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Research report

Environmental and behavioral controls of the expression of clozapine tolerance: Evidence from a novel across-model transfer paradigm

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HIGHLIGHTS

- Repeated clozapine treatment caused a tolerance effect in the avoidance conditioning model.
- Repeated clozapine treatment also caused a tolerance effect in the PCP hyperlocomotion model.
- The transfer of clozapine tolerance from one model to another is situational specific and time-dependent.

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ABSTRACT

Repeated administration of antipsychotic drugs induces a sensitization-like or tolerance-like effect in many behavioral tasks, including the conditioned avoidance response (CAR) and the phencyclidine (PCP)-induced hyperlocomotion, two rodent models with high predictive validity for antipsychotic activity. This study investigated the impacts of contextual and behavioral variables on the expression of clozapine tolerance using a recently validated across-model transfer paradigm (Zhang and Li, 2012 [1]). Male Sprague-Dawley rats were first repeatedly treated with clozapine (2.5–10.0 mg/kg, sc) in the CAR model or PCP (1.6 mg/kg, sc)-induced hyperlocomotion model for five consecutive days. They were then tested for the expression of clozapine tolerance in another model for another 5 days. Finally, all rats were switched back to the original model and tested again for the expression of clozapine tolerance. When tested in the PCP model, rats previously treated with clozapine in the CAR model did not show an immediate weaker inhibition of PCP-induced hyperlocomotion than those treated with clozapine for the first time, but showed a significantly weaker inhibition over time. In contrast, when tested in the CAR model, rats previously treated with clozapine in the PCP model showed an immediate weaker disruption of avoidance response than those treated with clozapine for the first time, but this weaker effect diminished over time. These results suggest that the expression of clozapine tolerance is strongly modulated by the test environment and/or selected behavioral response. Clozapine tolerance and its situational specificity may be related to the drug's low extrapyramidal motor side effect, its superior therapeutic efficacy and/or emergence of clozapine withdrawal syndrome.

1. Introduction

Clozapine (CLZ) is the prototypical atypical antipsychotic drug with superior efficacy in treating the negative symptoms (e.g. social withdrawal, anhedonia) and cognitive deficits (e.g. attentional deficits) associated with schizophrenia, and patients who respond poorly to other antipsychotic medications [2–5]. Animal studies find that CLZ differs from other antipsychotics in many ways. For example, repeated treatment of haloperidol elicits dopamine supersensitivity (up-regulation of D_2^{High} receptors), whereas repeated treatment of CLZ has little effect on D_2^{High} up-regulation [6,7]. Neuroanatomically, CLZ, but not haloperidol, shows greater selectivity for the mesolimbic dopamine system than for the nigrostriatal dopamine system [8,9]. Behaviorally, repeated treatment of haloperidol, olanzapine and risperidone tends to cause a sensitization [1,10–12], whereas repeated CLZ causes a tolerance [11,13]. For

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instance, in a motor function and attention test, Stanford and Fowler [14] reported that CLZ-treated rats exhibited tolerance to the drug's suppressive effect on the amount of time that rats were in contact with a force-sensing target disk. In contrast, haloperidol-treated rats displayed little tolerance on this measure. In a fixed ratio 5 lever pressing test, Trevitt et al. [15] found that both haloperidol and CLZ significantly suppressed lever pressing. However, with repeated injections, haloperidol enhanced its suppression, while CLZ decreased it. In a lever pressing for food reward task, Varvel et al. [16] and Villanueva and Porter [17] also found that repeated haloperidol produced a significant increase in response duration, while repeated CLZ produced a decrease. CLZ-induced tolerance has also been observed in a drug discrimination task [18,19].

The conditioned avoidance response (CAR) and phencyclidine (PCP)-induced hyperlocomotion are two tests commonly used to study antipsychotic drugs. Both tests have high predictive validity for antipsychotic efficacy, as acute administration of antipsychotic drugs, but not anxiolytics or antidepressants selectively disrupts avoidance response, and suppresses PCP-induced hyperlocomotion [20–25]. In recent years, we have examined the long-term consequences of repeated CLZ treatment in both tests. First, rats are repeatedly treated with different doses of CLZ for 5 days then their avoidance responses and PCP-induced hyperlocomotion are recorded. This period is termed the *induction* phase. Two days later, all rats are given a challenge dose of CLZ (5.0 mg/kg) to assess the expression of CLZ tolerance (termed the *expression* phase). In the CAR model [11,13], we found that although repeated administration of CLZ produces an inhibition of avoidance responses persistently during the induction phase (no apparent tolerance), in the expression phase when all rats are challenged with a low dose of CLZ, rats previously treated with CLZ made significantly *more* avoidances than those who are treated with this drug for the first time, indicative of CLZ tolerance. Similarly, in the PCP hyperlocomotion model, repeated CLZ treatment dose-dependently and persistently inhibits PCP-induced hyperlocomotion in the induction phase (no apparent tolerance) [23], but exhibits a tolerance effect in the expression phase as rats previously treated with CLZ have a weaker inhibition of PCP-induced hyperlocomotion than those treated with CLZ for the first time. The overall pattern of CLZ effect in both models is in sharp contrast to the effects of haloperidol, olanzapine and risperidone which cause a sensitization [1,11,13].

Despite being widely demonstrated, experimental conditions that govern the expression of CLZ tolerance are still poorly understood. For example, the extent to which the expression of CLZ tolerance is modulated by contextual cues and behavioral variables is not known. How CLZ's action on avoidance responses and on PCP-induced hyperlocomotion, in turn, influences the rate and extent of its tolerance development is also not known. In this study, we examined how the environmental cues and behavioral responses affect the expression of CLZ tolerance using a novel across-model transfer paradigm [1,26]. Our general approach was to repeatedly treat animals with CLZ in one model to induce a tolerance process, then to test its expression in another model, and finally to retest its expression back in the original model. We recently used this paradigm and determined that the expression of haloperidol and olanzapine sensitization in the CAR and PCP models is strongly influenced by test environment and/or selected behavioral response [1]. This study extended this line of research into CLZ. If CLZ tolerance results from inevitable neurobiological adaptations produced by the direct pharmacological actions of the drug, it should persist into another model. However, if the contextual cues and behavioral responses associated with different models have a powerful control on the expression of CLZ tolerance, it should not be detectable when the model is changed.

2. Materials and methods

2.1. Animals

Adult 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 6:30 a.m. and 6:30 p.m.). Room temperature was maintained at 22 ± 1 °C with a relative humidity of 45–60%. 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

Clozapine (CLZ, gift from NIMH drug supply program) was dissolved in 1.2% glacial acetic acid in distilled sterile water [11,27]. Phencyclidine hydrochloride (PCP, gift from the NIDA Chemical synthesis and Drug Supply Program) was dissolved in 0.9% saline. All drugs were administrated subcutaneously in a volume of 1.0 ml/kg body weight. In the first experiment (from CAR to PCP), we tested three doses of CLZ (2.5, 5.0, 10.0 mg/kg) to assess the possible dose-dependent nature of CLZ tolerance. These doses of CLZ produce a reliable disruption on avoidance responding and inhibit the PCP-induced hyperlocomotion [23,24]. Furthermore, CLZ at 5.0 and 10.0 mg/kg gives rise to a clinically comparable range (40–60%) of striatal dopamine D_2 occupancy [27]. Based on findings from the first experiment and our previous work [23], we tested CLZ at 5.0 mg/kg in the second experiment (from PCP to CAR). The dose of PCP (1.6 mg/kg, sc) was based on our own study [1] and others [28].

2.3. Apparatus

2.3.1. Two-way avoidance conditioning apparatus

Eight identical two-way shuttle boxes custom designed and manufactured by Med Associates (St. Albans, VT) were used. Each box was housed in a ventilated, sound-insulated isolation cubicle (96.52 cm *W* × 35.56 cm *D* × 63.5 cm *H*). Each box was 64 cm long, 30 cm high (from grid floor), and 24 cm wide, and was divided into two equal-sized compartments by a partition with an arch style doorway (15 cm high × 9 cm wide at base). A barrier (4 cm high) was placed between the two compartments, so the rats had to jump from one compartment to the other. The grid floor consisted of 40 stainless-steel rods with a diameter of 0.48 cm, spaced 1.6 cm apart center to center, through which a scrambled footshock (Unconditioned stimulus, US, 0.8 mA, maximum duration: 5 s) was delivered by a constant current shock generator (Model ENV-410B) and scrambler (Model ENV-412). The rat location and crossings between compartments were monitored by a set of 16 photobeams (ENV-256-8P) affixed at the bottom of the box (3.5 cm above the grid floor). Illumination was provided by two houselights mounted at the top of each compartment. The conditioned stimulus (CS, i.e. 76 dB white noise) was produced by a speaker (ENV 224 AMX) mounted on the ceiling of the cubicle, centered above the shuttle box. Background noise (approximately 74 dB) was provided by a ventilation fan affixed at the top corner of each isolation cubicle. All training and testing procedures were controlled by Med Associates programs running on a computer.

2.3.2. Motor activity monitoring 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. Experiments

2.4.1. Experiment 1: transferability of CLZ tolerance from the CAR model to the PCP hyperlocomotion model and back to the CAR model

This experiment examined whether the tolerance induced by repeated CLZ treatment in the CAR model expressed in the PCP-induced hyperlocomotion model. The experiment comprised the following three phases: avoidance training/repeated CLZ treatment in the CAR, tolerance expression test in the PCP hyperlocomotion model, and tolerance expression retest back in the CAR model.

2.4.1.1. Avoidance training/repeated CLZ treatment in the CAR. Eighty-eight rats (in 2 batches) were first handled and habituated to the CAR boxes for 2 days (20 min/day). They were then trained for conditioned avoidance responding for a total of 10 sessions over a 2-week period. Each session consisted of 30 trials, with inter-trial intervals randomly varying between 30 and 60 s. Every trial started with a presentation of a white noise (CS) for 10 s, followed by a continuous scrambled foot shock (0.8 mA, US, maximum duration = 5 s) on the grid floor. If a subject crossed from one compartment into the other within the 10 s of CS presentation, it avoided the shock and this shuttling response was recorded as avoidance. If the rat remained in the same compartment for more than 10 s and made a crossing only after receiving the