ELSEVIER

European Journal of Pharmacology 356 (1998) 189–192

ejp

Short communication

The cataleptogenic effects of the neuroleptic nemonapride are attenuated by its 5-HT_{1A} receptor agonist properties

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Received 16 April 1998; revised 13 July 1998; accepted 17 July 1998

Abstract

The effects of the 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)-cyclohexanecarboxamide (WAY 100635) on catalepsy induced by the dopamine D_2-like receptor antagonist/5-HT_{1A} receptor agonist nemonapride were examined and compared to its effects on catalepsy induced by neuroleptics that have low affinity for 5-HT_{1A} receptors. Nemonapride induced catalepsy in both cross-legged position and bar tests at low, but not at high doses. Pretreatment with WAY 100635 (0.63 mg/kg) reinstated catalepsy at higher doses of nemonapride, indicating that the 5-HT_{1A} receptor agonist properties of nemonapride are responsible for its inability to produce catalepsy at high doses. Additionally, WAY 100635 enhanced significantly the effects of low doses of nemonapride, and of the dopamine D_2-like receptor antagonists raclopride and haloperidol. The present data indicate that the 5-HT_{1A} receptor agonist properties of nemonapride attenuate its ability to induce catalepsy at higher doses, and suggest further that tonic 5-HT_{1A} receptor activation may modulate neuroleptic-induced catalepsy.

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

1. Introduction

Although conventional neuroleptics ameliorate positive symptoms in schizophrenia, they also induce extrapyramidal side-effects. Both of these effects are thought to be mediated by blockade of dopamine D_2-like receptors, but their expression can be modulated by other systems, e.g., serotonin (for recent review, see Kinon and Lieberman, 1996). Recently, attention has been focused on the potential role of the 5-HT_{1A} receptor in attenuating extrapyramidal side-effect liability. In preclinical studies, stimulation of this receptor markedly attenuates the effects of neuroleptics in several models for extrapyramidal side-effects, e.g., neuroleptic-induced dystonia in non-human primates (Liebman et al., 1989), catalepsy (for review, see Wadenberg, 1996), and the forelimb retraction time in the paw test (Ellenbroek et al., 1994). Even though many of the clinically available neuroleptics have low affinity at 5-HT_{1A} receptors (Leysen et al., 1993), the interest in 5-HT_{1A} receptors in neuroleptic research is illustrated by the development of novel mixed dopamine/5-HT compounds, such as ziprasidone and mazapertine, which have high affinity at 5-HT_{1A} receptors (Reitz et al., 1994; Seeger et al., 1995). A neurochemical study from our laboratory indicated that the recently marketed neuroleptic and dopamine D_2-like receptor antagonist nemonapride has prominent agonist properties at 5-HT_{1A} receptors both in vitro and in vivo (Assié et al., 1997). Here, we studied whether these properties ameliorate its cataleptogenic effects by examining the ability of 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) to alter the effects of nemonapride on catalepsy measured using the cross-legged position and bar tests. To examine the specificity of these interactions, we compared the effects of nemonapride with those of the neuroleptics raclopride and haloperidol, which have low affinity at 5-HT_{1A} receptors (Leysen et al., 1993).

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0014-2999/98/$19.00
PII: S0014-2999(98)00536-6

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Ico: OFA SD (I.O.P.S. Caw), Iffa Credo, Lyon, France) weighing 180 ± 20 g on arrival, were individually housed in plastic hanging cages with a grid floor (21 × 31 × 18 cm; W × L × H). A minimum of a five-day acclimatization period was allowed before animals were used in experiments. Animals were housed in an environmentally controlled room (21 ± 1°C; humidity: 55 ± 5%) on a 12 h:12 h light:dark cycle (lights on at 0700 h), with food (AO4, UAR, Epinay/s/Orge, France) and filtered water (0.22 μ) continuously available. Animals were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996) and the European Directive 86/609 and the protocol (No. 15) was carried out in compliance with French regulations and with local ethical committee guidelines for animal research.

2.2. Procedure

Catalepsy was measured using two different tests (e.g., Kleven et al., 1996), i.e., the cross-legged position test, and the bar test. In the cross-legged position test, the hindlimbs were placed over the ipsilateral forelimbs and the time during which an animal remained in this position was determined up to a maximum of 30 s. In the bar test the forelimbs of the rat were placed on a 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. The number of animals tested in each group was nine for nemonapride and seven for raclopride and haloperidol.

2.3. Analysis of data

The two dependent variables used in this study, i.e., the duration of catalepsy in the cross-legged position test and the bar test, were analyzed separately. The cataleptogenic effects of nemonapride were analyzed with a one-way analysis of variance followed by Dunnett's post-hoc tests for individual comparisons with saline-treated controls, with $P < 0.05$ considered to be statistically significant. Interactions between WAY 100635 and the neuroleptics were analyzed with a two-way analysis of variance, with factors pretreatment and dose, followed by Dunnett's post-hoc tests for individual comparisons ($P < 0.05$).

2.4. Drugs

The following drugs were used: haloperidol (Sigma, St. Louis, MO, USA), raclopride tartrate (Research Biochemicals, Natick, MA, USA), nemonapride (YM-09151-2) and WAY 100635 di-HCl (provided by J.L. Maurel, Centre de Recherche Pierre Fabre). Raclopride and WAY 100635 were dissolved in distilled water, and haloperidol and nemonapride were dissolved in distilled water with a drop of acetic acid, after which the pH was adjusted to 5–7 with a solution of 4% sodium hydroxide. All drugs were administered subcutaneously (s.c.) in a volume of 10 ml/kg. Doses refer to the free base. WAY 100635 or saline was administered at 70 min, and a neuroleptic or saline at 55 min before the first set of catalepsy tests.

3. Results

Nemonapride significantly induced catalepsy in the cross-legged position test and in the bar test in a dose-dependent, but apparently non-monotonic manner (Fig. 1, upper panels). Pretreatment with WAY 100635 (0.63 mg/kg) enhanced significantly the effects of low doses of

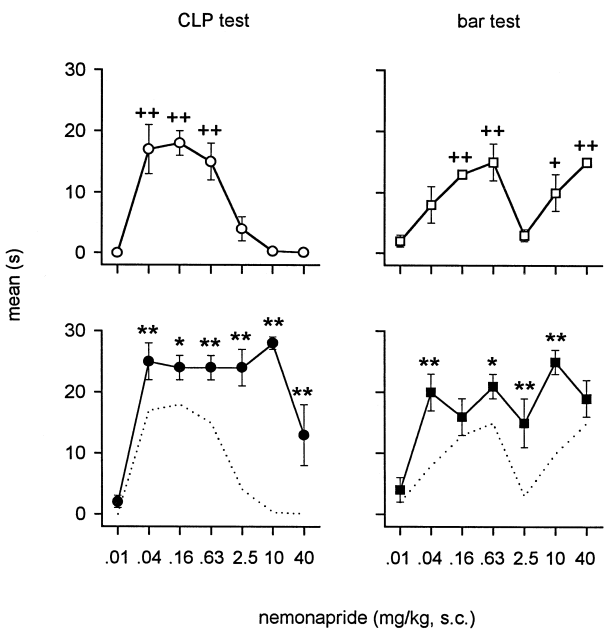

Fig. 1. The effects of the 5-HT_{1A} receptor antagonist WAY 100635 on catalepsy induced by the dopamine D_2-like antagonist/5-HT_{1A} receptor agonist nemonapride. Upper panels: effects of nemonapride alone (open symbols) in the cross-legged position test (left) and the bar test (right). Lower panels: effects of WAY 100635 (0.63 mg/kg) in combination with nemonapride (closed symbols) in the cross-legged position and the bar test. The control group treated with nemonapride alone (upper panels) was replotted as dotted lines. Shown are the mean values ± S.E.M. ($n = 9$/group). + $P < 0.05$, + + $P < 0.01$ compared with animals treated with saline only (mean values ± S.E.M.: 0.0 ± 0.0 s for the cross-legged position test and 0.10 ± 0.10 s for the bar test) based on Dunnett's post-hoc tests with one-tailed probability; * $P < 0.05$, * * $P < 0.01$ compared with animals treated with the corresponding dose of nemonapride (and saline), based on Dunnett's post-hoc tests with one-tailed probability, because we hypothesized that WAY 100635 would enhance nemonapride-induced catalepsy.

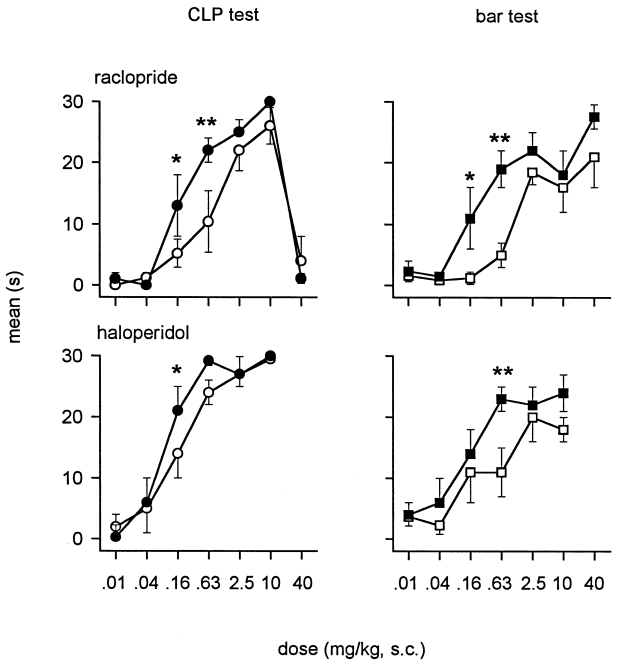

Fig. 2. The effects of the 5-HT_{1A} receptor antagonist WAY 100635 on catalepsy induced by the dopamine D_2-like receptor antagonists raclopride and haloperidol. Values represent the effects of saline (open symbols) or WAY 100635 (0.63 mg/kg; closed symbols) in combination with a neuroleptic in the cross-legged position test (left panels) and the bar test (right panels). Shown are the mean values ± S.E.M. ($n = 7$/group). $*\ P < 0.05$, $**\ P < 0.01$ compared with animals treated with the corresponding dose of the neuroleptic (and saline), based on Dunnett's post-hoc tests with two-tailed probability.

nemonapride (0.04–0.63 mg/kg) and prevented the decrease in catalepsy at higher doses of nemonapride (Fig. 1, lower panels). When administered in combination with saline, WAY 100635 (0.63 or 2.5 mg/kg) did not induce catalepsy (data not shown).

Raclopride dose-dependently increased catalepsy in both tests, although like nemonapride, the highest dose (40 mg/kg) was ineffective in the cross-legged position test (Fig. 2, upper panels). Pretreatment with WAY 100635 enhanced significantly the effects of low doses of raclopride (0.16–0.63 mg/kg), but did not reinstate catalepsy in the cross-legged position test at the highest dose. Haloperidol dose-dependently increased catalepsy in both tests (Fig. 2, lower panels). Pretreatment with WAY 100635 enhanced significantly the effects of intermediate doses of haloperidol (0.16 and 0.63 mg/kg for the cross-legged position and bar test, respectively).

4. Discussion

In this study, the neuroleptic nemonapride induced less catalepsy at higher doses and it was demonstrated that its ability to activate 5-HT_{1A} receptors was responsible for this phenomenon. Thus, these findings suggest that the recently identified 5-HT_{1A} receptor agonist properties of nemonapride (Assié et al., 1997) are involved in its cataleptogenic effects. Additionally, the 5-HT_{1A} receptor antagonist WAY 100635 enhanced the cataleptogenic effects induced by dopamine D_2-like receptor blockade, suggesting that tonic 5-HT_{1A} receptor activation may play a modulatory role in the effects of neuroleptics on catalepsy.

The present results suggest that 5-HT_{1A} receptor agonist properties play a role in the cataleptogenic effects of nemonapride because (1) dose–response functions of nemonapride were not monotonic, i.e., the effects of nemonapride decreased at high or intermediate doses, (2) the descending part of the dose–response functions occurred at doses similar to those shown to have 5-HT_{1A} receptor agonist properties in vivo (Assié et al., 1997), and (3) pretreatment with the 5-HT_{1A} receptor antagonist WAY 100635 reinstated nemonapride-induced catalepsy at higher doses. The latter effect was specific for nemonapride because WAY 100635 did not alter the lack of cataleptic effects of the highest dose of raclopride in the cross-legged position test. Taken together, it can be concluded that the 5-HT_{1A} receptor agonist properties of nemonapride attenuated its ability to induce catalepsy at higher doses.

Another important finding of the present study was that pretreatment with WAY 100635 enhanced catalepsy induced by dopamine D_2-like receptor selective doses of nemonapride (Assié et al., 1997), and by low doses of the dopamine D_2-like receptor antagonists raclopride and haloperidol. The latter finding agrees with preliminary results reported by Bartoszyk et al. (1996) showing that cotreatment with WAY 100635 (0.1 mg/kg, s.c.) slightly, but significantly, enhanced haloperidol (1.0 mg/kg, s.c.)-induced catalepsy. Because in the present study, WAY 100635 alone had no effect on catalepsy, the most likely explanation for its effects on catalepsy induced by dopamine D_2-like receptor antagonists is that it blocked tonic 5-HT_{1A} receptor activation. That is, neuroleptic-induced catalepsy is sensitive to 5-HT_{1A} receptor stimulation (for review, see Wadenberg, 1996) and it is conceivable that tonic 5-HT_{1A} receptor activation (cf. Cadogan et al., 1994; Routledge, 1996) may be sufficient to alter the cataleptogenic effects of neuroleptics. Although it remains to be demonstrated, a possible implication of the present findings is that neuroleptics with additional 5-HT_{1A} receptor antagonist properties may have enhanced extrapyramidal side-effects under some conditions, due to blockade of tonic 5-HT_{1A} receptor activation.

In clinical studies, nemonapride was reported to have therapeutic efficacy in schizophrenia and to produce relatively mild extrapyramidal side-effects (e.g., Kudo et al., 1989; Mori et al., 1989). Because catalepsy is thought to model extrapyramidal side-effects, and because of the present finding that the cataleptogenic effects of nemonapride were attenuated by its 5-HT_{1A} receptor agonist properties, it is conceivable that the 5-HT_{1A} receptor agonist

properties of nemonapride contribute to its relatively low level of extrapyramidal side-effects in the clinic. On the other hand, the finding that the substituted benzamide nemonapride has mild extrapyramidal side-effects does not differentiate it from a number of other substituted benzamides such as sulpiride (e.g., Kudo et al., 1989), which appear to lack 5-HT_{1A} receptor agonist properties (Leysen et al., 1993). Furthermore, the dopamine D_2-like receptor antagonist properties of nemonapride appear at doses approximately 16–64 lower than those having 5-HT_{1A} receptor agonist properties (Assié et al., 1997). Thus, even though the present findings suggest that the 5-HT_{1A} receptor agonist properties of nemonapride may attenuate its ability to induce extrapyramidal side-effects, it is not clear whether the doses used in the reported clinical studies are high enough for its 5-HT_{1A} receptor agonist properties to play an important role. It will be of interest to determine whether or not in vivo 5-HT_{1A} receptor agonist properties are evident at clinically relevant doses of mixed dopamine D_2-like receptor antagonist/5-HT_{1A} receptor agonists. Nonetheless, our results indicate that the cataleptogenic effects of nemonapride are modified as a result of its 5-HT_{1A} receptor activity.

Acknowledgements

The authors would like to thank Martine Alquier and Catherine Grevoz-Barret for their technical assistance and Joël Besnard for assistance with data management and analysis.

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