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Short communication

Effect of the amisulpride isomers on rat prolactinemia

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Abstract

The effects on rat serum prolactin level of the two isomers constituting the racemic form of amisulpride were compared. (*S*−)-amisulpride induced hyperprolactinemia at lower doses ($ED_{50} = 0.09 \pm 0.01$ mg/kg) than racemic- ($ED_{50} = 0.24 \pm 0.03$ mg/kg) and (*R*+)-amisulpride ($ED_{50} = 4.13 \pm 0.05$ mg/kg), in accord with their affinities for pituitary dopamine D_2 receptor ($K_i = 3.8 \pm 0.2$, 6.4 ± 0.2 and 143.3 ± 2.3 nM, respectively). At doses twice the ED_{50}, (*S*−)-amisulpride produced a maximal increase in prolactin level similar to that of the racemic form ($403 \pm 21\%$ and $425 \pm 15\%$, respectively), but higher than that of (*R*+)-amisulpride ($198 \pm 8\%$). These results suggest that the hyperprolactinemia induced by the racemic-amisulpride is mostly due to its (*S*−)-isomer.

Keywords: Antipsychotic; Benzamide; Prolactin

1. Introduction

The substituted benzamide amisulpride is a selective dopamine D_2/D_3 receptor antagonist currently used in the treatment of different mental disorders. Clinical studies indicated that amisulpride, at high doses, is efficacious in reducing the positive symptoms of schizophrenia (Delker et al., 1990), while at low doses, it is successfully employed in the treatment of dysthymia and of the negative symptoms of schizophrenia (Boyer et al., 1995; Lecrubier et al., 1997). Although clinical evidence indicated that amisulpride shared some common characteristics with other atypical antipsychotics, it was shown that, similarly to haloperidol, amisulpride even when administered at low doses causes hyperprolactinemia in patients (Wetzel et al., 1994). This side effect was associated to the blockade of dopamine D_2 receptors at the pituitary level (Wetzel et al., 1994; Grunder et al., 1999).

The pharmaceutical form of amisulpride currently available is constituted by a racemic mixture made by equivalent amounts of two enantiomers (*R*+ and *S*−). Recently, the two isomers have been isolated and biochemical studies, carried out in our laboratory on transfected cell membranes, indicated that (*S*−)-amisulpride binds to the dopamine D_2 receptor with higher affinity than racemic-amisulpride, while the (*R*+)-isomer showed a weak affinity for the dopamine D_2 receptor and a selectivity for the dopamine D_3 receptor (Castelli et al., 2001). Subsequently, we found that high doses of (*S*−)-amisulpride induced catalepsy in rat, while (*R*+)-amisulpride did not produce motor impairment and reduced the (*S*−)-amisulpride-induced catalepsy, suggesting that the (*R*+)-isomer was not devoid of an intrinsic pharmacological activity (Marchese et al., 2002). In the present study, we wanted to investigate whether, in the rat, the effects on serum prolactin level induced by the two amisulpride isomers and by the racemic form were in direct relation with their affinities for the dopamine D_2 receptor of the pituitary gland.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley albino young adult rats (Charles River, Calco, Italy) weighting 100–125 g were kept on a

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12:12-h dark/light cycle (7:00 a.m./7:00 p.m.) with food and tap water available ad libitum. All experimental protocols were approved by the Ethical Committee at the University of Cagliari and performed in strict accordance with the E.C. regulation for care and use of experimental animals (EEC No. 86/609).

2.2. Drugs and chemicals

Racemic-amisulpride hydrochloride and its isomeric forms—(*S* −)-amisulpride tartrate and (*R*+)-amisulpride phosphate—were kindly supplied by Sanofi~Synthélabo (Bagneaux, France). [^{3}H]YM-09151-2 (specific activity 85 Ci/mmol) was from NEN Life Science Product (Boston, MA, USA).

2.3. [^{3}H]YM-09151-2 homogenate binding

Rats were killed by decapitation, pituitaries were rapidly dissected and homogenized in 100 volumes of ice cold 50 mM Tris–HCl buffer, 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 resuspended and centrifuged two more times at 48,000 × *g* for 20 min at 4 °C. The final pellets were suspended in 70 volumes of ice cold Tris–HCl buffer containing the 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM EDTA and 5.7 mM ascorbic acid, pH 7.4.

[^{3}H]YM-09151-2 binding was determined by the method developed by Niznik et al. (1985). Briefly, 200 μl (100–150 μg protein depending on the experiment) of homogenated pituitary membranes was added to the incubation medium containing 25 pM [^{3}H]YM-09151-2 and different concentrations of the compounds tested (8–10 concentrations for each compound). Non-specific binding was determined in presence of (*S* −)-sulpiride (10 μM). After 60 min incubation at 25 °C in the dark, samples were filtered through Whatman GF-B filter 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 4 ml of scintillation fluid (Ultima Gold MV, Packard). Saturation and competition curves were analyzed using a computer program (Kell 6.0, Biosoft, U.K.). All the experiments were performed in triplicate and each result expressed as mean ± S.E.M of five independent experiments. Protein content was determined using the Bio-Rad Dc Kit (Bio-Rad Laboratories, Munich, Germany) and following manufacturer's instructions.

2.4. Serum prolactin determination

Rats (8–10 for each group) were treated (s.c.) with vehicle or different doses of the amisulpride forms, dissolved in 25 μl glacial acetic acid and tamponated (pH 6.4) using a solution 0.1 M of sodium bicarbonate in distilled water (vehicle solution). After 30 min from injection, the rats were decapitated and ~ 800 μl of blood collected. Blood was stored at 4 °C for a maximum of 30 min and serum separated after centrifugation at 3000 × *g* and 4 °C for 8 min. To avoid stress, rats were handled and habituated to environment for three consecutive days before decapitation.

Serum prolactin levels were measured using a rat prolactin radioimmunoassay kit (Biocode, Liege, Belgium) and following manufacturer's instructions. The detection limit of the rat prolactin radioimmunoassay was 0.5 ng/ml and the intra-assay coefficient of variation was less than 5%.

Results were expressed as ng/ml or as a percent vs. vehicle-treated rats. The experimental procedure was repeated three times for ED_{50} (mean ± S.E.M.) determination and statistical analyses.

2.5. Statistical analysis

The statistical significance of the effect of any compound was evaluated by one-way analysis of variance (ANOVA). When a significant ($P < 0.05$) interaction was demonstrated, the Newman–Keuls post hoc test was used to compare the effect of the different drugs.

3. Results

3.1. [^{3}H]YM-09151-2 homogenate binding

The binding of [^{3}H]YM-09151-2 to pituitary membranes was clearly saturable and the specific binding represented the 95% of total binding at half the maximum specific binding while the ligand depletion was lower than 10%. Scatchard plots of the data from the saturation experiments could be fitted to a single site model with a K_d of 23.5 ± 0.3

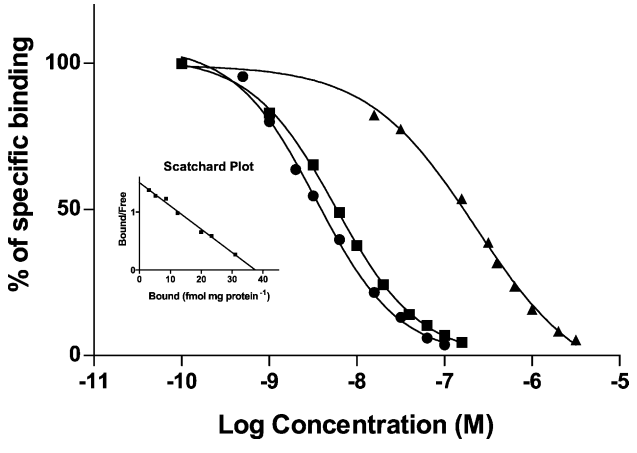

Fig. 1. Inhibition of [^{3}H]YM-09151-2 binding to rat pituitary membranes by racemic-amisulpride (■), (*S* −)-amisulpride (●) or (*R*+)-amisulpride (▲). Data are from one competition experiment out of five and are expressed as the percentage of binding in the absence of competitor. Inset shows the Scatchard plot form one of three independent saturation experiments.

pM and a B_{max} of 38.3 ± 5.1 fmol/mg protein (Hill coefficient 0.997 ± 0.08). The comparative study of the K_i of the amisulpride forms obtained from the competition curves (Fig. 1) indicated a significant difference between groups (one-way ANOVA $F(2,12) = 76.4$, $P < 0.01$) and showed that racemic-amisulpride ($K_i = 6.4 \pm 0.2$ nM) bound pituitary D_2 receptors with higher affinity than (*R*+)-amisulpride ($K_i = 143.3 \pm 2.3$ nM) ($P < 0.01$) but lower than (*S* −)-amisulpride ($K_i = 3.8 \pm 0.2$ nM) ($P < 0.05$).

3.2. Serum prolactin levels

The basal level of rat serum prolactin was 9.8 ± 0.5 ng/ml, in accord to what was indicated and obtained using the standard rat serum provided by the radioimmunoassay kit and indicating that rat stress condition was avoided, as confirmed by the low variability of the serum blood levels observed in vehicle-treated rats. The comparative study of the ED_{50} for the different amisulpride forms showed a significant difference between groups (one-way ANOVA $F(2,6) = 349$, $P < 0.01$) and indicated that racemic-amisulpride ($ED_{50} = 0.24 \pm 0.03$ mg/kg) induced rat hyperprolactinemia at doses significantly lower than (*R*+)-amisulpride ($ED_{50} = 4.13 \pm 0.05$ mg/kg) ($P < 0.01$) but higher than (*S* −)-amisulpride ($ED_{50} = 0.09 \pm 0.01$ mg/kg) ($P < 0.05$). As shown in Fig. 2, at doses twice the ED_{50}, racemic-amisulpride induced a maximal increase of prolactin level vs. vehicle-treated rats similar to that of the (*S* −)-amisulpride (425 ± 15% and 403 ± 21%, respectively), but higher

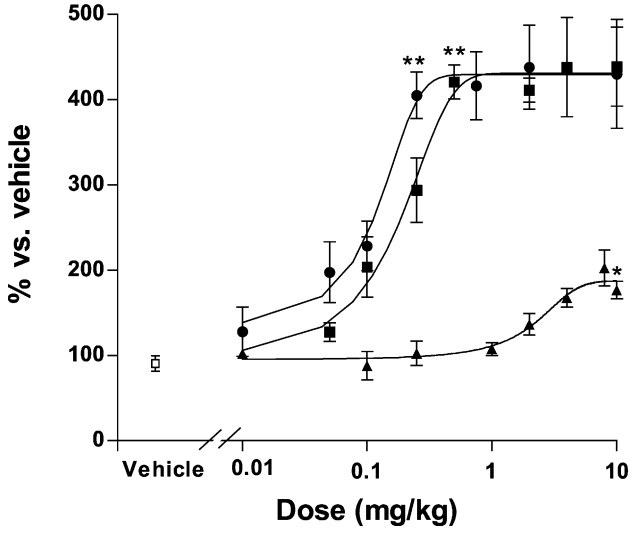

Fig. 2. Effect of different doses of racemic-amisulpride (■), (*S* −)-amisulpride (●) or (*R*+)-amisulpride (▲) on rat serum prolactin level. Data are expressed as percentage of serum prolactin level with respect to vehicle-treated rats (□) (9.8 ± 0.5 ng/ml). The graph is representative of one of three independent experiments, each point represents the mean ± S.E.M. of ten rats. Significant differences of racemic-amisulpride (0.5 mg/kg), (*S* −)-amisulpride (0.2 mg/kg) and (*R*+)-amisulpride (10 mg/kg) vs. vehicle-treated rats were obtained using one-way ANOVA followed by Newmann–Keuls test for multiple comparisons (* $P < 0.05$ and ** $P < 0.01$ vs. vehicle-treated rats).

($P < 0.01$) than that of (*R*+)-amisulpride (198 ± 8%) (one-way ANOVA $F(2,6) = 169$, $P < 0.01$).

4. Discussion

The present results showed that racemic- and (*S* −)-amisulpride have a very high propensity to induce hyperprolactinemia in rats. The increase of the rat serum prolactin levels was dose-dependent in a range of doses (0.1–1 mg/kg) that may be related to the antidepressant effect in humans (30–100 mg/day) (Perrault et al., 1997). Consistently, this preclinical finding well correlates to what has been reported in humans, where, even at the low doses employed in the treatment of dysthymia, the racemic form of this substituted benzamide produces a high surge in prolactinemia (Wetzel et al., 1994). Interestingly, a high frequency of at least one adverse event of the endocrine type is often reported in the clinical trial with amisulpride (Lecrubier et al., 1997; Ravizza, 1999) and mostly in dysthymic patient (~ 17%) (Lecrubier et al., 1997; Ravizza, 1999) with respect to patients affected by schizophrenia (~ 5%) (Wetzel et al., 1998; Peuskens et al., 1999), this in spite of the fact that a much higher dose is used in the latter group. This apparent contradiction may be explained by the fact that studies in dysthymia are performed in population of patients with a higher female to male ratio (mean 62% vs. 46%) with respect to individuals affected with schizophrenia. Female subjects are more sensitive to the endocrine effects of amisulpride with respect to male individuals (Grunder et al., 1999). In our study, we found that high doses of racemic-amisulpride (8–10 mg/kg), corresponding to antipsychotic doses in humans, produced a surge of prolactinemia.

The present results on the serum prolactin levels following treatment with the two amisulpride enantiomers showed that the (*S* −)-isoform of amisulpride increased serum prolactin level at lower doses than (*R*+)-amisulpride. Moreover, (*S* −)-amisulpride induced an increase of serum prolactin level at doses significantly lower (about two times) than the racemic form, indicating that the hyperprolactinemia induced by the racemic-amisulpride should be mostly attributed to the amount of (*S* −)-amisulpride contained in the mixture.

Although racemic- and (*S* −)-amisulpride do not discriminate between dopamine D_2 and D_3 receptor subtypes (Castelli et al., 2001), the effect on prolactin secretion is likely mediated only by the blockade of dopamine D_2 receptors, since the effect induced by amisulpride on rat prolactinemia is mimicked by other dopamine D_2 receptor antagonists (Kakigi et al., 1992) but not by dopamine D_3 receptor antagonists (Reavill et al., 2000). In line with this hypothesis, we found that the rank order of potency in inducing hyperprolactinemia for the different forms of amisulpride well fitted with the data obtained from in vitro studies, showing that racemic-amisulpride binds to

the pituitary dopamine D_2 receptor with an affinity about two times lower than that of (*S* −)-amisulpride and more than 10 times higher with respect to that of (*R*+)-amisulpride.

Interestingly, (*R*+)-amisulpride induced only a one fold maximal increase of serum prolactin level when compared to control animals, suggesting that (*R*+)-amisulpride possesses weak antagonistic properties at the dopamine D_2 receptor level in vivo. Studies carried out using the racemic form of amisulpride showed that the mixture acts as full antagonist (Schoemaker et al., 1997), suggesting that the two isomers that constitute the racemate might be antagonists as well. However, the possibility that the (*R*+)-isomer may act as a partial or inverse agonist cannot be excluded.

Other atypical antipsychotics showing weak affinity for the dopamine D_2 receptor, such as clozapine and olanzapine, have low propensity to induce hyperprolactinemia (Markianos et al., 2001). However, clozapine and olanzapine possess an antagonistic activity at other receptors binding sites (i.e. serotonergic 5-$HT_{2A/2C}$ receptor and α_2 adrenoceptor), which, when stimulated, have been hypothesized to increase prolactin levels (Albinsson et al., 1994; Kapoor et al., 1993). Considering the doses of (*R*+)-amisulpride administered, an involvement of other receptors different from those of the dopaminergic system cannot be excluded (Marchese et al., 2002).

In conclusion, the present results indicated that the hyperprolactinemia induced by racemic-amisulpride was mainly due to its (*S* −)-isomer and that, in vivo, (*S* −)-amisulpride might be the active isomer of the racemic mixture at the pituitary dopamine D_2 receptor level. On the other hand, the (*R*+)-isomer does not seem to be completely inactive with respect to prolactin induction, suggesting that further investigations are needed in order to complete the understanding of its full pharmacological profile.

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