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Dose determination of haloperidol, risperidone and olanzapine using an in vivo dopamine D_2-receptor occupancy method in the rat

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Abstract

The purpose of the present study was to determine antipsychotic doses that achieve 80% striatal dopamine D_2-receptor occupancy for haloperidol, risperidone and olanzapine in rats. Wistar rats were treated with normal saline vehicle (controls), haloperidol (0.25 and 0.5 mg/kg/day), risperidone (3, 5 and 6 mg/kg/day) and olanzapine (5 and 10 mg/kg/day) for 7 days via osmotic minipumps. Striatal and cerebellar tissue were collected and in vivo dopamine D_2-receptor occupancies were determined using ^{3}H-raclopride. The doses required to achieve dopamine D_2-receptor occupancy of 80% in 11- and 24-week old rats were: haloperidol 0.25 mg/kg/day, risperidone 5 mg/kg/day and olanzapine 10 mg/kg/day.

Keywords: Dopamine D_2-receptor occupancy; In vivo; Raclopride; Dosage; Olanzapine; Risperidone

1. Introduction

Antipsychotic drugs have a significant affinity for dopamine D_2-receptors (Seeman, 2002). The therapeutic effects of most antipsychotic drugs, such as haloperidol, risperidone and olanzapine, are mediated predominantly by the central blockade of dopamine D_2-receptors (Seeman, 2002). Moreover, it has been shown that therapeutic doses are associated with dopamine D_2-receptor occupancy reaching 80% (Farde et al., 1988). Hence, the aim of this study was to determine clinically relevant doses of haloperidol, risperidone and olanzapine in rats. In this study, in vivo dopamine D_2-receptor occupancy by the antipsychotic drugs was determined using ^{3}H-raclopride (a substituted benzamide) as the radioligand, because raclopride has a high affinity and is selective for dopamine D_2-receptors (Köhler et al., 1985). This is analogous to other in vivo methods, such as positron emission tomography (PET), that are used in human occupancy studies (Farde, 1986; Lammertsma et al., 1996). An important issue to consider in animal studies is the short half-life of antipsychotic drugs in rats compared with humans (Table 1). In order to achieve dopamine D_2-receptor occupancy in the clinically relevant range throughout the duration of treatment with the drugs in this study, steady-state drug levels were achieved in the rats by using subcutaneous continuous infusion minipumps for 7 days.

2. Materials and methods

2.1. Animals

Adult male Wistar rats were divided into two groups, 11-week old and 24-week old animals. All animals were housed in groups of two per cage in a temperature-controlled room (25 °C) with a 12-h light/dark cycle. Food and water were available ad libitum. Animals were weighed weekly and their behaviour was

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Table 1
Half-life of antipsychotics in humans and rats (adapted from Kapur et al., 2003)

Drug	Half-life in humans	Half-life in rats (strain)
Haloperidol	12–36 h	1.5 h (Sprague–Dawley)
Risperidone	20–24 h	1.0 h (Wistar)
Olanzapine	21–54 h	2.5 h (Sprague–Dawley)

monitored daily. All animal procedures were approved by The University of Queensland Animal Care and Ethics Committee and comply with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.2. Drugs and chemicals

Haloperidol (Sigma Pharmaceuticals, Melbourne, Vic, Australia) and olanzapine (gift from Eli Lilly, Sydney, NSW, Australia) were dissolved in normal saline (0.9% NaCl) and risperidone (gift from Janssen-Cilag, Sydney, NSW, Australia) was dissolved in 1% acetic acid. All drugs were administered by Alzet osmotic minipumps at a rate of 10.0 μl/h (model 2ML1, Bioscientific, Sydney, NSW, Australia). ^{3}H-raclopride (specific activity 2224 MBq/μmol; PerkinElmer Life Sciences, Boston, MA, USA) was used as the competitive dopamine D_2-receptor antagonist.

2.3. Surgical procedure

Rats underwent surgery for implantation of an Alzet osmotic minipump. Rats were anesthetized using halothane before surgery. A small incision was made on the back and pumps were inserted into the subcutaneous space. Eleven-week old animals (279±6.9 g; n=39) received pump delivery of haloperidol (0.25 and 0.5 mg/kg), risperidone (3, 5 and 6 mg/kg), olanzapine (5 and 10 mg/kg) or normal saline (0.9% NaCl) for 7 days. These doses were selected using data published by an independent study (Kapur et al., 2003) and our preliminary data. Twenty-four week old animals (591±12 g; n=16) received pump delivery of 0.25 mg/kg/day haloperidol, 5 mg/kg/day risperidone, 10 mg/kg/day olanzapine, or normal saline (0.9% NaCl) for 7 days.

2.4. In vivo radioligand binding

Thirty minutes before sacrifice, all animals received an intravenous injection of ^{3}H-raclopride (7.5 μCi/rat, in a volume of 0.4 ml of normal saline; 125 pmol ^{3}H-raclopride per rat) via the lateral tail vein. All rats were sacrificed by decapitation. Brains were immediately removed, and the left and right striata (for determination of specific binding) and the cerebellum (for determination of non-specific binding) were isolated. Tissue samples were weighed and placed in 20 ml glass scintillation vials. The collected tissue samples were dissolved with 2 ml of Solvable (PerkinElmer Life Sciences) and gently shaken for 24 h. Thereafter, 10 ml of Aquasure liquid scintillant (PerkinElmer Life Sciences) was added and allowed to mix for a further 24 h. ^{3}H was determined by liquid scintillation spectrometry, using a Packard liquid scintillation counter (PerkinElmer Life Sciences).

2.5. Determination of dopamine D_2-receptor occupancy

Dopamine D_2-receptor binding occupancy was determined using the cerebellum as a reference tissue that does not contain specific binding sites (Lammertsma et al., 1996; Lammertsma and Hume, 1996). Specific binding was obtained for each animal as the difference between radioactivity of the striatum and cerebellum. ^{3}H determinations in striatal and cerebellar samples were obtained and expressed as dpm/mg tissue weight. Dopamine D_2-receptor occupancy was determined using the following equations, which are valid under the conditions used, i.e. tracer amount of 125 pmol ^{3}H-raclopride per rat (Wadenberg et al., 2000; Laruelle, 2000):

$$\text{Dopamine } D_2 \text{ Receptor Binding Potential } (D_2\text{BP}) = \frac{\text{striatum(dpm/mg)} - \text{cerebellum(dpm/mg)}}{\text{cerebellum(dpm/mg)}}$$

$$\text{Dopamine } D_2 \text{ Receptor Occupancy } (\%) = 100 \times (D_2\text{BP}_{\text{control}} - D_2\text{BP}_{\text{drug}})/D_2\text{BP}_{\text{control}}$$

2.6. Statistical analysis of data

Data were analyzed using GraphPad Prism Software (GraphPad, San Diego, USA) and GraphPad Instat (GraphPad). In all experiments, data were expressed as means±SEM. One-factor analysis of variance (ANOVA), followed by Tukey post hoc tests, was used to compare the effects of the various doses of the three drugs used in the 11-week old animals. The effects of single doses of the three drugs in 11-week old animals were compared with 24-week old animals by 2-factor ANOVA. One sample t-tests were used to determine whether the dopamine D_2-receptor occupancies for specific doses of any given drug were significantly greater than 65% or less than 80%.

3. Results

An in vivo dopamine D_2-receptor occupancy method, using ^{3}H-raclopride binding, was used to establish doses for haloperidol, risperidone and olanzapine to give receptor occupancy values in the range of 65–80% in 11-week and 24-week old male Wistar rats. ^{3}H-raclopride binding in the striatum of the saline treated control animals was 59.7±9.1 fmol/g for 11-week old animals (n=6) and 47.7±5.0 fmol/g for 24-week old animals (n=4). These values are significantly greater than the non-specific ^{3}H-raclopride binding measured in samples of cerebellum from the same animals (10.9±0.5 fmol/g for 11-week old animals, $P<0.01$, and 11.0±1.6 fmol/g for 24-week old animals $P<0.01$; paired t-test).

In 11-week old rats, there were significant differences between the dopamine-D_2 receptor occupancy values for the three drugs (haloperidol, risperidone and olanzapine) at the doses tested (one-factor ANOVA, $P<0.001$). Subsequent post

hoc tests, showed that: (i) The effects of haloperidol at 0.5 mg/kg/day and 0.25 mg/kg/day were not significantly different ($P>0.05$); (ii) Risperidone had a significantly greater effect at 6 mg/kg/day than at 3 mg/kg/day ($P<0.05$), but there was no difference between the effects at 5 and 6 mg/kg/day ($P>0.05$); (iii) Olanzapine had a significantly greater effect at 10 mg/kg/day than at 5 mg/kg/day ($P<0.001$). Haloperidol at 0.25 mg/kg/day and 0.5 mg/kg/day, risperidone at 5 mg/kg/day and 6 mg/kg/day and olanzapine at 10 mg/kg/day resulted in dopamine D_2-receptor occupancies that were not significantly different from 80% ($P>0.05$, one sample *t*-tests). The doses that were selected for further studies were: 0.25 mg/kg/day for haloperidol, 5 mg/kg/day for risperidone and 10 mg/kg/day for olanzapine, at which there were no statistically significant differences in dopamine D_2-receptor occupancy values for the three drugs ($P>0.05$, post hoc tests).

Experiments were also carried out with 24-week old male Wistar rats using the doses of haloperidol, risperidone and olanzapine that were established in the study using 11-week old rats. These further experiments were carried out to verify the effectiveness of these doses in mature rats. The data for the 24-week old rats (Fig. 1) showed that haloperidol (0.25 mg/kg/day), risperidone (5 mg/kg/day) and olanzapine (10 mg/kg/day) all resulted in dopamine D_2-receptor occupancy values that were significantly greater than 65% ($P<0.05$, one sample *t*-tests) and not significantly different from 80% ($P>0.05$, one sample *t*-tests). There were also no significant differences between the occupancies for the three drugs at these dosage levels ($P>0.05$, 2-factor ANOVA). The dopamine D_2-receptor occupancy values for each drug were not statistically significantly different in 24-week old rats compared with 11-week old rats ($P>0.05$, 2-factor ANOVA).

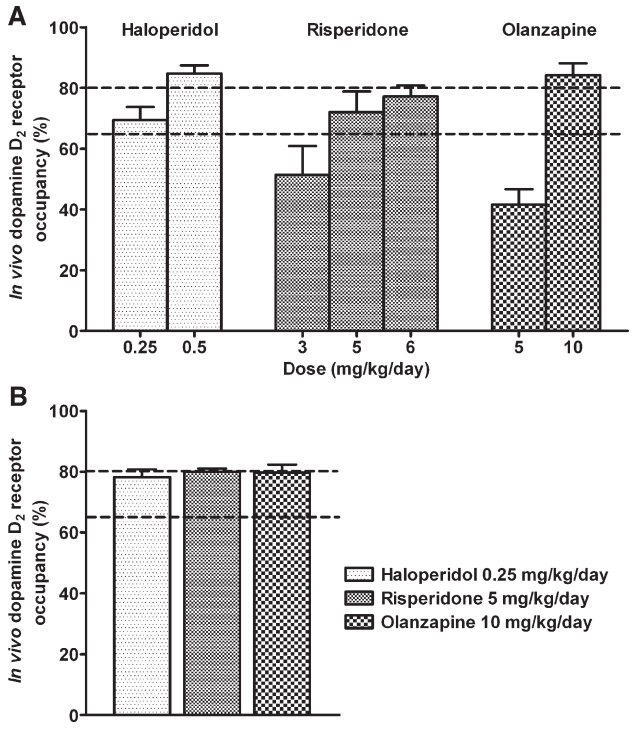

Fig. 1. Dopamine D_2-receptor occupancies of haloperidol, risperidone and olanzapine in 11-week old (A, $n=4–5$) and 24-week old (B, $n=4$) male Wistar rats, following 7 days of treatment using Alzet osmotic minipumps. Data presented as mean±SEM for *n* rats. Horizontal lines indicate the dopamine D_2-receptor occupancy range of 65% to 80%.

4. Discussion

The aim of this study was to determine clinically relevant doses of haloperidol, risperidone and olanzapine that achieve levels of striatal dopamine D_2-receptor occupancy of 80% in rats, using an in vivo radioligand binding method. Our results indicated that in 11-week and 24-week old male Wistar rats 65% to 80% dopamine D_2-receptor occupancy is achieved with haloperidol at 0.25 mg/kg/day, risperidone at 5 mg/kg/day and olanzapine at 10 mg/kg/day.

Previous studies that have determined dopamine D_2-receptor occupancy in rats include those by Sumiyoshi et al. (1994), Schotte et al. (1996), Zhang and Bymaster (1999), Kapur et al. (2003) and Assié et al. (2005). The variability in results between these studies could be a consequence of the strain of rat used in the study, the route and frequency of administration of the antipsychotic drugs and/or the method of determining dopamine D_2-receptor occupancy. The in vitro method of determining dopamine D_2-receptor occupancy used by Schotte et al. (1996) is more variable and less sensitive compared with the in vivo method (Kapur et al., 2001) used in this study. Another confounding factor is that the radioligand was administered subcutaneously in some in vivo dopamine D_2-receptor occupancy studies, such as Zhang and Bymaster (1999), whereas intravenous administration of the radioligand as used in this study allows equilibrium concentrations at the dopamine D_2-receptors to be reached more rapidly. Previous studies, except Kapur et al. (2003), also used single doses of antipsychotic drugs, and hence do not provide an indication of the dopamine D_2-receptor occupancy achieved with chronic dosing. Since it is known that most antipsychotics have a half-life averaging between 4 to 6 times less in rats than in humans (Table 1), the continual presence of the antipsychotic drug at therapeutically active levels was achieved in the present study and that of Kapur et al. (2003) by chronic dosing of rats using osmotic minipumps. It has been shown that, in the case of antipsychotics, although plasma concentrations decline with time after single dose administration, brain dopamine D_2-receptors are occupied to a greater extent than indicated by their plasma levels (Tauscher et al., 2002). Hence, it is highly likely that, because of the longer half-life of the drugs in human patients, the dopamine D_2-receptor occupancy by the drugs will be maintained at levels in the therapeutic range of 80% in the clinical situation. This was modelled in the present study by continuous minipump infusion of antipsychotic drugs.

We required dopamine D_2-receptor occupancy data for subsequent studies on the chronic effects of antipsychotic drugs in Wistar rats. The study conducted by Kapur et al. (2003) is the most comparable to the present study, but Sprague–Dawley rats were used. The results from the present study compared with that of Kapur et al. (2003) showed that the doses of haloperidol

and olanzapine to achieve 65% to 80% dopamine D_2-receptor occupancy were unaffected by the strain of rat or age.

PET studies on dopamine D_2-receptor occupancy by antipsychotic drugs in humans provide comparable data to in vivo dopamine D_2-receptor occupancy determinations in animal studies, and both methods are dependent on use of an appropriate radioligand. A long-standing problem in PET studies has been that butyrophenones (such as spiperone) and benzamides (such as raclopride) yield significantly different densities for dopamine D_2-receptor binding sites (Seeman et al., 1992). More specifically, it was shown that benzamides label 30% to 100% more dopamine D_2-receptor binding sites than butyrophenones (Seeman et al., 1992). This difference can be explained by ^{3}H-spiperone labelling monomers, while ^{3}H-raclopride labels both dimers and monomers (Inoue et al., 1999; Zawarynski et al., 1998; Seeman et al., 1992). Hence, the density of ^{3}H-raclopride-labelled sites would be expected to exceed the density of ^{3}H-spiperone-labelled sites (Inoue et al., 1999; Zawarynski et al., 1998; Seeman et al., 1992) and is a better reflection of the density of total dopamine D_2-receptor binding sites (Inoue et al., 1999). Another difference between ^{3}H-spiperone and ^{3}H-raclopride is the lack of receptor specificity for the former ligand. For instance, it was shown that ^{3}H-raclopride has a high affinity and selectivity for dopamine D_2-receptors, whereas ^{3}H-spiperone binds to both dopamine D_2-receptors and serotonin 5-HT_2-receptors (Farde et al., 1985; Köhler et al., 1985). Another possible explanation for different dopamine D_2-receptor occupancies obtained with the different radioligands might be that ^{3}H-raclopride has a shorter dissociation half-time in comparison to ^{3}H-spiperone in the striatum (Inoue et al., 1999; Bischoff and Gunst, 1997; Köhler et al., 1985), so that much shorter equilibration times are required when ^{3}H-raclopride is used as the radioligand. Taken together, these factors led us to the conclusion that ^{3}H-raclopride is a more suitable ligand for the study of dopamine D_2-receptor occupancy than ^{3}H-spiperone. In addition, the present study examined dopamine D_2-receptor occupancy of typical and atypical antipsychotics and hence required a ligand that was highly selective for dopamine D_2-receptors only. The dose-occupancy results of the present study in rats may only be comparable to dose-occupancy results from human PET studies using raclopride as the radioligand.

In conclusion, using an in vivo receptor occupancy method to determine antipsychotic doses in animal studies that achieves 80% dopamine D_2-receptor occupancy level provides a rationale for selecting doses that are comparable to those determined in clinical settings by PET studies using benzamide radioligands.

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