enhanced the cataleptic effects of lower, dopamine D_2-like receptor selective, doses of nemonapride, as well as those of the dopamine D_2-like receptor antagonists raclopride and haloperidol (Prinssen et al., 1998). Here, we replicated the catalepsy-enhancing effects of WAY 100635 for nemonapride and raclopride, in animals that were treated repeatedly with saline, suggesting that this is a robust phenomenon. In contrast, we found that WAY 100635 lost its ability to enhance the cataleptic effects of low doses of nemonapride and raclopride in animals treated repeatedly with the 5-HT synthesis inhibitor *p*-chlorophenylalanine methyl ester. Because *p*-chlorophenylalanine methyl ester strongly decreases basal 5-HT concentrations in vivo (e.g., O'Connell et al., 1991), this suggests that WAY 100635 enhances the cataleptic effects of antipsychotics by blocking 5-HT_{1A} receptor activation. This 5-HT_{1A} receptor activation appears to be caused by tonic 5-HT release because dopamine receptor antagonists do not enhance 5-HT levels (e.g., Ferré and Artigas, 1995; Assié et al., 1997). A blockade of tonic 5-HT_{1A} receptor activation underlying the catalepsy-enhancing effects of WAY 100635 is conceivable, because tonic 5-HT_{1A} receptor activity has been demonstrated in animals during periods of active arousal (for review, see Routledge (1996)), and because antipsychotic-induced catalepsy is very sensitive to 5-HT_{1A} receptor stimulation (for review, see Wadenberg (1996)). These results indicate that tonic 5-HT_{1A} receptor activation may play a role in antipsychotic-induced catalepsy.

If indeed blockade of tonic 5-HT_{1A} receptor activation with a 5-HT_{1A} receptor antagonist enhances antipsychotic-induced catalepsy, it could be reasoned that depletion of

5-HT should have done the same. However, because the 5-HT_{1A} receptors that modulate catalepsy are thought to be located somatodendritically (e.g., Invernizzi et al., 1988), thereby regulating 5-HT neuronal activity, this issue is likely to be complex. In future studies, we will address this issue in detail.

The present results have implications not only for the role of tonic 5-HT_{1A} receptor activation in catalepsy, but also for the role of the 5-HT_{1A} receptor agonist properties of nemonapride (Assié et al., 1997) in its cataleptogenic effects (Prinssen et al., 1998). That is, in animals repeatedly treated with *p*-chlorophenylalanine methyl ester, nemonapride induced less catalepsy at higher doses, similar to findings in non-pretreated animals (Prinssen et al., 1998). And, again, pretreatment with WAY 100635 reinstated catalepsy at these higher doses, which was even more striking in the present study because WAY 100635 did not alter the cataleptic effects of lower doses. One exception was the effect of the highest dose of nemonapride in the CLP test, which was not significantly altered by WAY 100635. Knowing that the effects of WAY 100635 are surmountable by high doses of 5-HT_{1A} receptor agonists (cf. Kleven and Koek, 1998), the highest dose of nemonapride may also have surmounted the effects of WAY 100635. That such surmountable antagonism would affect more readily the CLP test is in agreement with findings that the effects of antipsychotics in this test are attenuated more extensively by weak exogenous 5-HT_{1A} receptor activation (i.e., by administration of a low dose of a 5-HT_{1A} receptor agonist) than those in the bar test (cf. Prinssen et al., 1999). Together, these data indicate that the 5-HT_{1A} receptor agonist properties of nemonapride alter its cataleptogenic effects at high doses. Nevertheless, given the ca. 60-fold separation between the first dose of nemonapride to produce significant catalepsy and that on the descending limb of the dose–response curve (Prinssen et al., 1998), the doses used in clinical studies may not be high enough for its 5-HT_{1A} receptor agonist properties to play an important role. The recent finding that nemonapride rapidly induces acute dystonia in more than half of the schizophrenic patient population (Kondo et al., 1999), comparable to conventional antipsychotics such as haloperidol, supports the idea that, at clinical doses, its 5-HT_{1A} receptor agonist properties do not play an important role.

In summary, we replicated the finding that WAY 100635 enhances catalepsy induced by dopamine-selective doses of antipsychotics under control conditions, but did not find such enhancement in 5-HT-depleted animals, suggesting that the effects of WAY 100635 were due to blocking tonic 5-HT_{1A} receptor activation that inhibits antipsychotic-induced catalepsy. Given the predictive validity of catalepsy, and inasmuch as such tonic activation may also exist in humans, these results suggest that 5-HT_{1A} receptor antagonist properties could worsen the extrapyramidal side-effects of antipsychotics. However, it is difficult to predict how 5-HT_{1A} receptor blockade, basal 5-HT levels, and antipsychotic treatment will interact in the clinic, because both basal 5-HT activity (for review, see Breier (1995)) and 5-HT_{1A} receptor density (e.g., Hashimoto et al., 1991) may be altered in patients with schizophrenia. Nevertheless, the present results caution against the addition of 5-HT_{1A} receptor antagonists to antipsychotic treatment, because of possible increased extrapyramidal side-effects.

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