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Effects of olanzapine, sertindole and clozapine on learning and memory in the Morris water maze test in naive and MK-801-treated mice

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ABSTRACT

Cognitive dysfunction in schizophrenia is associated with functional disease symptoms. The beneficial effects of second generation antipsychotic drugs on cognitive function in schizophrenic patients are controversial. In this study, we investigated the effects of the second generation antipsychotics olanzapine, sertindole and clozapine on cognitive function in the Morris water maze task in naive or MK-801-treated animals. Male balb-c mice were treated subchronically with olanzapine (1.25, 2.5 and 5 mg/kg, i.p.), sertindole (0.63, 1.3, 2.5 mg/kg, s.c.) or clozapine (0.5 and 1 mg/kg, i.p.), and cognitive deficits were induced by MK-801 (0.2 mg/kg, i.p.) administration. Water maze performance was expressed as escape latency to find the hidden platform, the time spent in target quadrant, the mean distance to platform and the swim speed. In naive mice olanzapine impaired water maze performance, whereas sertindole and clozapine had no effect while the MK-801-induced cognitive impairment was reversed by the second generation antipsychotics — olanzapine, sertindole and clozapine at the doses used. These results revealed that while olanzapine had some disturbing effects on cognitive functions in naive animals; olanzapine, sertindole and clozapine might improve cognitive deficits in schizophrenic patients.

1. Introduction

For many years, positive psychotic symptoms were the primary target in the treatment of schizophrenia. However, schizophrenia is also associated with cognitive dysfunction (Green et al., 2000). Cognitive impairment in schizophrenia is common, and it decreases the quality and function of life (Addington and Addington, 1999); these impairments manifest primarily as disruptions in working memory as well as learning and attention. Cognitive dysfunctions indicate the initial illness and lead to the development of psychotic symptoms (Tollefson, 1996). These problems also diminish the ability of the patient to care for oneself and increase the frequency of hospitalization, thus increasing the cost to society (Sevy and Davidson, 1995). Therefore, effective treatment of cognitive deficits in schizophrenic patients may greatly impact the patient's quality of life.

One current hypothesis is that these cognitive impairments are caused by hypofunction of the N-methyl-D-aspartate (NMDA) receptor (Krystal et al., 1994). NMDA receptor antagonists, such as ketamine and phencyclidine, induce schizophrenia-like symptoms in healthy subjects, including positive, negative, and cognitive symptoms (Krystal et al., 1994). NMDA receptor antagonists also disturb learning and memory functions in animals that are similar to those seen in schizophrenia; these agents are useful for establishing animal models of cognitive impairment (Wass et al., 2006; Didriksen et al., 2007).

It is assumed that atypical antipsychotic therapy is more effective to treat cognitive dysfunction, in comparison to classical antipsychotics. In clinical studies, the second generation atypical antipsychotic agents clozapine, ziprasidone, quetiapine and olanzapine improved cognitive impairment, whereas typical agents, such as haloperidol, had no effect (Harvey and Keefe, 2001; Purdon et al., 2001). In preclinical cognitive tests, varying results have been observed with atypical antipsychotics on normal cognitive functions. In some studies, antipsychotics disturbed cognitive functions, whereas these agents produced no effect in other studies (Didriksen, 1995; Skarsfeldt, 1996; Didriksen et al., 2006).

The Morris water maze task (MWM) is a visual spatial learning and memory task that depends on the coordinated action of several brain regions and neurotransmitter systems (D'Hooge and De Deyn, 2001). Specific deficits were found in animals with damage to hippocampus, striatum, basal forebrain, cerebellum and several neocortical areas, and specific roles in MWM performance have been proposed for these

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regions. McNamara and Skelton (1993) reviewed the involvement of different neurotransmitter and modulator systems in spatial learning, and suggested that only the cholinergic, glutamatergic, and some peptidergic systems may really be required for this kind of learning, whereas systems using γ-aminobutyric acid (GABA), opioids or biogenic amines are either detrimental or unrelated to these functions. Performance in relies on several cognitive functions, including learning, working and long-term memory, retention, and attention, all of which are deficient in schizophrenia (Morris, 1984). MK-801 is a NMDA receptor antagonist which is widely used as an animal model of psychosis and induces a variety of cognitive disturbances related to schizophrenia. MK-801 can disrupt normal Morris water maze performance similar to cognitive deficits seen in schizophrenic patients (Enomoto et al., 2008). It impairs learning and memory functions that depend on the hippocampus and the amygdala (Jafari-Sabet, 2006).

Olanzapine is an atypical antipsychotic drug commonly used for the treatment of schizophrenia and other psychosis (Fulton and Goa, 1997). Atypical antipsychotics, such as olanzapine, exert less selective activity on various neurotransmitter receptors (i.e., moderately potent antagonists at $5HT_2$ receptors with lesser and approximately equal potency at D1, D2, and α1 receptors (Seeman, 2002)). Radioligand binding studies have shown that olanzapine has high affinity for several neuronal receptors, including DA D_1, D_2, D_4, 5-HT_{2A}, 5-HT_{2C}, α1-adrenergic, and histaminic H_1 and muscarinic receptors (Bymaster et al., 1996). Sertindole is an antipsychotic drug with a unique pharmacological profile (Arnt and Skarsfeldt, 1998). It is a phenylindole-derived, non-sedating antipsychotic drug with high affinity for only the dopamine D2, serotonine 5-HT2 and α1-adrenergic receptors. Sertindole demonstrated a favourable safety and tolerability profile, and was not associated with sedation, anticholinergic or histaminergic side effects, nor excessive weight gain and its effects on cognitive function in humans have not been investigated extensively.

Clozapine is the reference drug for atypical antipsychotics, and it acts on multiple receptors within the brain, including dopamine D4, serotonin 5-HT2a and 5-HT2c, norepinepherine α1 and α2, acetylcholine and muscarinic and histamine H1 receptors (Coward, 1992).

In this study, we investigated the effects of the second generation atypical antipsychotics olanzapine, sertindole and clozapine on spatial learning and memory both in naive and MK-801-treated mice using the Morris water maze test.

2. Materials and methods

2.1. Animals

Male, inbred balb/c ByJ mice (MAM TUBİTAK, Gebze-Kocaeli-Turkey) aged 7 weeks were used in this study. The animals were habituated to the laboratory for 2 weeks before experimentation. Mice were kept 4 to 5 per cage at 21±2 °C under a 12-h light/dark cycle (lights on at 8.00 a.m.). Tap water and food pellets were available ad libitum. All animals used for the experiments were naïve to the experiments. Each mouse was tested individually and used only once. All procedures were in compliance with the European Community Council Directive of 24 November 1986 and were approved by the Kocaeli University Ethics Committee (Number: AEK 1/2, Kocaeli, Turkey).

2.2. Morris water maze test

The Morris water maze was a circular pool (90 cm diameter and 30 cm height) filled with water (22 °C) to a depth of 14 cm and rendered opaque by the addition of small black balls. The pool was located in a dimly lit, soundproof test room with a various visual cues, including a white-black colored poster on the wall, a halogen lamp, a camera and the experimenter. The maze was divided into four quadrants, and three equally spaced points served as starting positions around the edge of the pool. The order of the release positions varied systematically throughout the experiment. A circular escape platform (6 cm diameter and 12 cm high) was located in one quadrant 1 cm above the water surface during the familiarization session and 1 cm below the water surface during the other sessions.

Video tracking was conducted with a video camera focused on the full diameter of the pool. Navigation parameters were analyzed by the Ethovision 3.1 video analysis system (Noldus, The Netherlands). The mice were trained in the Morris water maze five times daily (familiarization session, S_1, S_2, S_3, S_4). The following parameters were evaluated during each trial: escape latency (s) to find the hidden platform, the time spent in target quadrant (s), the mean distance to platform (cm) and the swim speed (cm/s). The escape latency, the time spent in target quadrant and the distance to platform calculations were used as measures for the development of spatial memory whereas swim speed was used to evaluate motor functions.

One familiarization and four acquisition sessions were performed using the Morris water maze. During the familiarization session and acquisition phase of the experiment, each mouse was given three trials. The delay between the trials was 60 s, and a 1-day interval was used between each session. For each trial, the mouse was taken from the home cage and placed into the water maze at one of three randomly determined locations with its head facing the center of the water maze. After the mouse had found and climbed on to the platform, the trial was stopped, and the escape latency was recorded. If the mouse did not climb onto the platform in 60 s, the trial was stopped, and the experimenter guided the mouse to the platform; the escape latency of 60 s was recorded.

Twenty-four hours after the last acquisition session, a 'probe trial' was used to assess the spatial memory retention of the location of the hidden platform. During this trial, the platform was removed from the maze and the mouse was allowed to search the pool for 60 s. The percent of time spent in each quadrant was recorded.

2.3. Experimental design

Olanzapine (1.25, 2.5 and 5 mg/kg), clozapine (0.5 and 1 mg/kg) and MK-801 (0.2 mg/kg) were administered intraperitoneally (i.p.) 60, 30 and 30 min before testing, respectively, whereas sertindole (0.63, 1.3, and 2.5 mg/kg) was injected subcutaneously (s.c.) 60 min before starting the test. The probe trial was performed on the sixth day of the test. The number of animals per group ranged from 6 to 10.

2.4. Drugs

MK-801 was purchased from Sigma (St. Louis, USA). Olanzapine was a gift from Biofarma (İstanbul, Turkey), sertindole was a gift from John Arth (Lundbeck Company, Denmark) and clozapine was a gift from the Adeka (Samsun, Turkey). Olanzapine and clozapine were dissolved in saline supplemented with 0.1 M hydrochloric acid. Sertindole was dissolved in distilled water supplemented with 0.1 M hydrochloric acid. MK-801 was dissolved in saline. All drugs were freshly prepared and given in a volume of 0.1 ml per 10 g body weight of mice. The control groups received the same volume of vehicle. The effective dose of each drug was selected according to pervious behavioral and neurochemical studies (Skarsfeldt, 1996; Didriksen et al., 2006, 2007).

2.5. Statistics

Repeated measures ANOVA and a post-hoc Tukey test were used to evaluate the effect of drug on performance in the MWM test. Wilcoxon paired t-test was used to compare the differences between the scores on the first and the last day of testing. Two way analysis of

variance (ANOVA) was used to determine significant differences between results from the probe trials. The post-hoc Dunnett test was performed when equal variances were assumed and a Dunnett's T_3-test was used when equal variances were not assumed in the comparison of the groups.

Data are expressed as the mean values ± SEM. Differences were considered to be statistically significant when P was less than 0.05.

3. Results

3.1. Effects of olanzapine, sertindole and clozapine on escape latency in naive mice in the Morris water maze test

There was a significant difference in escape latency in the 2nd, 3rd and 4th sessions during the evaluation of olanzapine (1.25, 2.5 and 5 mg/kg) in all groups [Two way ANOVA post-hoc Dunnett's t-test; $F(7,57)=5.16$, $p<0.001$; $F(7,57)=3.37$, $p=0.004$; $F(7,57)=4.88$, $p<0.001$; respectively; Fig. 1A]. Olanzapine (2.5 mg/kg) significantly increased the escape latency during the 3rd and 4th sessions ($p<0.05$), whereas at 5 mg/kg, it significantly increased the escape latency during the 2nd and 4th sessions ($p<0.05$) compared to control indicating that it impaired water maze performance in naive mice. When the effect of drugs on escape latency within each session was compared, a significant difference was observed in the effects on the control and 1.25 mg/kg olanzapine-treated groups [$F(4,36)=3.46$; $F(4,36)=6.17$; respectively, $p<0.05$], whereas no significant difference was found between the other groups (Fig. 1A).

The effects of sertindole (0.63, 1.3, and 2.5 mg/kg) on the escape latency in all groups were evaluated in the MWM test; significant differences were found between the groups during the 2nd, 3rd and 4th sessions [Two way ANOVA post-hoc Dunnett's t-test; $F(7,43)=7.74$, $p<0.001$; $F(7,43)=3.83$, $p=0.003$; $F(7,43)=6.42$, $p<0.001$; respectively; Fig. 1B]. Sertindole did not affect the escape latency at the doses tested indicating that it did not disturb cognitive performance in naive mice. When the effect of drugs on the escape latency within each sessions was compared, a significant difference was found between the control, sertindole (1.3 and 2.5 mg/kg) and MK-801 + sertindole 2.5 mg/kg-treated groups [$F(4,28)=7.97$; $F(4,20)=6.41$; $F(4,20)=5.10$; $F(4,20)=6.61$, respectively; $p<0.05$], whereas a significant difference was not found in other groups (Fig. 1B).

The escape latency of animals were significantly different in the 2th, 3rd and 4th sessions of the MWM test in clozapine treated groups [Two way ANOVA post-hoc Dunnett's t-test; $F(5,33)=6.94$, $p<0.001$; $F(5,33)=7.41$, $p<0.001$; $F(5,33)=13.74$, $p<0.001$; respectively; Fig. 1C]. Clozapine did not affect the escape latency of naive mice at the doses tested in this study indicating that it had no effect on water maze performance. Clozapine (0.5 and 1 mg/kg) significantly decreased the escape latency within each session [$F(4,28)=11.18$; $F(4,20)=4.08$; $F(4,20)=5.51$, respectively; $p<0.05$], whereas the other treatments did not have significant effects (Fig. 1C).

3.2. Effects of olanzapine, sertindole and clozapine on escape latency in MK-801 injected mice in the Morris water maze test

MK-801 (0.2 mg/kg) caused a significant disruption of learning and memory, indicated by an increase in the escape latency compared to the control animals ($p<0.05$). Olanzapine had no effect on the escape latency in MK-801-treated mice ($p>0.05$) (Fig. 1A).

Sertindole significantly decreased the escape latency when administered at 0.63 mg/kg in the 2nd session ($p<0.05$) and at 2.5 mg/kg in the 2nd, 3rd and 4th sessions ($p<0.05$) in MK-801-treated mice. At 1.3 mg/kg, sertindole did not affect the escape latency in MK-801-treated mice (Fig. 1B).

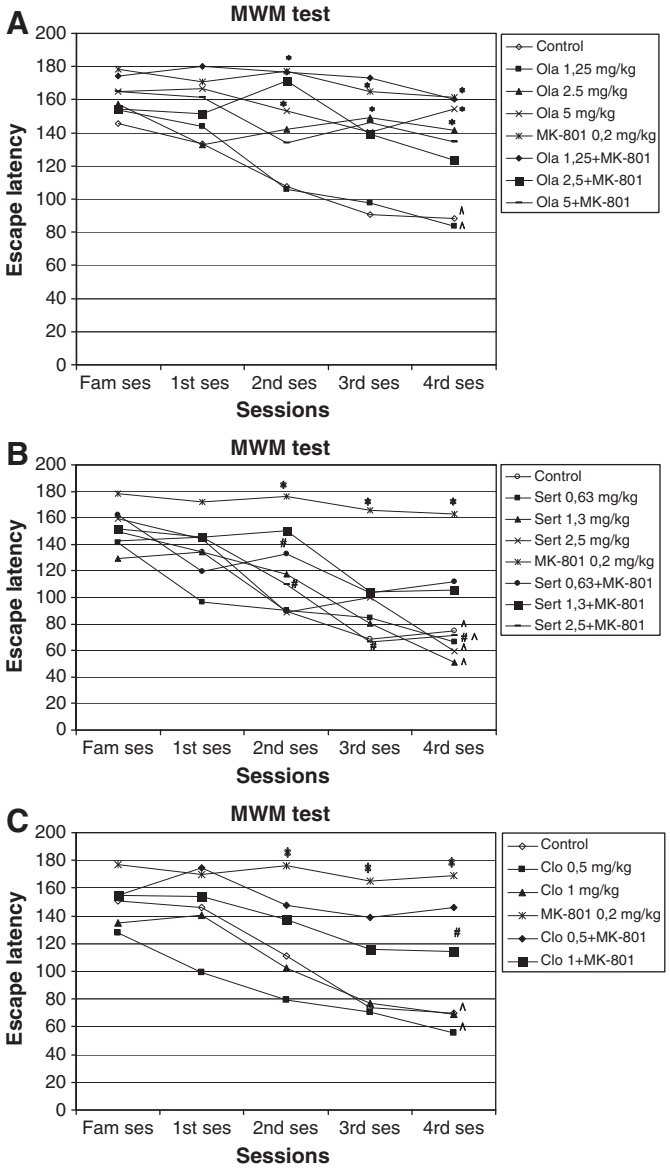

Fig. 1. Effects of antipsychotics on escape latency in the MWM test. (A) Olanzapine (1.25, 2.5 and 5 mg/kg,i.p) (B) sertindole (0.63, 1.3 and 2.5 mg/kg) (C) clozapine (0.5 and 1 mg/kg) or vehicle was administered daily 60, 60, and 30 min respectively before the first trial of the day for 5 days. MK-801 (0.2 mg/kg) was daily administered 30 min before the first trial of the day for 5 days alone or combined with antipsychotic drugs. Results are expressed as mean ± SEM. n = 8–10 per group. * $p<0.05$ vs. control group, #$p<0.05$ vs. MK-801 group, ^ $p<0.05$ the familiarization and the fourth sessions were compared for each drug group.

Clozapine significantly decreased the escape latency in the MK-801-treated mice when given at 1 mg/kg during the 4th session ($p<0.05$) (Fig. 1C).

3.3. Effects of olanzapine, sertindole and clozapine on time spent in target quadrant in naive mice in the Morris water maze test

A significant difference was observed between all olanzapine administered groups in the time spent in the target quadrant [Two way ANOVA post-hoc Dunnett's t-test; $F(7,57)=5.79$; $p<0.001$; Fig. 2A]. Olanzapine (2.5 and 5 mg/kg) significantly decreased the time spent in the escape platform's quadrant ($p<0.01$) in naive mice.

There was a significant difference between sertindole groups when the time spent in the escape platform quadrant during the probe trial of the MWM test was evaluated [Two way ANOVA post-

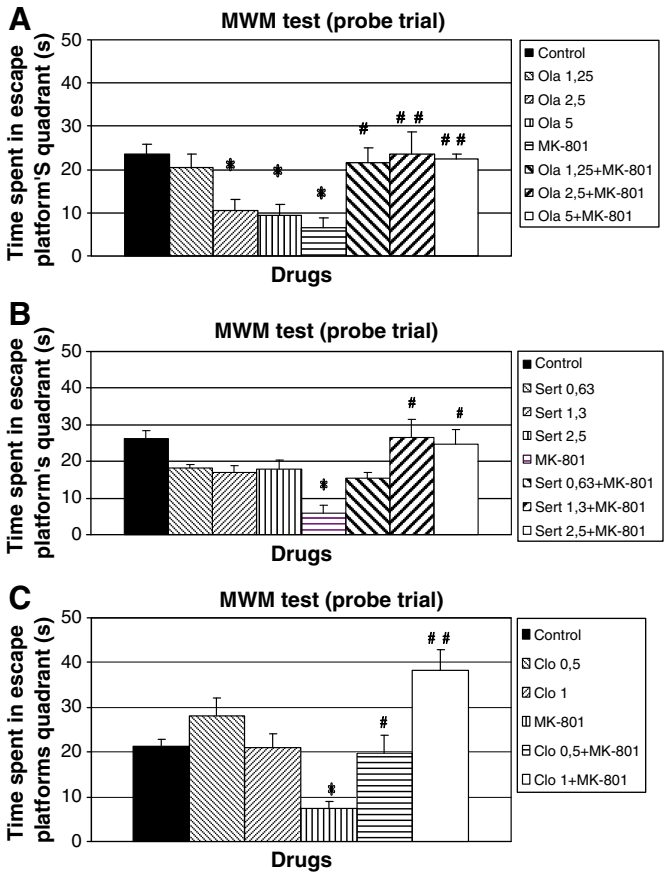

Fig. 2. Effects of antipsychotics on the time spent in escape platform's quadrant in the probe trial (60 s) of MWM test. (A) Ola: olanzapine (1.25, 2.5 and 5 mg/kg,i.p), (B)Sert: sertindole (0.63, 1.3 and 2.5 mg/kg), (C) Clo:clozapine (0.5 and 1 mg/kg) or vehicle was administered daily 60, 60, and 30 min respectively before the first trial of the day for 6 days. MK-801 (0.2 mg/kg) was daily administered 30 min before the first trial of the day for 6 days alone or combined with antipsychotic drugs. Results are expressed as mean ± SEM. n = 8–10. (A) * $p<0.01$ vs. control group, # $p<0.05$, ## $p<0.01$ vs. MK-801 group. (B) * $p<0.01$ vs. control group, # $p<0.01$ vs. MK-801 group. (C) * $p<0.05$ vs. control group, # $p<0.05$, ## $p<0.01$ vs. MK-801 group.

hoc Dunnett's t-test; $F(7,43)=6.48$; $p<0.001$; Fig. 2B]. Sertindole had no effect on the time spent in the escape platform quadrant in naive mice.

Also the time spent in the escape platform quadrant in clozapine injected groups differ during the probe trial of the MWM test [Two way ANOVA post-hoc Dunnett's t-test; $F(5,33)=10.09$; $p<0.001$; Fig. 2C]. Clozapine had no effect on the time spent in the escape platform quadrant in naive mice at the doses tested in this study.

3.4. Effects of olanzapine, sertindole and clozapine on time spent in target quadrant in MK-801 injected mice in the Morris water maze test

MK-801 (0.2 mg/kg) significantly decreased the time spent in the escape platform quadrant ($p<0.01$). Olanzapine (1.25, 2.5 and 5 mg/kg) significantly prolonged the time spent in the escape platform quadrant in MK-801-treated mice ($p<0.05$, $p<0.01$ and $p<0.01$, respectively) (Fig. 2A).

The time spent in the escape platform quadrant is significantly prolonged by sertindole (1.3 and 2.5 mg/kg) in MK-801-treated mice ($p<0.01$) (Fig. 2B).

Clozapine (0.5 and 1 mg/kg) significantly prolonged the time spent in the escape platform quadrant in MK-801-treated mice ($p<0.05$ and $p<0.01$, respectively) (Fig. 2C).

3.5. Effects of olanzapine, sertindole and clozapine on distance to platform in naive mice in the Morris water maze test

The mean distance to the platform in the probe trial of the MWM test was significantly different between the groups in olanzapine treated groups [Two way ANOVA post-hoc Dunnett's t-test; $F(7,57)=4.83$; $p<0.001$; Fig. 3A]. Olanzapine (2.5 and 5 mg/kg) significantly increased the mean distance to platform ($p<0.05$ and $p<0.01$, respectively) in naive mice suggesting that subchronic treatment of olanzapine disturbed learning and memory.

The mean distance to platform during the probe trial of the MWM test was significantly different between sertindole treated groups [Two way ANOVA post-hoc Dunnett's t-test; $F(7,43)=7.99$; $p<0.001$; Fig. 3B]. Sertindole significantly increased the mean distance to platform at 1.3 mg/kg ($p<0.05$), whereas it had no effect when administered at 0.63 and 2.5 mg/kg expressing that sertindole disturbed water maze performance in naive animals only at 1.3 mg/kg.

The mean distance to the platform was different between clozapine treated groups during the probe trial of the MWM test [Two way ANOVA post-hoc Dunnett's t-test; $F(5,33)=11.20$; $p<0.001$; Fig. 3C]. Clozapine had no effect on the behavior of the naive animals.

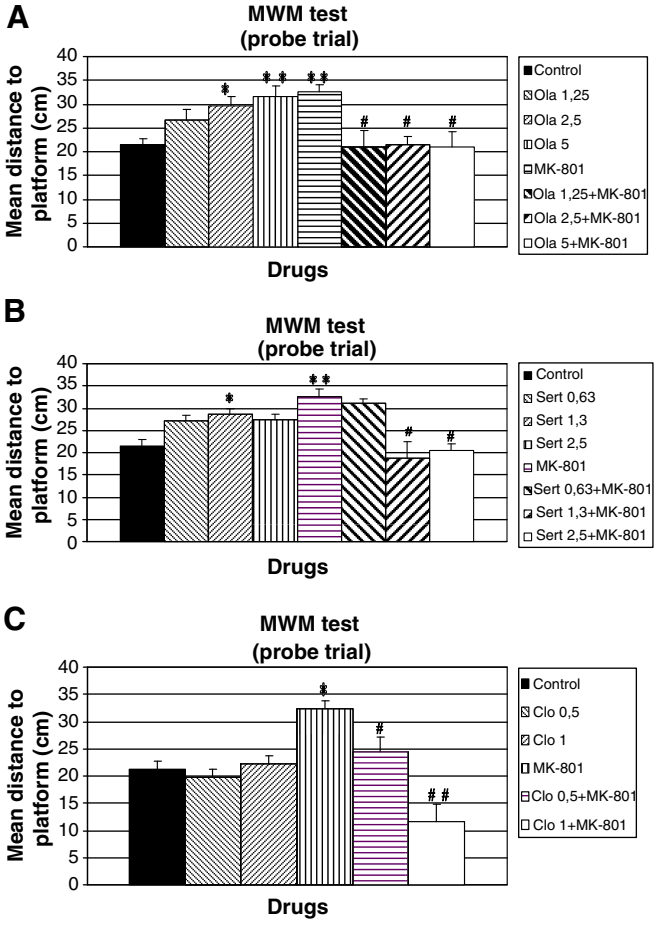

Fig. 3. Effects of antipsychotics on mean distance to platform in the probe trial (60 s) of MWM test. (A) Ola: olanzapine (1.25, 2.5 and 5 mg/kg,i.p), (B)Sert: sertindole (0.63, 1.3 and 2.5 mg/kg), (C) Clo:clozapine (0.5 and 1 mg/kg) or vehicle was administered daily 60, 60, and 30 min respectively before the first trial of the day for 6 days. MK-801 (0.2 mg/kg) was daily administered 30 min before the first trial of the day for 6 days alone or combined with antipsychotic drugs. Results are expressed as mean ± SEM. n = 8–10. (A,B) * $p<0.05$, ** $p<0.01$ vs. control group, # $p<0.05$ vs. MK-801 group. (C) * $p<0.01$ vs. control group, # $p<0.05$, ## $p<0.01$ vs. MK-801 group.

3.6. Effects of olanzapine, sertindole and clozapine on distance to platform in MK-801 injected mice in the Morris water maze test

MK-801 significantly increased the mean distance to the platform ($p<0.01$). Olanzapine (1.25, 2.5 and 5 mg/kg) significantly decreased the mean distance to the platform in MK-801-treated mice ($p<0.05$) (Fig. 3A) suggesting that it has some beneficial effects on cognitive performance.

Both sertindole (1.3 and 2.5 mg/kg) and clozapine (0.5 and 1 mg/kg) significantly decreased MK-801-induced increase in distance to platform ($p<0.05$) (Fig. 3B) and ($p<0.05$ and $p<0.01$, respectively) (Fig. 3C) suggesting that it has some beneficial effects on disturbed learning and memory.

3.7. Effects of olanzapine, sertindole and clozapine on motor function in naive and MK-801-treated mice

Each treatment group did not significantly differ in swimming speed in olanzapine treated groups [Two way ANOVA post-hoc Dunnett's t-test; $F(7,57)=2.22$; $p=0.05$]. Olanzapine (1.25, 2.5 and 5 mg/kg) or MK-801 (0.2 mg/kg) had no effect on swimming speed. The effect of olanzapine on motor function was expressed as swimming speed and was not different from the control group in MK-801-treated mice (Fig. 4A).

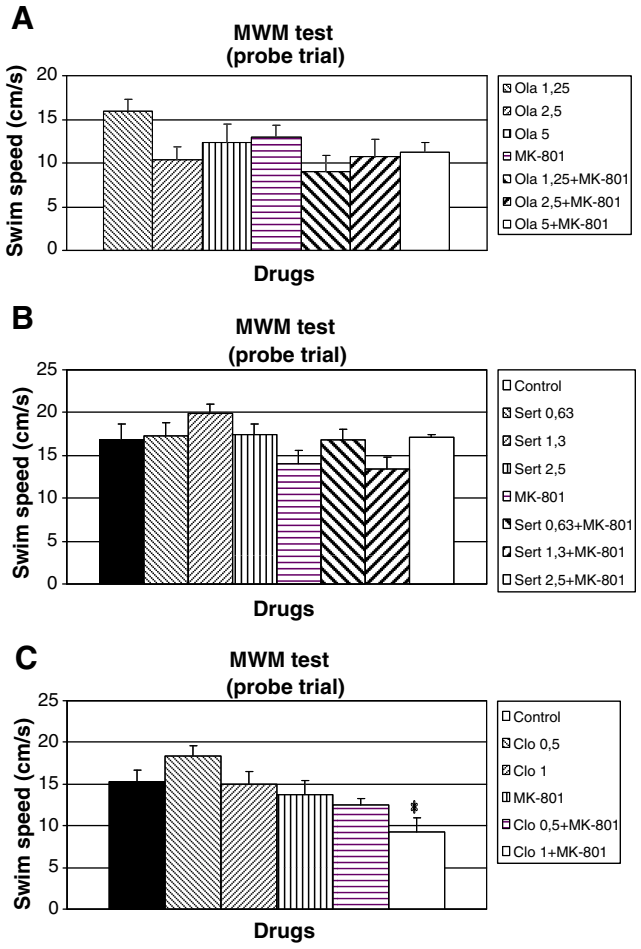

Fig. 4. Effects of antipsychotics on swimming speed in the probe trial (60 s) of MWM test. (A) Ola: olanzapine (1.25, 2.5 and 5 mg/kg, i.p.), (B)Sert: sertindole (0.63, 1.3 and 2.5 mg/kg), (C) Clo:clozapine (0.5 and 1 mg/kg) or vehicle was administered daily 60, 60, and 30 min respectively before the first trial of the day for 6 days. MK-801 (0.2 mg/kg) was daily administered 30 min before the first trial of the day for 6 days alone or combined with antipsychotic drugs. Results are expressed as mean ± SEM. n = 8–10. * $p<0.05$ vs. control group.

Drug administration did not significantly affect the swimming speed of the animals during the probe trial in sertindole treated groups [Two way ANOVA post-hoc Dunnett's t-test; $F(7,43)=2.05$; $p>0.05$]. Sertindole (0.63, 1.3 and 2.5 mg/kg) and MK-801 (0.2 mg/kg) had no effect on the swimming speed of mice (Fig. 4B).

A significant difference in the swimming speed of the animals was found between clozapine treated groups during the probe trial [Two way ANOVA post-hoc Dunnett's t-test; $F(5,33)=4.16$; $p<0.01$]. Clozapine (0.5 and 1 mg/kg) and MK-801 (0.2 mg/kg) had no effect on the swimming speed, whereas clozapine significantly decreased the swimming speed of MK-801-treated mice ($p<0.05$) (Fig. 4C).

4. Discussion

We investigated the effects of atypical antipsychotics on cognitive functions in naive and MK-801-treated mice. This study served as a pre-clinical evaluation of these drugs for use in patients with schizophrenia. The dose range for each drug was selected according to previous studies (Skarsfeldt, 1996) that determined the minimum effective dose to impair learning and memory (Skarsfeldt, 1996; Didriksen et al., 2006).

The main finding of this study is that the second generation atypical antipsychotic drug olanzapine disrupted spatial learning and memory in naive mice, whereas sertindole and clozapine had no effect. Since MK-801-induced impairment in learning and memory was reversed by olanzapine, sertindole and clozapine (1 mg/kg), these drugs may be effective for the treatment of cognitive dysfunctions in schizophrenia. The swimming speed was not affected, indicating that acute drug treatment had no effect on motor function.

The MWM test is a spatial and long-term memory task in which animals must use complex behavioral strategies to swim away from the pool wall, locate the platform, climb onto the platform, and remain on the platform (Cain, 1998). In a recent study, the NMDA receptor antagonists PCP and MK-801 impaired acquisition learning and reference memory in the MWM (Wass et al., 2006). In our study, MK-801 increased the escape latency during the acquisition session, decreased the time spent in the escape platform quadrant, and increased the mean distance traveled to the platform during the probe test; the drug treatment did not affect the swimming speed of mice. Because the position of the platform did not change throughout the experiment, these results indicate that MK-801 impaired the reference spatial memory.

In our study, antipsychotics were injected subchronically for six consecutive days. A decrease in the escape latency during each acquisition session reflects the memory of the learned task. Because the location of the escape platform was not changed throughout the experiment, our results reflect hippocampal-dependent reference memory. Repeated administration of drugs before each acquisition session and before the probe test was performed to evaluate the effects of olanzapine, sertindole and clozapine on learning and retrieval. Impaired learning and retrieval capacity are commonly reported in schizophrenia (Cairo et al., 2006). Accordingly, our study suggests that olanzapine, sertindole and clozapine improved MK-801-induced spatial learning and memory deficits in mice.

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 effectively treat psychosis without producing detrimental effects on cognition (Skarsfeldt, 1996; Arnt and Skarsfeldt, 1998; Didriksen et al., 2006). Haloperidol was also shown to disturb performance in the water maze (Skarsfeldt, 1996) and delayed-non-match to position performance (Didriksen, 1995) in rats. It is proposed that atypical antipsychotics are more effective at improving cognitive functions in comparison to classical antipsychotics; however, some studies report no effect of the drugs (Gallhofer et al., 1999). The majority of studies show that atypical antipsychotic drugs improve cognitive function (Stip et al.,

2003); however, studies of typical antipsychotics are controversial (Mortimer, 1997). For example, atypical antipsychotics, such as clozapine and olanzapine, attenuate cognitive deficits in schizophrenic patients in comparison to the effects of haloperidol (Smith et al., 2001).

Antipsychotics targeting the DA D_2 receptor may affect the positive symptoms of schizophrenia; however, the actions on non-DA D_2 receptors (DA D_1, D_3, and D_4), serotonin receptors (5-HT_{2A}, 5-HT_{1A}, 5-$HT_{3,6,7}$) and alpha-adrenergic receptors as well as other neurotransmitter receptors are hypothesized to be effective against the negative symptoms of schizophrenia (Sprague et al., 2004; Miyamoto et al., 2005). Effects on several of these receptors and, in particular, the balance between these effects is important for the reversal of MK-801-induced cognitive impairment. Several mechanisms may underlie the reversal of the MK-801-induced deficits by olanzapine, sertindole and clozapine.

The 5-HT_{2A} receptor regulates mesocortical dopamine projections, and the efficacy of atypical antipsychotics to block 5-HT_{2A} receptors within the prefrontal cortex may cause an increase in dopamine transmission and diminish cognitive dysfunctions in schizophrenic patients. Higher 5-HT_{2A}/dopamine D_2 receptor affinity is correlated with the successful treatment of the negative symptoms of schizophrenia (Altar et al., 1986), and these effects may be important for the relief of cognitive deficits. In recent studies, selective ligands for serotonin and adrenoceptors (e.g. WAY-100635, M100907, idazoxan, and others) have been examined in various NMDA receptor antagonist-induced animal models of cognitive impairment (Meneses, 2004; Marcus et al., 2005). In addition, post-training administration of the specific 5-HT_7 receptor antagonists SB-269970 and DR-4004 improved MK-801-induced memory impairment in the rat autoshaping task (Meneses, 2004). In our study, olanzapine, sertindole and clozapine reversed MK-801-induced deficits, possibly through interaction with serotonin receptors and adrenoceptors.

Sertindole has an equal preference for 5-HT_{2A} and dopamine D_2 receptors in vitro, whereas it exhibits minimal dopamine D_2 receptor blockade in vivo (Arnt and Skarsfeldt, 1998). In contrast to clozapine and olanzapine, sertindole has a very high affinity for 5-HT_6 receptors but fails to affect α_2-adrenergic, histaminergic, and muscarinic receptors (Arnt and Skarsfeldt, 1998). The 5-HT_6 receptor activation enhances cognition; this receptor is highly expressed in the hippocampus and cortex and interacts with cholinergic and glutamatergic systems. Receptor blockade has pro-cognitive effects and increases DA, glutamate, and acetylcholine concentrations in the frontal cortex (Hirst et al., 2006; Rodefer et al., 2006). Therefore, the marked efficacy of sertindole on cognition both in naive mice and MK-801-treated mice may be attributed to antagonism of 5-HT_6 receptors. Moreover, the role of α_2-adrenoceptors in cognition is not clear and needs further clarification.

Clozapine possesses a broad receptor binding profile, including all of the receptors mentioned earlier (Arnt and Skarsfeldt, 1998). The clozapine affinity ratio for 5-HT_{2A}/D_2 receptors is 9, and it does not produce extrapyramidal side effects due to low dopamine D2 receptor occupancy and potentially due to its anticholinergic activity (Arnt and Skarsfeldt, 1998).

Clozapine and olanzapine, in contrast to sertindole, block muscarinic and histaminergic H_1 receptors (Bymaster et al., 1999), blockade of which impairs cognitive performance. Clozapine exerts weak partial agonist/antagonist activity on different subtypes of muscarinic receptors (Weiner et al., 2004). In our study, clozapine had no effect on cognition in naive mice, and it improved MK-801-induced cognitive impairment. This finding may result from agonistic activity of clozapine and desmethyl-clozapine, its primary metabolite, on some muscarinic receptor subtypes (Weiner et al., 2004), whereas olanzapine, a muscarinic antagonist, elicits an opposite effect (Bymaster et al., 1999). Muscarinic M_1 agonism may improve cognition, whereas antimuscarinic activity potentially worsens cognitive function. Therefore, the effects of clozapine depend on a balance between the plasma and brain concentration of the compound (Weiner et al., 2004; Didriksen et al., 2007). Moreover, the discrepancy between studies may be due to aspects of methodology, such as tasks, training schedule, route of administration and dose.

It is well known that the muscarinic receptor antagonist scopolamine and the histamine H1 receptor antagonists pyrilamine and diphenhydramine induce learning and memory impairment in the MWM (D'Hooge and De Deyn, 2001) and/or RAM tests (Noda et al., 1995; Taga et al., 2001) in normal rats. Therefore, sertindole may not impair learning and memory in normal animals in the MWM test because it does not bind to either M_1 or H_1 receptors. Olanzapine has high binding and blocking activity against muscarinic M_1 receptors (Bymaster et al., 1996). In addition, orally-administered clozapine also blocks muscarinic M1 receptor-mediated behaviors (Moore et al., 1992) even though its metabolite N-desmethylclozapine has agonistic actions at the receptor (Li et al., 2005).

Cognitive performance is also influenced by the antihistaminic effects of drugs. There is a correlation between increased histamine occupancy and decreased cognitive performance. Clozapine potently antagonizes histamine H_1 receptors, which limits its cognitive improving effects. Therefore, many variables determine the efficacy of clozapine, which may also depend on the specific task tested.

Olanzapine is similar to clozapine in structure but possesses a different receptor binding profile. It exhibits nearly equal preference for 5-HT_{2A} and dopamine D2 receptors (Arnt and Skarsfeldt, 1998). In addition, it blocks muscarinic receptors. Despite its anticholinergic activity, olanzapine has been shown to improve cognition in schizophrenia (Meltzer and McGurk, 1999) and exerts beneficial effects on memory consolidation in rats in the delayed radial arm maze (Wolff and Leander, 2003). Olanzapine exhibits the strongest blockade of dopamine D_2 receptors compared to sertindole and clozapine, which may contribute to cognitive impairment in naive mice (Didriksen et al., 2006). As postulated, haloperidol, a dopamine D_2 receptor antagonist, also deteriorates cognitive functions in schizophrenics (Cutmore and Beninger, 1990) and normal volunteers (Beuzen et al., 1999) as well as disrupts water maze performance in rats (Skarsfeldt, 1996).

Antipsychotic agents alter glutamatergic neurotransmission by the modulation of glutamate release or by altering the density or subunit composition of glutamate receptors (Goff and Coyle, 2001). The glutamate system is dysregulated in schizophrenia. Of note, clinical studies have determined that inhibition of NMDA receptors by the noncompetitive antagonists PCP, MK-801 or ketamine causes schizophrenia-like symptoms in normal subjects and exacerbates psychotic symptoms in schizophrenics (Jentsch and Roth, 1999). Thus, animals treated with moderate doses of NMDA receptor antagonists, such as PCP, ketamine, or MK801, are used to model various aspects of schizophrenia; these animals exert schizophrenia symptoms, including hyperlocomotion, enhanced stereotypic behaviors, cognitive and sensorimotor gating deficits, and impaired social interactions (Lipska and Weinberger, 2000). Atypical antipsychotics exert differential effects on the NMDA receptor, in comparison to classical agents (Lidskey et al., 1993), and chronic antipsychotic drug treatment alters mRNA expression of NMDA receptor subunits.

In recent studies, olanzapine has been shown to increase ACh release via blockade of terminal muscarinic M_2 receptors (Johnson et al., 2005). These findings support the hypothesis that an increase in ACh release may be involved in the ameliorative effect of olanzapine. In addition, systemic clozapine administration can increase Ach release in the rat cortex and hippocampus (Huang et al., 2006). Clozapine-induced release Ach in the hippocampus is important because hippocampal Ach can alter spatial memory in the rat (Ogren et al., 1996). In a recent study, spatial impairment induced by a low dose of MK-801 was attenuated by cholinesterase inhibitors in mice (Csernansky et al., 2005). In addition, clozapine acts on

muscarinic M-4 receptors (Zorn et al., 1994), and the main metabolite of clozapine, N-desmethylclozapine, is a muscarinic M-1 receptor agonist. Therefore, it is possible that clozapine may enhance cholinergic transmission in the hippocampus, resulting in an attenuation of the cognitive deficit caused by NMDA receptor blockade (Weiner et al., 2004).

In conclusion, while olanzapine disturbed water maze performance, sertindole and clozapine induced no cognitive impairment in naive animals. Moreover the atypical antipsychotic drugs olanzapine, sertindole and clozapine might be clinically useful for the treatment of cognitive impairments in patients with schizophrenia.

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