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Repeated antipsychotic treatment progressively potentiates inhibition on phencyclidine-induced hyperlocomotion, but attenuates inhibition on amphetamine-induced hyperlocomotion: Relevance to animal models of antipsychotic drugs

Tao Sun [a,b], Gang Hu [a], Ming Li [b,*]

[a] Jiangsu Key Laboratory of Neurodegeneration, Department of Pharmacology, Nanjing Medical University, China
[b] Department of Psychology, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, USA

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ABSTRACT

Clinical observations indicate that antipsychotic action starts early and increases in magnitude with repeated treatment. Animal models that faithfully capture this time course of action are few. Inhibition of hyperlocomotion induced by amphetamine or phencyclidine has been widely used as a screening tool for the antipsychotic activity of a drug. We thus investigated whether repeated antipsychotic treatment could produce an early-onset and progressively increased antagonistic effect on amphetamine or phencyclidine-induced hyperlocomotion as a way of assessing the validity of such models in capturing time course of antipsychotic action. On each of the five consecutive test days, different groups of rats (n=6–7/group) received an initial injection of either haloperidol (0.01–0.10 mg/kg, sc), clozapine (5–20.0 mg/kg, sc), olanzapine (1.0 mg/kg, sc), chlordiazepoxide (10.0 mg/kg, ip) or vehicle (sterile water, sc) 30 min prior to a second injection of either amphetamine (1.5 mg/kg, sc) or phencyclidine (3.2 mg/kg, sc). Motor activity was subsequently monitored for 60 min after amphetamine or phencyclidine treatment. Repeated treatment of haloperidol, clozapine, or olanzapine progressively potentiated inhibition on repeated phencyclidine-induced hyperlocomotion and prolonged this action over the five consecutive days. In contrast, antipsychotic inhibition on repeated amphetamine-induced hyperlocomotion was gradually attenuated and shortened. Repeated treatment of chlordiazepoxide, a benzodiazepine anxiolytic, retained its inhibition on amphetamine-induced hyperlocomotion, but had no effect on phencyclidine-induced one. These results suggest that repeated phencyclidine-induced hyperlocomotion model based on repeated antipsychotic treatment regimen is capable of capturing the progressive increase pattern of antipsychotic treatment seen in the clinic and differentiating antipsychotics from anxiolytics; thus it may serve as a better model for the investigation of the neurobiological mechanisms of action of antipsychotic drugs and delineating the pathophysiology of schizophrenia.

1. Introduction

Recent years have witnessed a growing number of clinical studies suggesting that antipsychotic action starts early and increases in magnitude with repeated treatment (Agid et al., 2003, 2006; Emsley et al., 2006; Glick et al., 2006; Kapur et al., 2005; Leucht et al., 2005; Raedler et al., 2007). For example, Agid et al. (2003) examined 42 double-blind, comparator-controlled studies (>7000 patients) using a meta-analysis technique. They found that psychotic symptoms improved within the first week of treatment and showed a progressive improvement over subsequent weeks, with the overall pattern of improvement approximating an exponential curve. Leucht et al. (2005) analyzed a large homogeneous database of original patient data from 7 randomized, double-blind studies of the efficacy of amisulpride in patients with schizophrenia spectrum disorders and found the same results. More improvement occurs in the first few days than in any later period of equal duration (Leucht et al., 2005). These findings are in contrast to the traditional held belief that the onset of antipsychotic action is delayed and takes 2–3 weeks before the onset of therapeutic benefits is produced (Gelder et al., 2000).

This change in our clinical understanding demands a re-examination of the currently available animal models of antipsychotics drugs. Many models rely on the *acute* effects of antipsychotic treatment, including amphetamine-induced hyperlocomotion, the catalepsy and paw test to prepulse inhibition, latent inhibition, and social interaction (Arnt, 1982; Ellenbroek et al., 1987; Hoffman and Donovan, 1995;

* Corresponding author. 238 Burnett Hall, Department of Psychology, University of Nebraska-Lincoln, Lincoln, NE 685888, USA. Tel.: +1 402 472 3144.
E-mail address: mli2@unl.edu (M. Li).

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Sams-Dodd, 1999; Swerdlow et al., 2000; Weiner, 2003). Because of the limitation of acute treatment regimen, none of these models provides a relevant model of time course of antipsychotic effect. On the other hand, models that have used chronic treatment regimens, such as "depolarization block" (Grace and Bunney, 1986), antipsychotic-induced Fos expression (Robertson and Fibiger, 1992), social behavior (Sams-Dodd, 1998), or the chronic prepulse inhibition model (Andersen and Pouzet, 2001), have often examined behavioral or physiological changes *after* a certain period of treatment has elapsed (e.g., ~21 days after the first drug administration), instead of during the chronic treatment period. Thus, they are limited in tracking changes that occur during the treatment period.

We recently developed a rat conditioned avoidance responding model based on a repeated treatment regimen and examined its validity in modeling the time course of antipsychotic effect (Li et al., 2007). We found that rats repeatedly treated with haloperidol, olanzapine, or risperidone exhibited a decrease in avoidance responding starting on the first day of treatment. Over the seven daily test sessions, their avoidance responding displayed a progressive, across-session decline, which recovered after treatment was stopped. In contrast, rats treated with chlordiazepoxide or vehicle maintained a high level of avoidance responding throughout the entire testing period. Thus the repeated treatment conditioned avoidance responding model seems capable of mimicking several key features of clinical effects of antipsychotics, such as early-onset, progressive accumulation, asymptote, and drug-discontinuation relapse.

The present study represents another attempt to develop clinically relevant animal models of antipsychotic activity that capture important hallmarks of clinical features of antipsychotic treatment along the time course of antipsychotic treatment in the clinic. The objective was to investigate whether repeated antipsychotic treatment, as opposed to anxiolytic treatment, could produce an early-onset and progressively increased inhibitory effect on the amphetamine or phencyclidine-induced hyperlocomotion. Both amphetamine and phencyclidine-induced hyperlocomotion models are commonly used as screening tools for the detection of antipsychotic activity. When given *acutely*, all antipsychotics inhibit hyperlocomotor activity induced by *acute* administrations of amphetamine or phencyclidine (Arnt, 1995; Gleason and Shannon, 1997). However, little is known about the effects of *repeated* antipsychotic treatment on the motor activity induced by *repeated* amphetamine or phencyclidine treatment.

2. Materials and methods

2.1. Animals

A total of 144 male Sprague–Dawley rats (226–250 g upon arrival, Charles River, Portage, MI) were used. They were housed two per cage, in 48.3 cm×26.7 cm×20.3 cm transparent polycarbonate cages under 12-h light/dark conditions (light on between 6:30am and 6:30pm). Room temperature was maintained at 21±1 °C with a relative humidity of 55–60%. Food and water was available ad libitum. Animals were allowed at least one week of habituation to the animal facility before being used in experiments. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

2.2. Drugs

The injection solutions of haloperidol (5 mg/ml ampoules, Sabex Inc. Boucheville, Quebec, Canada) and chlordiazepoxide (Sigma-Aldrich, St. Louis, MO) were obtained by mixing drugs with sterile water. The injection solutions of d-amphetamine sulfate (Sigma-RBI) and phencyclidine hydrochloride (gift from National Institute on Drug Abuse Chemical Synthesis and Drug Supply Program) were obtained by mixing drugs with 0.9% saline. Clozapine (gift from the NIMH drug supply program) and olanzapine (Toronto Research Chemical Inc., Canada) were dissolved in 1.5% glacial acetic acid distilled water. Haloperidol, clozapine, olanzapine, amphetamine and phencyclidine were administered subcutaneously, whereas chlordiazepoxide was administered intraperitoneally.

2.3. Locomotor activity apparatus

Sixteen activity boxes were housed in a quiet room. The boxes were 48.3 cm×26.7 cm×20.3 cm transparent polycarbonate cages, which were similar to the home cages but were each equipped with a row of 6 photocell beams (7.8 cm between two adjacent photobeams) placed 3.2 cm above the floor of the cage. A computer detected the disruption of the photocell beams and recorded the number of beam breaks. All experiments were run during the light cycle.

2.4. Experiment 1: effects of repeated haloperidol and clozapine treatment on amphetamine-induced hyperlocomotion

In this experiment, we examined the effects of repeated haloperidol and clozapine treatment on amphetamine-induced hyperlocomotion. We chose three doses of haloperidol (0.01, 0.05 and 0.10 mg/kg) and clozapine (5, 10, and 20 mg/kg) which cover subclinical, optimal clinical, and supra-clinical doses based on the dopamine D_2 occupancy data (50%–75% occupancy) (Kapur et al., 2003a). Also, at the medium and high doses, both haloperidol and clozapine selectively disrupt avoidance responding —a validated behavioral index of antipsychotic activity (Li et al., 2004). The dose for amphetamine was 1.5 mg/kg, which is the common dose used in the literature (Arnt, 1995; Natesan et al., 2006; Sills et al., 2000).

2.5. Experimental procedure

Forty-eight rats were randomly assigned to one of eight groups (n=6/group): vehicle (water)+vehicle (saline, SAL), vehicle (water)+amphetamine, haloperidol (0.01 mg/kg)+amphetamine, haloperidol (0.05 mg/kg)+amphetamine, haloperidol (0.10 mg/kg)+amphetamine, clozapine (5.0 mg/kg)+amphetamine, clozapine (10.0 mg/kg)+amphetamine, and clozapine (20.0 mg/kg)+amphetamine. After two days of habituation to the testing room and the testing boxes (30 min/day for 2 days), on day 1, rats first received one of the following seven subcutaneous injections: vehicle (sterile water), haloperidol 0.01, 0.05, or 0.10 mg/kg, or clozapine 5.0, 10.0, or 20.0 mg/kg. They were then immediately placed in locomotor activity boxes for 30 min. At the end of the 30-min period, rats were taken out and injected with either vehicle (sc) or amphetamine (1.5 mg/kg, sc) and placed back in the boxes for another 60 min. Locomotor activity (number of photobeam breaks) was measured in 5 min intervals throughout the entire 90-min testing session. This procedure was repeated for another 4 days (a total of 5 testing days).

2.6. Experiment 2: effects of repeated haloperidol and clozapine treatment on phencyclidine-induced hyperlocomotion

Experiment 2 examined the effects of repeated haloperidol and clozapine treatment on phencyclidine-induced hyperlocomotion. The basic procedure was identical to that of Experiment 1 with the exception that phencyclidine (3.2 mg/kg, sc) was used. This dose of phencyclidine is shown to induce a robust hyperlocomotion effect without causing severe stereotypy (Gleason and Shannon, 1997; Kalinichev et al., 2008).

2.7. Experiment 3: effects of repeated olanzapine and chlordiazepoxide treatment on amphetamine or phencyclidine-induced hyperlocomotion

Experiment 3 examined the effects of repeated olanzapine and chlordiazepoxide treatment on amphetamine or phencyclidine-induced