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Effect of the amisulpride isomers on rat catalepsy

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Abstract

The substituted benzamide amisulpride is currently administered in its racemic form. In the present study, the biochemical and cataleptogenic profiles of the two enantiomers (*R*+ and *S*−) were compared with those of the racemic mixture. Displacement binding studies showed that the (*S*−)-isomer possesses an higher affinity for dopamine D2-like receptor (K_i 5.2 ± 0.4 nM) compared to (*R*+)-amisulpride (K_i 244 ± 12 nM) and to (*RS*)-amisulpride (K_i 9.8 ± 0.8 nM). In contrast, (*S*−)-amisulpride binds the α_2-receptor with an affinity (K_i 1528 ± 45 nM) lower than that of the (*R*+)-isomer (K_i 375 ± 34 nM) and of (*RS*)-amisulpride (K_i 783 ± 27 nM). The *bar test* was used to evaluate the catalepsy induced by each drug. (*RS*)-amisulpride induced catalepsy only at very high doses (> 100 mg/kg, s.c.) whereas, (*S*−)-amisulpride produced a catalepsy at a lower dose (30 mg/kg, s.c.) and (*R*+)-amisulpride did not produce any catalepsy up to the dose of 75 mg/kg. Interestingly, (*R*+)-amisulpride reduced the catalepsy induced by (*S*−)-amisulpride (50 mg/kg, s.c.) or haloperidol (0.3 mg/kg, s.c.), at the doses of 50 or 30 mg/kg, respectively. These results indicate that the weak cataleptic properties of (*RS*)-amisulpride might partially rely on its (*R*+)-isomer and provide a further explanation for the atypical properties of amisulpride as an antipsychotic.

Keywords: Antipsychotic; Benzamide; Extrapyramidal symptom

1. Introduction

Extrapyramidal symptoms, such as parkinsonism, akathisia, and acute dystonia are among the most common side effects of classical antipsychotic treatments. It is generally accepted that extrapyramidal symptoms and antipsychotic action of neuroleptics are due to the postsynaptic dopamine D_2 receptor blockade in the striatum and in the limbic system, respectively (Carlsson and Lindquist, 1963; Waldmeier and Maitre, 1976; Haraguchi et al., 1997). Atypical antipsychotics, such as clozapine, risperidone, olanzapine, show little or no propensity to induce extrapyramidal side effects when compared with conventional antipsychotics. It has been speculated that the atypical properties of these drugs could be related to their biochemical profile in which the dopamine D_2 receptor antagonism is associated with the blockade of other receptor systems, such as 5-HT receptors (5-HT_2) (Leysen et al., 1993, 1998; Meltzer, 1989a) and/or adrenoceptors (α_2) (Kalkman et al., 1998) receptors. Indeed, different authors indicated that a favorable ratio between the affinity for these receptors and the dopamine D_2 receptors antagonism may account for the atypical properties of an antipsychotic (Meltzer et al., 1989b; Kalkman et al., 1998).

Although the serotonergic and adrenergic antagonism could explain, at least in part, the low incidence of extrapyramidal symptoms caused by some atypical antipsychotics, it does not seem to play a role in the mechanism of action of the substitute benzamide class of compounds such as amisulpride. Clinical and behavioral studies indicated that amisulpride gives rise to a low incidence of extrapyramidal side effects in humans (Mann et al., 1984) and produces catalepsy only at very high doses in rats (Perrault et al., 1997). However, in vitro biochemical studies demonstrated

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that amisulpride is a selective antagonist for dopamine D_2 and D_3 receptors with a very weak binding affinity for α_2-adrenoceptor and 5-HT_2 receptors (Schoemaker et al., 1997). A possible explanation for the low incidence of extrapyramidal symptoms induced by amisulpride has therefore been related to its limbic selectivity in different experimental paradigms (Schoemaker et al., 1997; Bischoff, 1992). As reported by Schoemaker et al. (1997), this limbic selectivity might rely on a preferential dopamine D_3 receptor blockade with respect to the dopamine D_2 receptor subtype antagonism, making possible that a moderate dopamine D_2 receptor occupancy insufficient per se to exert extrapyramidal symptoms would concur to the limbic dopamine D_3 receptor blockade in producing the antipsychotic action.

The above mentioned studies were conducted using amisulpride in its racemic form. Recently, the two isomers, (*S*−)- and (*R*+)-amisulpride, that constitute the racemic mixture have been isolated and biochemical studies indicated that (*S*−)-amisulpride bound the dopamine D_2 receptor with higher affinity than racemic-amisulpride, while (*R*+)-amisulpride showed a certain selectivity for the dopamine D_3 receptor.

We have recently demonstrated that (*S*−)-amisulpride is the active isomer of amisulpride with respect to dopamine D_2 receptor affinity, while the (*R*+)-"inactive" isomer has an affinity for dopamine D_3 receptor in the nanomolar range (Castelli et al., 2001). In the present paper, the two isomers were characterized to better understand the phenomenon underlying the low propensity of the racemic form of amisulpride in producing catalepsy in the laboratory animals.

2. Materials and methods

2.1. Drugs and chemicals

Racemic (*RS*)-amisulpride hydrochloride and its isomeric forms: (*S*−)-amisulpride tartrate and (*R*+)-amisulpride phosphate were kindly supplied by Sanofi-Synthélabo (Bagneaux, France). Clozapine and haloperidol hydrochloride were purchased from Tocris (Avonmouth, Bristoll, UK) and risperidone from Sigma (St. Louis, MO, USA). [^{3}H]YM-09151-2 (85 Ci/mmol), [^{3}H]ketanserin (65.3 Ci/mmol) and [^{3}H]clonidine (74 Ci/mmol) were from NEN Life Science Products (Boston, MA, USA).

2.2. Animals

Male Sprague–Dawley albino rats (Charles River, Como, Italy) weighting 225–250 g were kept on a 12/12-h dark/light cycle with food and water available ad libitum. All experimental protocols were accepted by the Ethical Committee at the University of Cagliari and performed in strict accordance with the EC regulation for care and use of experimental animals (EEC No. 86/609).

2.3. Receptor binding studies

2.3.1. [^{3}H]YM-09151 (dopamine D2-like receptor antagonist) binding

Rats were killed by decapitation and striatum was rapidly dissected and homogenized in 100 volumes (w/v) of ice-cold 50 mM Tris–HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM EDTA and 5.7 mM ascorbic acid, pH 7.4, using a Polytron apparatus. The homogenates were centrifuged at 48,000 × *g* for 20 min at 4 °C and the resultant pellets suspended and centrifuged two more times at 48,000 × *g* for 20 min at 4 °C. The final pellets were suspended in 100 volumes (w/v) of the same buffer and 200 μl of striatal membrane homogenate (50–75 μg protein) was added to 2800 μl of the incubation medium containing 50 pM of [^{3}H]YM-09151 and different concentrations of the drugs tested (12 concentrations for each drug, ranging from 0.05 nM to 10 μM). These conditions were enough to avoid a ligand depletion higher than 10%. Non-specific binding was determined by adding (*S*−)-sulpiride (10 μM). After 60-min incubation at 24 °C in the dark, samples were filtered through Whatman GF/B filters using a Brandel 96-sample harvester apparatus (Brandel, Gaithersburg, MD, USA). Filters were rinsed four times with 4 ml of ice cold Tris–HCl buffer, pH 7.4. Radioactivity was measured in a liquid scintillation counter (Tricarb 2100, Packard, Meridien, USA) using 3 ml of scintillation fluid (Ultima Gold MV, Packard).

2.3.2. [^{3}H]Ketanserin (5-HT_{2A} receptor antagonist) binding

Cortex was homogenized in 40 volumes (w/v) of ice cold Tris–HCl buffer (50 mM, pH 7.7) and centrifuged at 48,000 × *g* for 20 min at 4 °C. Pellets were washed twice by resuspension in the same buffer followed by centrifugation at 48,000 × *g* for 20 min at 4 °C. The final pellets were then stored at − 80 °C for a period no longer than 1 week.

When needed, pellets were suspended in 40 volumes of ice-cold Tris–HCl buffer and the membranes were incubated in the presence of [^{3}H]ketanserin (0.5 nM) for 15 min at 37 °C. In competition binding studies, different concentrations of each drug tested were used (12 concentrations ranging from 0.01 nM to 10 μM). Non-specific binding was assessed by adding 10 μM of methysergide. The assay was stopped by adding 4 ml of cold buffer followed by a rapid filtration through Whatman GF/B filters. The filters were then washed twice with 4 ml of ice-cold buffer and the entrapped radioactivity was counted by a liquid scintillation counter, as described above.

2.3.3. [^{3}H]Clonidine (α_2-adrenoceptor agonist) binding

Rat cortex was rapidly dissected and homogenized in 10 volumes (w/v) of sucrose (0.32 M in 50 mM Tris–HCl, pH 7.4 at 4 °C) using a Teflon-glass homogenizer. The homogenate was then centrifuged at 1000 × *g* for 10 min, at 4 °C. The resulting supernatants were pooled and centrifuged at 31,000 × *g* for 20 min, at 4 °C. The supernatants were

Table 1
K_i of different antipsychotics on [^{3}H]YM-09151 (D_2), [^{3}H]ketanserin (5-HT_{2A}) and [^{3}H]clonidine (α_2) binding

Drug	(Rat striatum), D_2, K_i (nM) ± S.E.M.	(Rat cortex), 5-HT_{2A}, K_i (nM) ± S.E.M.	(Rat cortex), α_2, K_i (nM) ± S.E.M.
(*RS*)-amisulpride	9.8 ± 0.4	>5000	783 ± 27
(*S*−)-amisulpride	5.2 ± 0.1[a]	>5000	1528 ± 45[a]
(*R*+)-amisulpride	244 ± 12[b]	>5000	375 ± 14[a]
Haloperidol	1.11 ± 0.04[b]	42.2 ± 0.81	>5000
Risperidone	3.5 ± 0.6[a]	0.6 ± 0.01	16 ± 3[b]

K_i of the different forms of amisulpride and haloperidol, risperidone and clozapine for different [^{3}H] ligands. Data represent mean (± S.E.M.) of four independent experiments. Statistical differences vs. (*RS*)-amisulpride were calculated using one-way ANOVA followed by Neumann–Keuls test for multiple comparison ([a]$P<0.05$ or [b]$P<0.01$ vs. (*RS*)-amisulpride).

discarded and each pellet resuspended in 10 volumes (w/v) of assay buffer (50 mM Tris–HCl, 2 mM $MgCl_2$, 250 μM ascorbic acid, pH 7.4). [^{3}H]clonidine binding was measured at equilibrium in 1 ml aliquots containing cortex membranes, which were incubated for 60 min at 25 °C in presence of 2 nM of [^{3}H]clonidine and different concentrations of the drugs tested (12 concentrations for each drug ranging from 1 nM to 10 μM). Norepinephrine (50 μM) was used to define the non-specific binding, considering its low affinity for imidazoline receptor. After 60 min of incubation at 25 °C, membranes were filtered using Whatman GF/B filters and washed four times with 4 ml of ice cold incubation buffer. The radioactivity was measured in 4 ml of scintillation fluid using a liquid scintillation counter, as described above.

All the experiments, independently from the radioligand used, were performed in triplicate and each result expressed as mean ± S.E.M of three independent experiments. Protein content was determined using the Bio-Rad Dc Kit (Bio-Rad Laboratories, Munich, Germany) and following manufacturer's instructions.

2.4. Bar test

Catalepsy, defined as the acceptance and retention of abnormal posture was assessed by the method of the *bar test*. Briefly, the forepaws of the rat (8 to 12 rats for each group) were placed on a 9-cm-high bar and the length of time during which the animal retained this position was recorded each 60 min after treatment and for a maximum of 6 h, by an observer blind to the treatment. The longest latency time of three consecutive trials was recorded. Rats

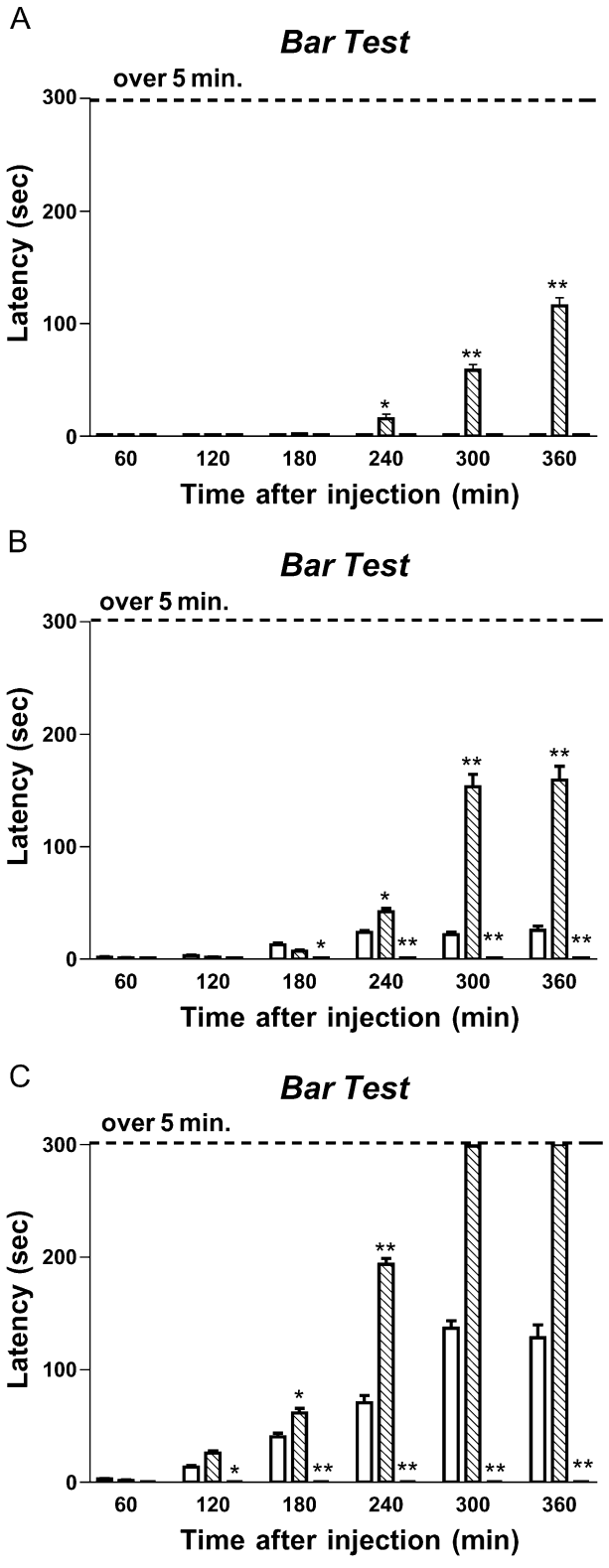

Fig. 1. Cataleptic effect of different doses of (*RS*)-, (*S*−)- and (*R*+)-amisulpride, determined during 360- at 60-min intervals. (A) Bars showing the different latency on *bar test* of rats treated with (*RS*)- (60 mg/kg, s.c.), (*S*−)- (30 mg/kg, s.c.) and (*R*+)-amisulpride (30 mg/kg, s.c.). (B) Rat catalepsy induced by (*RS*)- (100 mg/kg, s.c.), (*S*−)- (50 mg/kg, s.c.) and (*R*+)-amisulpride (50 mg/kg, s.c.). (C) Rat catalepsy induced by (*RS*)- (150 mg/kg, s.c.), (*S*−)- (75 mg/kg, s.c.) and (*R*+)-amisulpride (75 mg/kg, s.c.). Data represent mean values (± S.E.M.) of eight rats. Key to symbols: ▭ (*RS*)-amisulpride, ▨ (*S*−)-amisulpride, ▩ (*R*+)-amisulpride. Statistical differences vs. (*RS*)-amisulpride treated rats were calculated using two-way ANOVA followed by Neumann–Keuls test for multiple comparison (* $P<0.05$ and ** $P<0.01$ vs. (*RS*)-amisulpride treated rats).

were removed from the bar if their latency on *bar test* exceeded 300 s, (i.e. cut-off point of observation = 300 s).

Rats were treated with the different drugs, dissolved in 25 μl glacial acetic acid and tamponated (pH 7.2) using a solution 0.1 M of sodium bicarbonate in distilled water NaCl 0.9% and administered s.c., 60 min prior to testing. When the (*R*+)-amisulpride was administered in combination with (*S* −)-amisulpride or haloperidol, (*R*+)-amisulpride injections were carried out 15 min prior to (*S* −)-amisulpride administration or 60 min before testing (240 min after (*S* −)-amisulpride or 60 min after haloperidol administration). All the doses were referred to the free base form of the drugs.

2.5. Statistics

Analyses of saturation and competition curves for biochemical parameters, as well as the fitting of data to the appropriate binding model were performed by using the Kell 6.0 computer program (Biosoft, Cambridge, UK). Statistical analyses were performed using one-way or two-ways analysis of variance (ANOVA), when significance was found the Neumann–Keuls post-hoc test was applied and the level of significance was set at $P < 0.05$.

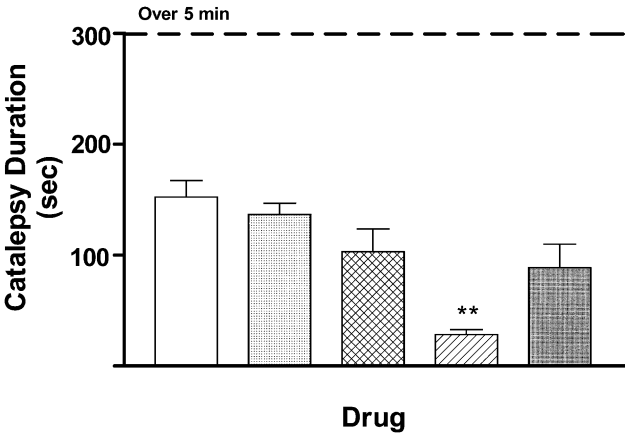

Fig. 3. Effect of different doses (*R*+)-amisulpride on (*S*−)-amisulpride (50 mg/kg, s.c.) induced catalepsy. *Bar test* scores were evaluated 300 min after (*S*−)-amisulpride injection. (*R*+)-amisulpride or vehicle was administered 60 min prior observation. Data represent mean values (± S.E.M.) of 12 rats. Statistical differences vs. (*S* −)-amisulpride plus vehicle treated rats were calculated using one-way ANOVA followed by Neumann–Keuls test for multiple comparison (** $P < 0.01$ vs. (*S*)-amisulpride plus vehicle treated rats). Key to symbol: (*S* −)-amisulpride plus vehicle ▭, (*R*+)-amisulpride (10 mg/kg, s.c.) ⬚, (*R*+)-amisulpride (30 mg/kg, s.c.) ▨, (*R*+)-amisulpride (50 mg/kg, s.c.) ▨, (*R*+)-amisulpride (75 mg/kg, s.c.) ▨.

3. Results

3.1. Receptor binding studies

As shown in Table 1, (*S* −)-amisulpride binds to striatal dopamine D_2 receptor with an affinity two times higher than the racemic form while (*R*+)-amisulpride has only a weak binding affinity for this receptor. (*S* −)-amisulpride showed an affinity for the dopamine D_2 receptor that was similar to that of risperidone and higher than that of clozapine. Very low affinities of the different forms of amisulpride were observed for 5-HT_{2A} receptor. (*R*+)-amisulpride showed a higher affinity for α_2-adrenoceptor with respect to (*S* −)- and (*RS*)-amisulpride.

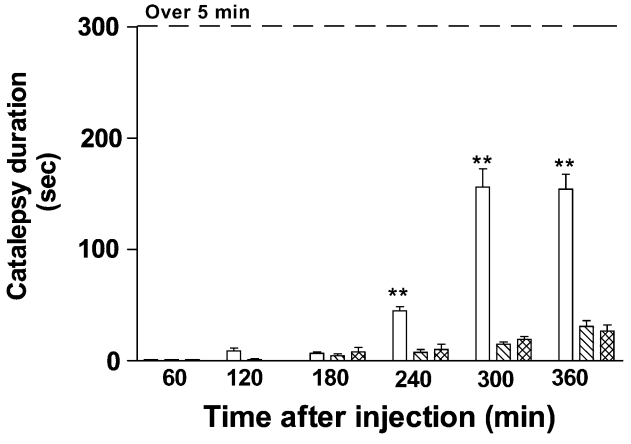

Fig. 2. Comparative effect of (*S* −)-amisulpride ▭ (50 mg/kg, s.c.), (*RS*)-amisulpride ▨ (100 mg/kg, s.c.) and co-administration of (*R*+) and (*S* −)-amisulpride (50 mg/kg, s.c.) ▨ on rat catalepsy. (*R*+)-amisulpride was administered 15 min prior (*S* −)-amisulpride injection. Bars represent mean values (± S.E.M.) of eight rats. Statistical differences vs. (*RS*)-amisulpride treated rats were calculated using two-way ANOVA followed by Neumann–Keuls test for multiple comparison (** $P < 0.01$ vs. (*RS*)-amisulpride treated rats).

3.2. Bar test

As shown in Fig. 1A, B, C, increasing amounts of racemic (*RS*)-amisulpride induced catalepsy only at very high doses (>100 mg/kg, s.c.). The maximal latency time in the *bar test* was recorded after 300 min from the injection and it remained constant for at least 1 h. When the catalepsy induced by different doses of (*RS*)-amisulpride was compared with respect to that obtained after equivalent doses of (*S* −)- and (*R*+)-amisulpride (Fig. 1A, B, C), the *bar test* latency was significantly different. (Two-way ANOVA in the different comparison groups: (*RS* −)-amisulpride 60 mg/kg and (*S* −)- and (*R*+)-amisulpride 30 mg/kg $F_{drug}(2, 21) = 363$, $P < 0.001$; (*RS* −)-amisulpride 100 mg/kg and (*S* −)- and (*R*+)-amisulpride 50 mg/kg $F_{drug}(2, 21) = 1303$, $P < 0.001$; (*RS* −)-amisulpride 150 mg/kg and (*S* −)- and (*R*+)-amisulpride 75 mg/kg $F_{drug}(2, 21) = 1821$, $P < 0.001$.) Rats treated with (*RS* −)-amisulpride (60 mg/kg) did not display any catalepsy, while an intense rat catalepsy was produced by (*S* −)-amisulpride (30 mg/kg) injection (Fig. 1A) ($P < 0.01$, after 5 h from drug administration).

Moreover, (*RS*)-amisulpride (100 and 150 mg/kg) induced a lower level of catalepsy when compared with equivalent doses of (*S*−)-amisulpride (50 and 75 mg/kg, respectively) (Fig. 1B and C) (for both comparisons, $P<0.01$ after 5 h from drug administration). (*R*+)-amisulpride did not produce catalepsy up the dose of 75 mg/kg (Fig. 1A, B, C).

As shown in Fig. 2, co-administration of (*R*+)- and (*S*−)-amisulpride (both 50 mg/kg) exerted a catalepsy similar to that of (*RS*)-amisulpride (100 mg/kg). Moreover, administration of different doses of (*R*+)-amisulpride significantly altered (*S*−)-amisulpride (50 mg/kg) induced catalepsy (one-way ANOVA: $F(4, 55)=8.083$, $P<0.01$) with a significant reduction after injection with 50 mg/kg of (*R*+)-amisulpride ($P<0.01$) (Fig. 3). However, the dose of 75 mg/kg of (*R*+)-amisulpride showed an anti-cataleptic action lower than 50 mg/kg, on (*S*−)-amisulpride (50 mg/kg) induced catalepsy (Fig. 3). Moreover, (*R*+)-amisulpride significantly altered (one-way ANOVA: $F(4, 55)=3.883$, $P<0.01$) the catalepsy induced by 0.3 mg/kg of haloperidol (Fig. 4), with a reduction of the rat latency on the *bar test* after administration of (*R*+)-amisulpride (30 mg/kg) ($P<0.01$). (*R*+)-amisulpride at the dose of 50 mg/kg did not affect the haloperidol-induced catalepsy (Fig. 4). Interestingly, the maximum effect of (*R*+)-amisulpride in reducing haloperidol induced catalepsy was reached 60 min after administration (data not shown), while (*S*−)-amisulpride induced the maximal cataleptogenic effect after 4 h from injection (Fig. 1A, B, C).

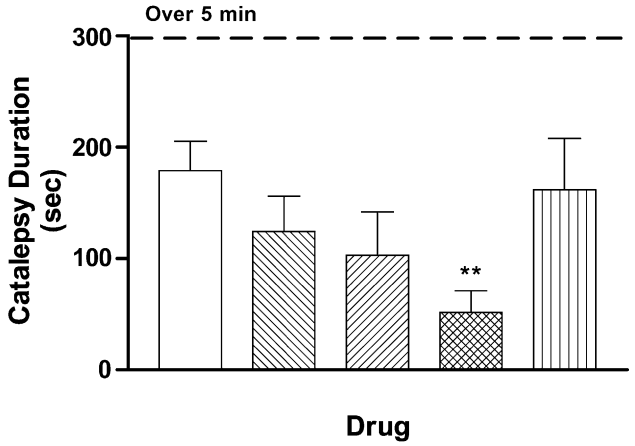

Fig. 4. Effect of different doses of (*R*+)-amisulpride on rat catalepsy induced by haloperidol (0.3 mg/kg, s.c.). Catalepsy observations were carried out 120 min after haloperidol injection and 60 min after administration of (*R*+)-amisulpride. Key to symbols: Haloperidol (0.3 mg/kg, s.c.) plus vehicle ▭, (*R*+)-amisulpride 5 mg/kg ▨ (*R*+)-amisulpride 15 mg/kg ▨, (*R*+)-amisulpride 30 mg/kg ▨, (*R*+)-amisulpride 50 mg/kg ▮. Data represent mean values (± S.E.M.) of 12 rats. Significant differences were obtained using one-way ANOVA followed by Neumann–Keuls test for multiple comparisons (** $P<0.01$ vs. haloperidol plus vehicle treated rats).

4. Discussion

The present results confirmed the low propensity of (*RS*)-amisulpride in producing catalepsy. As already observed (Perrault et al., 1997), catalepsy induced by (*RS*)-amisulpride occurred in rat only at very high doses (>100 mg/kg, s.c.) and after several hours from injection (3–4 h). The comparative study between (*S*−)- and (*RS*)-amisulpride on rat catalepsy indicated a peculiar characteristic of amisulpride, in which equivalent doses of the racemic and of the (*S*−)-isomer forms produced different effects. Indeed, administration of (*S*−)-amisulpride at the dose of 30 mg/kg induced catalepsy while no effect was observed with (*RS*)-amisulpride at the dose of 60 mg/kg. Moreover, higher doses of (*S*−)-amisulpride (50–75 mg/kg) induced a much stronger cataleptogenic effect than equivalent doses of (*RS*)-amisulpride (100–150 mg/kg). The comparative study of the in vitro affinity of (*S*−)- and (*RS*)-amisulpride for the dopamine D_2 receptor indicated that the binding properties of (*RS*)-amisulpride were basically due to the amount of (*S*−)-amisulpride contained in the mixture, since (*R*+)-amisulpride displayed only a weak binding for this receptor. Then, it appeared reasonable not to consider relevant a different dopamine D_2 receptor occupancy in vivo between (*S*−)- and (*RS*)-amisulpride, related to the affinity for the dopamine D_2 receptor. Moreover, the time courses of the catalepsy induced by (*S*−)- and (*RS*)-amisulpride did show a strong temporal correlation between the two amisulpride forms, suggesting an equal propensity of both compounds to cross the blood–brain barrier. However, the possibility remains that the (*R*+)-component of (*RS*)-amisulpride could compete with the (*S*−)-isomer and interfere with the crossing of the blood–brain barrier. However, we showed that the catalepsy induced by (*S*−)-amisulpride was antagonized by previously administered (*R*+)-amisulpride. This effect seemed to be due to specific anti-cataleptic properties of the (*R*+)-isomer. Indeed, (*R*+)-amisulpride was not only able to reduce the catalepsy induced by (*S*−)-amisulpride but it exerted the same action on haloperidol induced catalepsy as well. Then, it is feasible that the (*R*+)-amisulpride contained in the racemic mixture might be responsible for the weaker catalepsy observed after the administration of (*RS*)-amisulpride when compared to the (*S*−)-isomer. The effect of increasing doses of (*R*+)-amisulpride on the catalepsy induced either by (*S*−)-amisulpride or by haloperidol showed a U-shaped curve, suggesting the possibility that (*R*+)-amisulpride might act as a partial-agonist at level of dopamine D_2 receptor. By this mean, only at certain doses, (*R*+)-amisulpride could potentiate the effect of dopamine in counteracting the antagonistic action of (*S*−)-amisulpride and haloperidol on striatal dopamine D_2 receptors, decreasing catalepsy. Interestingly, haloperidol (0.3 mg/kg)-mediated catalepsy was reduced by lower doses of (*R*+)-amisulpride than that needed to antagonize the catalepsy induced by (*S*−)-amisulpride (50 mg/kg), probably depending on a different interaction of (*R*+)-amisulpride with the D_2 occupancy and dopamine level produced by the

two neuroleptics at those doses. An adjunctive hypothesis, that could explain the anti-cataleptic properties of (*R*+)-amisulpride, might rely on its α_2-adrenoceptor antagonism. Indeed, pre-treatment with the α_2-adrenoceptor antagonist yohimbine (1.25 to 10 mg/kg) reduced the catalepsy induced by haloperidol (Bende et al., 1990; Kalkman et al., 1998) and by (*S* −)-amisulpride (50 mg/kg) (data not shown). In vitro binding studies conducted in our laboratories, showed a weak affinity of (*R*+)-amisulpride for α_2-adrenoceptor that was about half of that of (*RS*)-amisulpride and similar to that of clozapine. However, it should be remarked that the (*R*+)-amisulpride prevented haloperidol-induced catalepsy only at high doses (15–30 mg/kg) while even low doses of yohimbine were able to produce an anti-cataleptic action (Bende et al., 1990). Considering the high doses needed to observe the anti-cataleptic properties of (*R*+)-amisulpride, this would suggest that α_2-blockade might occur also in vivo.

The unexpected characteristic of (*R*+)-amisulpride might imply that such otherwise unspecific isomer might possess additional behavioral effects useful to understand the atypical and/or antidepressant profile of its parent compound.

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