Abstract Rationale: Human positron emission tomography (PET) shows that striatal dopamine D₂ receptor occupancy predicts extrapyramidal side effects (EPS). Patients showed a clinical response with ≥65% D₂ occupancy, but EPS only when D₂ 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 D₁ 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 D₂ receptor blockade using a method of evaluating D₂ receptor occupancy similar in principle to that used in patients. Methods: In vivo binding of [¹¹C]-raclopride and [³H]-raclopride was compared. Doses of cold raclopride were chosen to provide a D₂ occupancy from 0 to 95%. The relationship between dose/time course of catalepsy and D₂ occupancy was assessed. Effects of raclopride on conditioned avoidance response (CAR) behavior were tested. Results: In vivo binding of [¹¹C]-raclopride compared to [³H]-raclopride was virtually the same. Using [³H]-raclopride, cold raclopride (0.01–0.2 mg/kg) produced 16–77% D₂ receptor occupancy and no catalepsy. Raclopride (0.5–2 mg/kg) produced 83–95% D₂ receptor occupancy and significant catalepsy. Raclopride (2 mg/kg) produced on average 95% and 87% D₂ receptor occupancy 1 and 2 h after administration, respectively, and maximum catalepsy. D₂ 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% D₂ occupancy, produced suppression of CAR. Conclusions: In vivo D₂ occupancy measurements in rats using [³H]-raclopride is analogous to using [¹¹C]-raclopride in human PET scanning. Suppression of CAR occurred at a D₂ occupancy of around 70–75%, and catalepsy at D₂ occupancy >80%. Results closely resembled human studies where 65–70% D₂ occupancy was required for antipsychotic response, while ≥80% D₂ 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% D₂ receptor blockade.

Key words [¹¹C]-Raclopride · [³H]-Raclopride · In vivo · D₂ receptor occupancy · Catalepsy · Conditioned avoidance response · Rat

Introduction

Despite the introduction of new antipsychotic compounds which bind to multiple brain receptors, the blockade of dopamine (DA) D₂ 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 D₂ 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 D₂ 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 D₂ receptor occupancy of patients with EPS was 82%.
while the average D₂ receptor occupancy of patients without EPS was 74%. It should also be noted that the patients with lower D₂ 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% D₂ receptor occupancy, clinical response is evident with 65–70% D₂ receptor occupancy, but only from 37 to 86% D₂ 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 D₂ receptor occupancy to obtain a reliable antipsychotic effect (≥65%) and doses that produce D₂ 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 D₂ 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 Tallarico 1998, 1999), that could lead to a systematic bias in determining D₂ 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 D₂ receptor occupancy and catalepsy in the same animal. Moreover, to bring the experimental design closer to the PET scan situation in humans, striatal D₂ receptor occupancy was determined through the in vivo (as opposed to ex vivo) binding of [¹¹C]-raclopride and [³H]-raclopride. To address the relationship between catalepsy and brain striatal D₂ receptor occupancy in the rat, a wide range of doses of cold raclopride (a selective dopamine D₂ receptor antagonist; Köhler et al. 1985) was used to provide a D₂ receptor occupancy ranging from 0 to 95%. Furthermore, we also assessed the relationship between the time course of catalepsy and D₂ 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 D₂ 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 D₂ 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 (19x101/2x8 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.


#### Drugs

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

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