Effects of olanzapine and clozapine on memory acquisition, consolidation and retrieval in mice using the elevated plus maze test

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Clozapine and olanzapine are antipsychotic drugs commonly used to treat schizophrenia and psychosis; however, few studies have investigated their effects on cognitive function using animal models. Thus, the effects of olanzapine and clozapine on memory acquisition, consolidation and retrieval were investigated in naïve mice using a modified elevated plus maze (mEPM) task. Olanzapine (0.15 and 0.30 mg/kg) and clozapine (0.5 and 1 mg/kg) were injected intraperitoneally (i.p.) into male Balb-c mice before training, immediately after training or before the second day of the trial. Our results showed that both olanzapine and clozapine disrupted the acquisition of spatial memory. In addition, clozapine impaired the consolidation of spatial memory, while olanzapine had no effect. Furthermore, olanzapine and clozapine significantly disrupted memory retrieval in naïve mice. Thus, these results at least suggest that olanzapine can be a superior treatment for schizophrenia compared to clozapine.

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1. Introduction

During the past decade, neurodevelopmental and cognitive function disorders have received increased attention in schizophrenia. The level of cognitive processing determines social and occupational function [21,36]. Atypical antipsychotics are commonly used to treat schizophrenia. Such drugs have a different mechanism than classical antipsychotic drugs and exert both 5-HT2 and D2 antagonistic effects with fewer extrapyramidal side effects [9,18]. Clozapine and olanzapine are effective and safe atypical antipsychotics commonly used for the treatment of psychosis, schizophrenia, psychotic depression and mania with bipolar disorder. Atypical antipsychotic drugs have important clinical advantages and improve cognitive function compared to typical antipsychotic drugs [10,12,28].

Improving cognitive function should be a characteristic of all newly developed drugs used for the treatment of schizophrenia with dementia. Limited studies that investigate the effects of atypical antipsychotics on memory processes in animal models have been conducted. Therefore, we determined the effects of commonly used atypical antipsychotic olanzapine on memory acquisition, consolidation and retention using the modified elevated plus maze test (mEPM) and compared these to the effects of the atypical antipsychotic clozapine.

2. Materials and methods

2.1. Animals

179 male inbred BALB/c ByJ mice (MAM TUBİTAK, Gebze, Kocaeli, Turkey) aged 7 weeks upon arrival to the laboratory were used in this study. Animals (4–5 per cage) were kept in the laboratory at 21 ± 1.5 °C with 60% relative humidity under a 12 h light/dark cycle (light on at 8.00 p.m.) for 2 weeks before experimentation. Tap water and food pellets were available ad libitum. All procedures involving animals were in compliance with the European Community Council Directive of 24 November 1986, and ethical approval was granted by the Kocaeli University Ethics Committee (number: AEK 10/6, Kocaeli, Turkey). All animals were naive to the experimental apparatus, and different animals were used for each test.

2.2. Modified elevated plus-maze test

Cognitive behavior was evaluated using the mEPM learning task, which measures spatial long-term memory [29]. The maze was
made of wood and consisted of two open arms (29 × 5 cm) surrounded by a short (1 cm) plexiglass edge to avoid falls and two enclosed arms (29 × 5 cm, 15 cm) arranged such that the two open arms were opposite to each other. The arms were connected by a central platform (5 × 5 cm). The maze was elevated 40 cm above the floor. The principle of this experiment is based upon the aversion of rodents to open spaces and heights. The animals prefer the enclosed, protected areas of the maze.

The procedure was performed as described previously [13–15,29]. During the acquisition session (day 1), each mouse was gently placed at the distal end of an open arm facing away from central platform. The time it took for the mice to move from the open arm to either of the enclosed arms (transfer latency) was recorded. Training (repeated exposure of animals to the open arms) shortened this parameter, possibly as a consequence of learning acquisition and retention. If the mouse did not enter the enclosed arm within 90 s, it was excluded from further experimentation. Animal entry into the enclosed arm required the animal to cross an imaginary line separating the enclosed arm from the central space with all four legs. After entering the enclosed arm, mice were allowed to move freely in the maze regardless of open and enclosed arms for 10 s. Mice were then returned to their home cage. The retention session followed 24 h after the acquisition session (on day 2). Mice were placed into the open arm, and the transfer latency was recorded again. Experiments were conducted between 10:00 and 14:00 h in a dimly lit, semi-soundproof room under a natural light.

2.3. Open field test

The effects of drugs on locomotor activity were measured using the open field test. This test is also known to examine anxiety-like behavior and to be sensitive to anxiolytic drug treatment [27] and was performed as previously described [3]. The testing apparatus consisted of a wooden box (33 cm × 33 cm × 30 cm) with an indirect red light. An animal was placed in the center of the test box. During a 5-min period, the total distance moved in the arena, the speed of the animal and the time spent in the center zone were recorded using Ethovision-XT software (Noldus).

2.4. Drug administration

Olanzapine was a gift from the Biofarma drug company (Istanbul, Turkey), and clozapine was a gift from the Adeka drug company (Samus, Turkey). Olanzapine and clozapine were dissolved in saline supplemented with 0.1 M hydrochloric acid. All drugs were freshly prepared and administered in a volume of 0.1 ml per 10 g body weight. The control groups received the same volume of vehicle. Olanzapine (0.15 and 0.30 mg/kg), clozapine (0.5 and 1 mg/kg) or vehicle was administered intraperitoneally (i.p.) 60 and 30 min, respectively, before the first session (training; day 1) testing memory acquisition, immediately after the first session (day 1) testing memory consolidation, and before the second session (day 2) evaluating memory retrieval in the mEPM test. Olanzapine (0.15 and 0.30 mg/kg), clozapine (0.5 and 1 mg/kg) or vehicle was administered intraperitoneally (i.p.) 60 and 30 min, respectively, before the open field test. The number of animals per group ranged from 6 to 10. The effective dose of each drug was selected according to previous behavioral and neurochemical studies [7,8,32].

2.5. Statistics

The Wilcoxon signed rank test was used to compare the differences between the first and the second transfer latencies in the mEPM test. To evaluate the differences among drug treatment groups during the first and second transfer latencies in the mEPM test, the Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) was used, followed by Dunn’s post hoc test. The total distance moved, the speed of the animal and the time spent in the center zone in the open field test were analyzed by one-way analysis of variance (ANOVA).

3. Results

3.1. Effects of olanzapine on memory acquisition, consolidation and retrieval in the modified elevated plus maze test

When olanzapine (0.15 and 0.3 mg/kg) was administered before the first session (training; day 1), there was no significant difference in first day latency (TL1) among the groups (p > 0.05). Olanzapine (0.15 and 0.3 mg/kg) significantly prolonged latency (TL2) on the second day compared to control group when the drug was administered before the acquisition session (Kruskal–Wallis H = 13.83; p < 0.05 and p < 0.01, respectively) (Fig. 1a).

Olanzapine (0.15 and 0.3 mg/kg) administered immediately after the first session (day 1) did not significantly affect memory consolidation (Kruskal–Wallis H = 0.95; p > 0.05, Fig. 1b).

Olanzapine administered before the second session (day 2) significantly prolonged TL2 (Kruskal–Wallis H = 9.66; p < 0.05) (Fig. 1c). In the comparison of TL1 and TL2 for each drug-treated group, TL2 was significantly decreased in the control group (p < 0.05), while this measure was not significantly different between the drug-treated groups (Fig. 1a–c).
3.2. Effects of clozapine on memory acquisition, consolidation and retrieval in the modified elevated plus maze test

Clozapine (0.5 mg/kg) administered before the acquisition session did not significantly affect the first session (day 1) latency ($p > 0.05$). However, clozapine (0.5 mg/kg) significantly prolonged the second session (day 2) latency (TL$_2$) compared to the control group when administered before the acquisition session (Kruskal–Wallis $H = 0.02; p < 0.05$) (Fig. 2a).

Administration of 1 mg/kg clozapine immediately after the acquisition session significantly prolonged TL$_2$ (Kruskal–Wallis $H = 7.88; p < 0.05$), while 0.5 mg/kg clozapine had no effect (Fig. 2b).

Clozapine (0.5 and 1 mg/kg) significantly prolonged TL$_2$ when administered before the retention trial (Kruskal–Wallis $H = 10.92, p < 0.05, p < 0.01$, respectively) (Fig. 2c).

In the comparison of TL$_1$ and TL$_2$ between each drug-treated group, TL$_2$ was significantly decreased in the control group ($p < 0.05$), while this measure was not significantly different between the drug-treated groups (Fig. 2a–c).

3.3. Effects of olanzapine and clozapine on locomotor activity and anxiety-like behavior in the open field test

There was no significant difference between groups when the effect of a single injection of olanzapine (0.15 and 0.3 mg/kg) or clozapine (0.5 and 1 mg/kg) on the total distance traveled (cm) in naive mice was evaluated using the open field test [one-way ANOVA; $F(4,35)=0.92; p>0.05$, Fig. 3a]. Moreover, drug treatment neither affected the time spent in the center zone [One-way ANOVA; $F(4,35)=0.1; p>0.05$, Fig. 3b], nor the speed of the animals [One-way ANOVA; $F(4,35)=0.29; p>0.05$, Fig. 3c] in the open field test.

4. Discussion

In our study, clozapine and olanzapine significantly prolonged latency in the second session (day 2) (TL$_2$) when administered before the acquisition trial. Administration of clozapine immediately after the acquisition trial significantly prolonged TL$_2$, while olanzapine had no effect. Clozapine and olanzapine significantly prolonged TL$_2$ when administered before the retention trial. Therefore, both olanzapine and clozapine disrupted the acquisition and retention of spatial memory in naive mice; however, olanzapine did not affect memory consolidation. Drug treatment affected neither locomotor activity nor the anxiety-like behavior, as assessed by the open field test.

Recent neurophysiological studies suggest that the hippocampo–prefrontal dialogue would be altered in schizophrenia models. Sigurdsson et al. [31] showed that oscillatory coupling
in the theta band is impaired in mice genetic models. Such coupling would be determinant for tagging neuronal assemblies in the two structures during learning [4] and that would favor reactivation (and presumably consolidation) during subsequent sleep episodes [26]. Interestingly, the initial hippocampo–prefrontal tagging requirement for long term memory trace storage has been beautifully shown in a recent paper [20].

The effects of antipsychotics on learning and memory are controversial. Haloperidol and risperidone impair cognition at doses used to treat psychosis, whereas clozapine and sertindole treat psychosis effectively without producing detrimental effects on cognition [2,7,32]. Wolff and Leander [38] concluded that olanzapine has a detrimental effect on learning but a beneficial effect on memory consolidation and/or retention, as assessed by the delayed radial arm maze test. Haloperidol and risperidone produced significant cognitive side effects at doses effective in psychosis models, while clozapine and sertindole were effective in the psychosis models without detrimental effects on cognition [2,7,32].

Second generation atypical antipsychotic drugs strongly affect the serotonin system and increase prefrontal dopamine levels; thus, these drugs alleviate negative symptoms and cognitive dysfunction in schizophrenia [1]. Clozapine possesses a broad receptor binding profile [2]. Clozapine does not produce extrapyramidal side effects due to low dopamine D2 receptor occupancy and potentially due to its anticholinergic activity [2]. Olanzapine is similar to clozapine in structure but possesses a different receptor binding profile. This drug exhibits a similar preference for the 5-HT2A and dopamine D2 receptors [2]. Despite its anticholinergic activity, olanzapine has been shown to improve cognition in schizophrenia [24]. It has been postulated that olanzapine has a detrimental effect on learning but beneficial effects on memory consolidation and/or retention [7]. Olanzapine is a more effective dopamine D2 receptor antagonist than sertindole and clozapine, which may contribute to cognitive impairment in naïve mice [7]. Clozapine and olanzapine block muscarinic and histaminergic H1 receptors [5], blockade of which impairs cognitive performance. Clozapine exerts weak partial agonist/antagonist activity on different subtypes of muscarinic receptors [37]. It is well known that the muscarinic receptor antagonist scopolamine and the histamine H1 receptor antagonists pyrilamine and diphenhydramine induce learning and memory impairment, as assessed by the Morris water maze [6] and/or radial arm maze tests [33] in rats.

Both clozapine and olanzapine may impair learning and memory in animals in the mEPM test because they bind to either M1 or H1 receptors. Olanzapine induces high affinity antagonism of the muscarinic M1 receptors [5]. In addition, orally administered clozapine also blocks muscarinic M1 receptor-mediated behaviors [25]. Clozapine potently antagonizes histamine H1 receptors, which limits its beneficial cognitive effects. There is a correlation between increased histamine occupancy and decreased cognitive performance [34]. Therefore, many variables determine the efficacy of clozapine and olanzapine, which may also depend on the specific test task.

The mEPM test is a simple method that evaluates spatial memory. Shortened transfer latency in the second trial is used as a parameter to measure the retention or consolidation of memory, and drug treatment prior to first day may be utilized to determine the effects on memory acquisition [30]. In our study, drugs were administered before the first session to evaluate the effects on memory acquisition. In addition, drugs were administered just after the first session to evaluate the effects on memory consolidation. Finally, drugs were administered just before the second session to evaluate the effects on memory retention. Evaluation of the effects of drug in the first trial may be confounded by non-specific effects, such as effects on anxiety, locomotion and motility [16]; however, these drugs had minimal non-specific effects on anxiety and locomotion, as assessed by the open field test.

Hall [11] originally described the open field test for the study of animal emotional behavior. In this procedure, an animal, usually a rodent, is placed in an unknown environment from which escape is prevented by surrounding walls [35]. The open field test is now one of the most popular procedures used to study animal psychology [3]. In such a situation, rodents prefer the periphery of the apparatus to the center of the open field. An increase in time spent in the center area without modification of total locomotion and vertical exploration can be interpreted as decreased anxiety. Behavioral studies have reported anxiolytic-like and anxiogenic-like effects as well as a lack of effects with the use of typical or atypical antipsychotic drugs in a broad range of animal models of fear or anxiety [17,19]. In recent studies, clozapine, olanzapine and chloridiazepoxide all induced anxiolytic effects when fear/anxiety was measured [22,23]. Our results confirmed that olanzapine and clozapine had no effect on anxiety, as assessed by the open field test. The discrepancy between studies may be due to aspects of methodology, such as the type of task tested, training schedule, route of administration and dose of the drug.

Determining the effects of antipsychotics on cognition is important to evaluate its clinical availability, usefulness and safety for the treatment of schizophrenic and dementia patients. In conclusion, both clozapine and olanzapine had detrimental effects on different aspects of memory, which are controlled by various receptor and neurotransmitter systems. These results suggest that olanzapine may be a superior treatment for schizophrenia compared to clozapine because olanzapine did not alter memory consolidation. As the improvement of cognitive function should be desired for drug therapy of schizophrenia, future studies investigating the effects of olanzapine and clozapine on cognition in schizophrenic animal models will evaluate whether these drugs may be safely used for the treatment of schizophrenic patients.

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References


