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ORIGINAL INVESTIGATION

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# Dopamine $D_2$ receptor occupancy predicts catalepsy and the suppression of conditioned avoidance response behavior in rats

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**Abstract** *Rationale:* Human positron emission tomography (PET) shows that striatal dopamine $D_2$ receptor occupancy predicts extrapyramidal side effects (EPS). Patients showed a clinical response with ≥65% $D_2$ occupancy, but EPS only when $D_2$ occupancy >78%. Catalepsy and the selective suppression of conditioned avoidance response (CAR) are often used as animal models to predict EPS and antipsychotic effect, respectively. However, the quantitative relationship between striatal $D_2$ occupancy and effects in these models is not known. *Objectives:* The present study intended to investigate the relationship between animal catalepsy, suppression of CAR, and $D_2$ receptor blockade using a method of evaluating $D_2$ receptor occupancy similar in principle to that used in patients. *Methods:* In vivo binding of [$^{11}$C]-raclopride and [$^3$H]-raclopride was compared. Doses of cold raclopride were chosen to provide a $D_2$ occupancy from 0 to 95%. The relationship between dose/time course of catalepsy and $D_2$ occupancy was assessed. Effects of raclopride on conditioned avoidance response (CAR) behavior were tested. *Results:* In vivo binding of [$^{11}$C]-raclopride compared to [$^3$H]-raclopride was virtually the same. Using [$^3$H]-raclopride, cold raclopride (0.01–0.2 mg/kg) produced 16–77% $D_2$ receptor occupancy and no catalepsy. Raclopride (0.5–2 mg/kg) produced 83–95% $D_2$ occupancy and significant catalepsy. Raclopride (2 mg/kg) produced on average 95% and 87% $D_2$ receptor occupancy 1 and 2 h after administration, respectively, and maximum catalepsy. $D_2$ occupancy at 4, 8 and 24 h was on average 58%, 46%, and 4%, respectively. No catalepsy was observed. Raclopride (0.2 mg/kg), estimated at 70–75% $D_2$ occupancy, produced suppression of CAR. *Conclusions:* In vivo $D_2$ occupancy measurements in rats using [$^3$H]-raclopride is analogous to using [$^{11}$C]-raclopride in human PET scanning. Suppression of CAR occurred at a $D_2$ occupancy of around 70–75%, and catalepsy at $D_2$ occupancy >80%. Results closely resembled human studies where 65–70% $D_2$ occupancy was required for antipsychotic response, while ≥80% $D_2$ occupancy led to EPS. Brain mechanisms involved in mediation of catalepsy in rats and EPS in humans might indeed be similar. Both suppression of CAR in rats and antipsychotic response in humans might share an underlying construct, i.e. the need for around 70% $D_2$ receptor blockade.

**Key words** [$^{11}$C]-Raclopride · [$^3$H]-Raclopride · In vivo · $D_2$ receptor occupancy · Catalepsy · Conditioned avoidance response · Rat

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## Introduction

Despite the introduction of new antipsychotic compounds which bind to multiple brain receptors, the blockade of dopamine (DA) $D_2$ receptors remains an important property by which antipsychotic medications are thought to exert their therapeutic effect (Seeman 1992; Seeman et al. 1997; Kapur et al. 1999, 2000). At the same time, as DA $D_2$ receptors are increasingly blocked, disturbing and incapacitating extrapyramidal side effects (EPS) emerge (see, e.g. Baldessarini 1990; Farde et al. 1992).

In recent years, several positron emission tomography (PET) studies in patients have shown that striatal $D_2$ receptor occupancy in humans is a reliable predictor for EPS with both typical as well as atypical antipsychotic treatment (Farde et al. 1992; Kapur et al. 1995, 2000). Thus for example, Farde et al. (1992) found that following treatment with traditional antipsychotics, the average $D_2$ receptor occupancy of patients with EPS was 82%,

while the average $D_2$ receptor occupancy of patients without EPS was 74%. It should also be noted that the patients with lower $D_2$ receptor occupancy who had no EPS were all responding well to treatment. Similarly, in a double-blind, randomized prospective study, Kapur et al. (2000) found that when patients are randomized from 37 to 86% $D_2$ receptor occupancy, clinical response is evident with 65–70% $D_2$ receptor occupancy, but only patients with a $D_2$ receptor occupancy >78% show EPS.The human data suggest that there is a threshold for EPS around 78–80%, and exceeding that threshold by even a few percent has a significant effect on EPS. Thus, it seems that the window between doses that produce sufficient $D_2$ receptor occupancy to obtain a reliable antipsychotic effect (≥65%) and doses that produce $D_2$ receptor occupancy at which EPS begin to emerge (~80%) is fairly narrow (Farde et al. 1992; Nordström et al. 1993; Kapur et al. 1996, 2000). This might at least partly explain why it is often difficult to find an optimal therapeutic dose without EPS.

It is not clear, however, whether such a threshold of $D_2$ receptor occupancy also exists in analogous test models in rats. The catalepsy test for rats is a common and widely used preclinical screening test for the EPS liability of potentially antipsychotic drugs. Although catalepsy is usually assessed following acute drug administration, the test has proven to be a reliable predictor for the propensity of an antipsychotic drug to induce EPS (i.e. pseudoparkinsonism; dystonia) in humans (see, e.g. Elliott et al. 1990; Wadenberg 1996).

While it has been suggested that catalepsy is related to the propensity of a drug to induce striatal DA receptor blockade (Sanberg 1980; Elliott et al. 1990), this has never been systematically and quantitatively investigated. Since the catalepsy test is so often relied upon as a predictor of human EPS, this is an important issue to clarify as it would help to define further the validity of this test.

Receptor occupancy in animals is usually determined by means of an ex vivo autoradiography technique (see, e.g. Schotte et al. 1996; Mijnster et al. 1998). During this procedure, a significant amount of the drug under investigation dissociates from the receptors (unpublished data in our laboratory). Since it has been shown that drugs differ in the rate at which they dissociate from receptors (Seeman and Tallerico 1998, 1999), that could lead to a systematic bias in determining $D_2$ receptor occupancy. A microPET apparatus for small animals has recently been presented (Cherry et al. 1998). However, this technique is still in its infancy, and it will probably be some time before such a technique becomes common practice in laboratories.

In an effort to bring the preclinical animal test situation closer to the clinical test situation, the present study was designed to measure $D_2$ receptor occupancy and catalepsy in the same animal. Moreover, to bring the experimental design closer to the PET scan situation in humans, striatal $D_2$ receptor occupancy was determined through the in vivo (as opposed to ex vivo) binding of [$^{11}$C]-raclopride and [$^{3}$H]-raclopride. To address the relationship between catalepsy and brain striatal $D_2$ receptor occupancy in the rat, a wide range of doses of cold raclopride (a selective dopamine $D_2$ receptor antagonist; Köhler et al. 1985) was used to provide a $D_2$ receptor occupancy ranging from 0 to 95%. Furthermore, we also assessed the relationship between the time course of catalepsy and $D_2$ receptor occupancy. For comparison, catalepsy measurements were performed using both the inclined grid (Ahlenius and Hillegaart 1986) and the bar (Kuschinsky and Hornykiewicz 1972) tests.

Finally, to get a preliminary idea of how the $D_2$ receptor occupancy necessary to produce an antipsychotic-like effect in the conditioned avoidance response (CAR) test (an animal screening test for potentially antipsychotic compounds; see, e.g. Wadenberg and Hicks 1999) compares to the $D_2$ receptor occupancy necessary to produce catalepsy, the effect of different doses of raclopride on CAR behavior was also tested.

## Materials and methods

### Animals

Adult male Sprague-Dawley rats, 200–225 g, were purchased (Charles River, Montréal, Canada). The animals were housed, two per cage, in (19×101/2×8 in) transparent polycarbonate cages (Lab Products Inc., Seaforth, Delaware, USA) under reversed light/dark conditions using a 12-h on/off schedule (lights off 08:00 a.m.). Room temperature was maintained at 21±1°C with a relative humidity of 55–60%. Food and water were available ad libitum. The animals were allowed 1 week of adaptation to laboratory conditions before being used in experiments.

Principles of animal care (The Animals for Research Act 1968–1969 of the Province of Ontario, RSO, 1990, c A22; 1994 c. 27; and NIH Publication No 85-23, revised 1985) were followed.

### Drugs

Raclopride tartrate (Astra, Södertälje, Sweden), was dissolved in physiological saline, and given subcutaneously in a volume of 2 ml/kg body weight. [$^{3}$H]raclopride (NEN Life Sciences, Boston, Mass., USA), and [$^{11}$C]-raclopride (synthesized at the PET Centre, CAMH, Clarke Divison, Toronto, Canada) were used as radioligands and administered intraveneously into the tail.

### General procedures

#### *Injections and measurements*

The rats were randomized to ten different doses of raclopride (0.01–2.0 mg/kg), and a vehicle treated group. All rats were given the cold raclopride subcutaneously 60 min before death, and had the [$^{3}$H]-raclopride (7.5 μCi/rat; in a volume of 0.4 ml 0.9% NaCl solution) injected through a lateral tail vein 30 min before death. The rats were tested for catalepsy 50 min after subcutaneous injection (i.e. 10 min before death) (see Table 1). Animals were killed

**Table 1** General time schedule for the experiments

| –60 | –30 | –10 | 0 (min) |
|---|---|---|---|
| Raclopride (SC) | [$^{3}$H] or [$^{11}$C]-raclopride (IV) | Catalepsy | Decapitation |

by decapitation, and the brains were immediately removed and placed on ice.

In the time-effect experiment, all animals assigned to later time intervals were also observed 1 h after administration. This was done to mimic the repeated testing situation in time-effect studies.

## Standardisation of procedure for measurement of occupancy using [$^{11}$C]-raclopride and [$^{3}$H]-raclopride

One of the main objectives of this study was to use a method of occupancy determination in rats that was analogous to the one used in human studies (Farde et al. 1988a; Kapur et al. 1999). Human studies involve the intravenous injection of [$^{11}$C]-raclopride followed by PET scanning, which provides the data on [$^{11}$C]-raclopride counts in different brain regions. The ratio of counts in the (striatum minus cerebellum)/cerebellum during the period of pseudoequilibrium is used as an estimate of the $B_{max}/K_d$ of [$^{11}$C]-raclopride for dopamine $D_2$ receptors (Farde et al. 1988a; Kapur et al. 1999). This is justified on the grounds that the cerebellar (C) counts reflect non-specific binding and free ligand, whereas the striatal (S) counts reflect specific binding of the ligand to $D_2$ receptors in addition to the non-specific and free ligand binding. Using these assumptions, it can be shown that the (S-C/C) ratio reflects the ratio of $B_{max}/K_d$, where $B_{max}$ is the total number of $D_2$ receptors and $K_d$ is the affinity of the ligand (Farde et al. 1986, 1988a; Ito et al. 1998; Kapur et al. 1999). This measure is often referred to as the binding potential (BP) (Farde et al. 1988a). To obtain measures of antipsychotic-induced occupancy, the $D_2$ BP of a patient on an antipsychotic drug is then compared to that obtained in an appropriate control.

In pilot studies (unpublished data) we injected [$^{11}$C]-raclopride in rats and killed them at different time intervals, from 15 to 60 min post-injection to obtain the time course of specific and non-specific binding. These data showed that the specific binding (i.e. striatum minus cerebellum) reached equilibrium between 20 and 30 min, as has been reported previously (Köhler et al. 1985; Hume et al. 1992). Therefore we chose 30 min post-injection as the optimal time for killing.

### *Comparison of [$^{11}$C]-raclopride and [$^{3}$H]-raclopride*

One of the difficulties of [$^{11}$C]-raclopride, the ligand used in human PET studies, is that it requires synthesis by cyclotron and has a very short half-life (20.4 min). As a result, it is not feasible as a standard procedure for rodent studies and would not be accessible to most other laboratories. Therefore, we wanted to examine whether [$^{3}$H]-raclopride, which is commercially available and has a long half-life, would give similar results. To make a relevant comparison, 20 animals were assigned to either vehicle ($n$=4) or raclopride in eight stratified doses (0.031–8 mg/kg, $n$=16). The occupancy of cold raclopride was then measured using both [$^{3}$H]-raclopride and [$^{11}$C]-raclopride in the same animal as described below. The dose of the cold raclopride was injected 1 h prior to killing. At 30 min prior to death, the rat received a simultaneous injection of 1 mCi [$^{11}$C]-raclopride (specific activity 600–1000 mCi/μM) and 7.5–10 μCi [$^{3}$H]-raclopride (78 Ci/mM) via a heated (45°C) tail vein. The rats were killed and the striata and cerebellum dissected from each brain. In each rat, the left and the right striata were separately dissected and were randomly assigned for counting of [$^{11}$C]-raclopride or [$^{3}$H]-raclopride. The cerebellum was dissected, homogenized, and divided into halves, one for [$^{11}$C]-raclopride and the other for [$^{3}$H]-raclopride.

The tissue for determination of occupancy using [$^{11}$C]-raclopride was transferred into polypropylene tubes and was immediately counted for gamma-emission using a CobraII Auto-Gamma counter (Canberra-Packard, Guelph, Canada). Determination of $D_2$ receptor occupancy in tissue using [$^{3}$H]-raclopride was carried out according to the procedure described below.

### *Dissection and tissue counting*

The rats were decapitated and the striata and cerebella were rapidly dissected. The cerebellum was homogenized with a small spatula, and approximately one-third (50–100 mg) of this was sampled. The left and right striata were pooled into a single sample (~60 mg). Tissue samples were collected in previously weighed 20 ml glass scintillation vials. The vials were then weighed with tissue and 2 ml Solvable (Canberra Packard, Canada) was added to the vials. The vials were kept on an automated shaking tray, and gently agitated for 24 h at 23°C. Thereafter, 5 ml of Aquasure (formerly Formula 965; Canberra Packard, Canada) scintillation fluid was added, and the mixture was allowed to mix for another 24 h. Quantitation of [$^{3}$H] radioactivity was determined by liquid scintillation spectrometry using a Beckman LS5000 CE liquid scintillation counting system at 50% efficiency. Striatal and cerebellar counts were obtained and expressed as disintegrations per min/mg (DPM/mg) for future calculations.

### *Calculation of $D_2$ binding potential and receptor occupancy*

With each ligand the $D_2$ receptor binding potential was obtained for each of the animals as S-C/C. The value for the control group was pooled, and the occupancy in each rat was then determined using the same formula as used in human studies (Farde et al. 1988a; Kapur et al. 1999):

$$\%\text{Occupancy}=100\times(D_2BP_{control}-D_2BP_{indiv}/D_2BP_{control})$$

### *Catalepsy measurements*

For comparison, both the grid and the bar catalepsy tests were used. Animals were first observed on an inclined (60°) grid, and thereafter with their front paws placed on a bar elevated 10 cm above the floor in the observation cage. To establish a reliable baseline, the first 30 s were excluded from the actual rating time to avoid effects of exploratory motor activity due to a new environment. The time the rat remained in the same position (grid test), or removed both their forelimbs from the bar (bar test) was then measured for a maximum of 2.5 min. The catalepsy was scored from 0 to 5 according to the time (square root transformation) the animal remained immobile (min): 0=0–0.08, 1=0.09–0.35, 2=0.36–0.80, 3=0.81–1.42, 4=1.43–2.24, 5=≥2.25 min, i.e., if the rat remained immobile for >2.25 min it was scored as 5 etc. (see, e.g. Ahlenius and Hillegaart 1986).

### *Conditioned avoidance response behavior*

Rats were trained and tested in a computer-assisted two-way active avoidance (shuttlebox) apparatus equipped with a tilting grid floor, with microswitch detection, connected to a high resistance power supply. The boxes were divided into two compartments of equal size by a partition with one opening. Upon presentation of the 80 dB white noise conditioned stimulus (CS), the animals had 10 s to move from one compartment of the shuttlebox into the other. If the rat remained in the same compartment for more than 10 s, an intermittent electric shock, the unconditioned stimulus (UCS), was presented in the grid floor until an escape was performed. If the animal did not respond within 50 s of the shock period, the trial was terminated (escape failure). Intertrial intervals varied at random between 20 and 40 s. The following variables were recorded: *avoidance* (response to CS within 10 s); *escape* (response to CS+UCS); *escape failures* (failure to respond); and *intertrial crosses*. The animals were trained for 5 consecutive days. Experimental manipulations were always preceded by a pretest. All pretest and experimental sessions were run for 10 min (for further details, see Wadenberg et al. 1998). The same animals were tested repeatedly according to a change-over design (Li 1964) serving as their own controls.

### Statistics

For catalepsy studies, the statistical analysis was performed by means of the Kruskal-Wallis one-way ANOVA by ranks followed by the Mann-Whitney *U*-test for comparisons with vehicle treated controls. For CAR studies, the Friedman two-way ANOVA by ranks, followed by the Wilcoxon matched-pairs signed-ranks test for comparisons with control conditions was used (Siegel and Castellan 1988). Data for dose and time relationships between $D_2$ receptor occupancy and catalepsy were analyzed by correlation analysis (Statistica 5.1, StatSoft, Inc.).

## Results

### Dose and time effects of raclopride on catalepsy in rats

In the experiment relating dose to $D_2$ receptor occupancy and catalepsy, raclopride (0.01–0.2 mg/kg, SC) did not produce catalepsy. Raclopride (0.5–2.0 mg/kg, SC) produced a statistically significant (KW=7.83, $P<0.05$) catalepsy, that reached its highest score (5) in animals treated with raclopride 2.0 mg/kg ($P<0.05$ compared to vehicle treated animals) (Table 2).

In the experiment relating time course of catalepsy to $D_2$ receptor occupancy, raclopride (2 mg/kg)-induced catalepsy reached its peak at 1 h after administration. Animals assigned to the 2-h observation time still scored maximum catalepsy. Animals assigned to the 4-, 8-, and 24-h observation times did not show any cataleptic effects at any of these observation times (Table 2 ). Vehicle treated controls for the different treatment groups did not show any cataleptic behavior at any observation time (data not shown).

Results from treatment with doses of raclopride below 0.06 mg/kg are not shown, since they did not add any further information.

### Grid versus bar test

For comparison, animals were observed for catalepsy both in the grid and the bar test. A preceding experiment showed no measurement differences between animals first observed on the bar, and animals first observed on the grid (data not shown). There was a slight tendency for animals to score somewhat lower in the bar test. However, the median scores in the two tests were close to identical (Table 2 ), and the statistical correlations did not differ as a function of test method.

### Comparison of $D_2$ receptor occupancy measurement accuracy between [$^{11}$C]-raclopride and [$^{3}$H]raclopride in the rat

Data from all 20 animals (see Materials and methods) was available, and the $D_2$BP obtained by the two techniques was virtually identical [$F(1,18)=3753$, $r=0.998$, $P<0.0001$]. The occupancies in the 16 animals treated with raclopride ranged from 0 to 97%, were highly correlated [$F(1,14)=281$, $r=0.976$, $P<0.0001$], and the difference between the two techniques was on average less than 2%. As a result, all further experiments were carried out using [$^{3}$H]raclopride.

### Dose-effect relationship between $D_2$ receptor occupancy and catalepsy following raclopride administration in rats

Raclopride (0.01–0.2 mg/kg, SC, –1 h) produced a dose-dependent increase in $D_2$ receptor occupancy ranging from 16 to 77%. No catalepsy was observed in this dose range (see also above). Raclopride (0.5–2 mg/kg, SC, –1 h) produced a dose-dependent increase in $D_2$ receptor occupancy ranging from 83 to 95%. The increase in $D_2$ receptor occupancy was paralleled by a corresponding increase in cataleptic intensity that reached its maximum following treatment with raclopride 2 mg/kg. There was a statistically significant positive correlation between percent $D_2$ receptor occupancy and degree of catalepsy ($r=0.80$; $P<0.05$) (Fig. 1).

**Table 2** Dose and time effects of the dopamine $D_2$ receptor antagonist raclopride on catalepsy as measured by the grid and the bar test, respectively. Catalepsy scores are shown as medians (±semi-interquartile range) based on four or five animals per treatment group. Statistical evaluation of catalepsy was performed by the Kruskal-Wallis one-way ANOVA by ranks, followed by the Mann-Whitney *U*-test (Siegel and Castellan Jr 1988) for comparisons with vehicle treated controls (upper panel)

$^{ns}P>0.05$, $^{*}P<0.05$

| Catalepsy | | | | |
|---|---|---|---|---|
| Raclopride (mg/kg) | Grid (scores) | (Time range, min) | Bar (scores) | (Time range, min) |
| 0 | 0.0±0.3 | 0.03–0.25 | 0.0±0.0 | 0.00–0.00 |
| 0.06 | 0.0±0.3$^{ns}$ | 0.02–0.10 | 0.0±0.0$^{ns}$ | 0.00–0.00 |
| 0.12 | 0.0±0.5$^{ns}$ | 0.05–0.50 | 0.0±0.0$^{ns}$ | 0.00–0.00 |
| 0.2 | 0.5±0.3$^{ns}$ | 0.06–0.12 | 0.0±0.0$^{ns}$ | 0.00–0.06 |
| 0.5 | 3.5±0.3$^{*}$ | 1.25–1.70 | 0.0±0.0$^{ns}$ | 0.00–0.35 |
| 1.0 | 2.5±0.8$^{ns}$ | 0.15–1.85 | 2.5±1.3$^{ns}$ | 0.00–2.50 |
| 2.0 | 5.0±0.0$^{*}$ | 2.50–2.50 | 5.0±0.0$^{*}$ | 2.50–2.50 |
| Raclopride (2 mg/kg, h) | | | | |
| 1 | 5.0±0.0 | 2.50–2.50 | 5.0±0.0 | 2.50–2.50 |
| 2 | 5.0±1.0 | 0.15–2.50 | 4.0±1.8 | 0.20–2.50 |
| 4 | 0.0±0.8 | 0.00–0.45 | 0.0±0.0 | 0.00–0.00 |
| 8 | 1.5±2.5 | 0.00–2.50 | 0.0±0.0 | 0.00–0.05 |
| 24 | 0.0±0.0 | 0.02–0.07 | 0.0±0.0 | 0.00–0.00 |

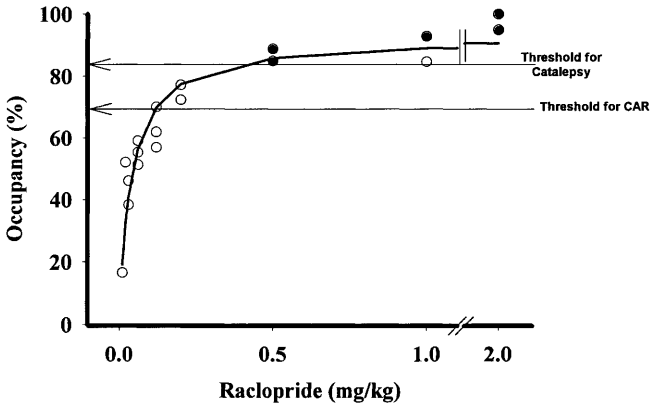


**Fig. 1** Dose-effect relationship between percent $D_2$ receptor occupancy and catalepsy following raclopride administration in rats. Non-cataleptic animals are presented as *open circles* and cataleptic animals as *filled circles*, each circle representing one animal (total $n$=23). There was a statistically significant ($r$=0.80; $P$<0.05) positive correlation between catalepsy and percent $D_2$ receptor occupancy. Only animals with a $D_2$ receptor occupancy >80% were scored as cataleptic

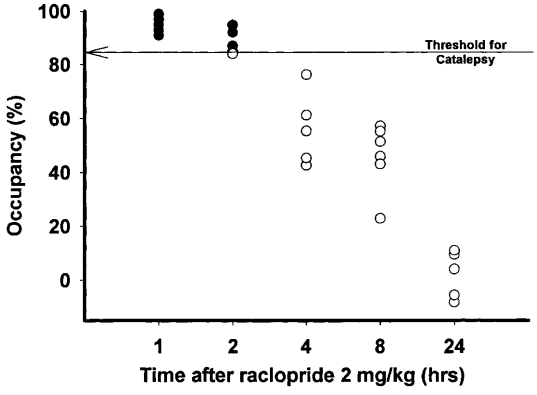


**Fig. 2** Time-effect relationship between percent $D_2$ receptor occupancy and catalepsy following a single dose of raclopride in rats. Non-cataleptic animals are presented as *open circles* and cataleptic animals as *filled circles*, each circle representing one animal (total $n$=26). There was a statistically significant ($r$=0.85; $P$<0.05) positive correlation between catalepsy and percent $D_2$ receptor occupancy. Only animals with a $D_2$ receptor occupancy >80% were scored as cataleptic

## Time-effect relationship between $D_2$ receptor occupancy and catalepsy following a single dose of raclopride in rats

Raclopride (2 mg/kg, SC) produced an average of 95% and 87% $D_2$ receptor occupancy 1 and 2 h after administration, respectively. This level of occupancy was associated with maximum catalepsy scoring. There was a time dependent decrease in $D_2$ receptor occupancy resulting in an average occupancy of 58% (4 h), 46% (8 h), and 4% (24 h), respectively. At these later observation times no catalepsy was observed. There was a statistically significant correlation between percent $D_2$ receptor occupancy and degree of catalepsy over time ($r$=0.85; $P$<0.05) (Fig. 2).

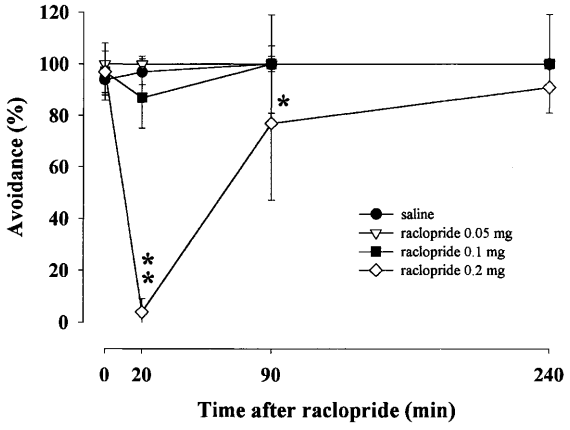


**Fig. 3** Effects of raclopride on the conditioned avoidance response (*CAR*) behavior in rats. The selective DA $D_2$ receptor antagonist raclopride (0.05, 0.1 or 0.2 mg /kg, SC) was administered 20 min before the first observation time. The animals were observed 20, 90 and 240 min after administration. Shown are medians ±semi-interquartile range based upon repeated observations of the same eight animals serving as their own controls in a change-over design (Li 1964). Statistical evaluation was performed by means of the Friedman two-way ANOVA followed by the Wilcoxon matched-pairs signed-ranks test (Siegel and Castellan Jr 1988) for comparisons with vehicle treated animals. $^{*}P$<0.05, $^{**}P$<0.01

In summary, doses of raclopride that produced a $D_2$ receptor occupancy >80% produced catalepsy. Catalepsy was observed as long as $D_2$ occupancy stayed at >80%. As the $D_2$ occupancy decreased over time below 80%, the catalepsy remitted.

## Effects of raclopride on the conditioned avoidance response (CAR) behavior in rats

Based on the results presented above, raclopride 0.05, 0.1 and 0.2 mg/kg were chosen as doses that would be expected to produce a $D_2$ receptor occupancy of approximately 55, 65 and 75%, respectively. Raclopride 0.2 mg/kg produced a statistically significant suppression of CAR at the 20 ($P$<0.004) and 90 ($P$<0.02) min observation times. The animals were back to normal avoidance performance at the 240 min observation time. There was a non-significant tendency for suppression of CAR by raclopride 0.1 mg/kg at the 20 min observation time. Raclopride 0.05 mg/kg had no effect on CAR. There were no escape failures at any dose or time (Fig. 3).

# Discussion

The DA $D_2$ receptor antagonist raclopride produced a dose-dependent, statistically significant catalepsy 1–2 h after administration. There were no significant differences between catalepsy scorings in the bar compared to the inclined grid test. There was a significant positive correlation between $D_2$ receptor occupancy and catalepsy

over dose as well as over time. However, while there was a gradual increase in $D_2$ receptor occupancy over dose, the cataleptic response tended to show more of an all-or-none profile beginning around 83% $D_2$ receptor occupancy (or 0.5 mg/kg raclopride). A similar tendency was observed in time course of action. The catalepsy was observed as maximal at 1 and 2 h after administration, i.e. when $D_2$ receptor occupancy was >80%. Over time (i.e. at 4, 8 and 24 h after administration) as $D_2$ receptor occupancy dropped below this threshold, no catalepsy was observed.

In planning the present experiments, we wanted to bring the $D_2$ receptor occupancy measurement technique closer to the in vivo PET situation. Two main requirements needed to be met: the radioligand had to be injected intraveneously into the living animal; the radioligand needed to be the same as, or be able to substitute for, the PET ligand. Furthermore, to get an accurate quantification of $D_2$ receptor occupancy, behavioral testing of the animals should preferably be performed as close to the optimal time for killing as possible. [$^{11}$C]-raclopride, usually used as a radioligand in PET, is expensive to use. Due to the short half-life, it also puts a time strain on the experimental schedule. Therefore, a pilot test was performed to investigate whether the $D_2$ receptor radio-ligand [$^{3}$H]-raclopride could substitute for [$^{11}$C]-raclopride. It was found that the $D_2$ receptor binding potential obtained by the two techniques was close to identical, and that the difference in results obtained by the two techniques was on average less than 2%. Thus, using [$^{3}$H]-raclopride made it possible systematically to investigate and quantify the relationship between $D_2$ receptor occupancy and catalepsy over dose and time in the rats.

To the best of our knowledge, this is the first study systematically to quantify the relationship between $D_2$ receptor occupancy and catalepsy in rats. The finding that this relationship is indeed a very good predictor of human $D_2$ receptor occupancy and EPS further strengthens the validity of catalepsy as a screening test for EPS liability of potentially antipsychotic agents. In addition, the time course of action of raclopride was also well reflected by the catalepsy scores. This seems to indicate that, not only does catalepsy closely describe human striatal $D_2$ receptor occupancy and EPS following antipsychotic treatment, but that catalepsy also is a reliable indicator of gradual dissociation of the antipsychotic agent from $D_2$ receptors. Taken together, the results suggest that brain mechanisms involved in the mediation of catalepsy in rats and EPS in humans might indeed be similar.

To facilitate comparisons between laboratories, catalepsy was assessed by means of the two most common test settings: the inclined grid and the bar tests. A preceding pilot study revealed no behavioral differences between animals first observed on the bar compared to animals first observed on the inclined grid. Therefore, to simplify the design of the present testing schedule, animals were observed first on the inclined grid and then on the bar. Catalepsy scorings of the present study in the inclined grid compared to the bar test were virtually identical. Thus, future studies using either one of the tests should be generating very similar results.

Finally, the conditioned avoidance response (CAR) is a commonly used test to predict antipsychotic activity. The CAR test has significant predictive validity, some construct validity, but little face validity (see, e.g. Wadenberg and Hicks 1999). However, since antipsychotic drugs are presumed to exert their therapeutic effect mainly via an action at the DA mesocorticolimbic system (see, e.g. Carlsson 1988; Owens and Risch 1995), it seems reasonable to believe that this pathway is also involved in CAR behavior. The nucleus accumbens$_{shell}$ ($NAS_{shell}$) is an important substructure of the DA mesocorticolimbic system (Heimer et al. 1993), presumed to be involved in the pathophysiology of schizophrenia, and in behavior arising from emotion and motivation (Mogenson et al. 1980). Local application of the DA $D_2$ receptor antagonist (–)sulpiride into the $NAS_{shell}$, but not the dorsolateral neostriatum, suppresses CAR (Wadenberg et al. 1990). Furthermore, the atypical antipsychotic clozapine, that does not produced EPS in humans or catalepsy in animals, also produces a selective suppression of CAR following systemic injections (Wadenberg et al. 1993), as well as following local application into the $NAS_{shell}$ (see Wadenberg and Hicks 1999). The CAR test, therefore, seems primarily to involve DA mesocorticolimbic, rather than DA nigrostriatal pathways that mediate extrapyramidal motor functions. Accordingly, it would have been more informative to measure accumbal $D_2$ occupancy, in addition to striatal $D_2$ occupancy, to examine if any differences exist between the two. However, it should be pointed out that this issue has been carefully examined before in ex-vivo autoradiographic studies, and no evidence for differential or preferential occupancy in extrastriatal regions was found for any of a series of typical and atypical antipsychotics examined (Schotte et al. 1996).

Antipsychotic drugs produce a selective suppression of a previously acquired avoidance behavior in the animals, while they improve delusions and hallucinations in humans. Furthermore, the CAR test is an acute single dose test in rats, whereas in humans improvement follows only after repeated dosing. Despite these differences, it is of interest to note that the minimum antipsychotic-like dose effective in the CAR test causes around 70–75% $D_2$ receptor occupancy. This is considerably lower than the >80% $D_2$ receptor occupancy needed for the induction of catalepsy. Furthermore, this relationship is virtually identical to results from human studies, which find that the threshold for clinical response is at the level of 65–70% $D_2$ receptor occupancy, while EPS emerges when $D_2$ receptor occupancy exceeds 80%. Thus, it seems that both suppression of CAR in rats and antipsychotic response in humans share an underlying construct – the need for around 70% $D_2$ receptor blockade. This may in part explain why this seemingly unrelated test predicts human therapeutic outcome so well.

In the present study, the selective DA $D_2$ receptor antagonist raclopride was used as a preclinical prototype

for an antipsychotic agent. Although raclopride is currently not used as an antipsychotic in the clinic, it clearly displays antipsychotic activity. Thus, open label clinical trials have reported that raclopride (4–16 mg/day) showed good antipsychotic efficacy (patients were rated "very much", or "much", improved), was well tolerated, and had a relatively safe side effect profile (Farde et al. 1988b; Cookson et al. 1989). Furthermore, in addition to its antipsychotic-like effects in the CAR test, raclopride (0.1–0.3 mg/kg) also dose-dependently reverses apomorphine-induced disruption of the prepulse inhibition of the acoustic startle reflex, PPI, in rats (Swerdlow et al. 1991; Varty and Higgins 1995; Wadenberg and Pais, unpublished data), another screening test for antipsychotic activity (see Swerdlow and Geyer 1998).

In summary, $D_2$ receptor occupancy can be measured in rats using [$^3$H]-raclopride with methods analogous to those using [$^{11}$C]-raclopride in PET scanning in humans. Catalepsy is associated with >80% striatal $D_2$ receptor occupancy. Catalepsy is also a reliable indicator of gradual dissociation of the antipsychotic agent from $D_2$ receptors. The results suggest that brain mechanisms involved in the mediation of catalepsy in rats and EPS in humans indeed might be similar. Finally, antipsychotic-like suppression of CAR can be observed at a $D_2$ receptor occupancy between 70 and 75%, while catalepsy emerges only when $D_2$ receptor occupancy is >80%. These figures are remarkably analogous to human studies where 65–70% $D_2$ receptor occupancy is found to be required for clinical antipsychotic response, while 80% $D_2$ receptor occupancy or greater leads to EPS.

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