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Involvement of N-methyl-D-aspartate receptors in the antidepressant-like effect of 5-hydroxytryptamine 3 antagonists in mouse forced swimming test and tail suspension test

Nastaran Kordjazy [1], Arya Haj-Mirzaian [1], Shayan Amiri, Sattar Ostadhadi, Hossein Amini-khoei, Ahmad Reza Dehpour *

[a] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran

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ABSTRACT

Recent evidence indicates that 5-hydroxytryptamine 3 (5-HT3) antagonists such as ondansetron and tropisetron exert positive behavioral effects in animal models of depression. Due to the ionotropic nature of 5-HT3 and N-methyl-D-aspartate (NMDA) receptors, plus their contribution to the pathophysiology of depression, we investigated the possible role of NMDA receptors in the antidepressant-like effect of 5-HT3 receptor antagonists in male mice. In order to evaluate the animals' behavior in response to different treatments, we performed open-field test (OFT), forced swimming test (FST), and tail-suspension test (TST), which are considered as valid tasks for measuring locomotor activity and depressive-like behaviors in mice. Our data revealed that intraperitoneal (i.p.) administration of tropisetron (5, 10, and 30 mg/kg) and ondansetron (0.01, and 0.1 μg/kg) significantly decreased the immobility time in FST and TST. Also, co-administration of subeffective doses of tropisetron (1 mg/kg, i.p.) or ondansetron (0.001 μg/kg, i.p.) with subeffective doses of NMDA receptor antagonists, ketamine (1 mg/kg, i.p.), MK-801 (0.05 mg/kg, i.p.) and magnesium sulfate (10 mg/kg, i.p.) resulted in a reduced immobility time both in FST and TST. The subeffective dose of NMDA (NMDA receptor agonist, 75 mg/kg, i.p.) abolished the effects of 5-HT3 antagonists in FST and TST, further supporting the presumed interaction between 5-HT3 and NMDA receptors. These treatments did not affect the locomotor behavior of animals in OFT. Finally, the results of our study suggest that the positive effects of 5-HT3 antagonists on the coping behavior of mice in FST and TST are at least partly mediated through NMDA receptors participation.

1. Introduction

Nowadays, depression is one of the most common psychiatric disorders (Kessler et al., 2009) and has become a public health concern (Silva et al., 2014), since it is associated with high morbidity and mortality (Cakmakci et al., 2013). Because of the high prevalence of depression (Compton et al., 2006), introducing new antidepressant agents with favorable pharmacological properties and lower side effects is of benefit.

Antidepressant effect of some 5-hydroxytryptamine subtype 3 (5-HT3) receptor antagonists has been demonstrated in animal models. Tropisetron and ondansetron exhibited antidepressant properties in behavioral tests such as forced swimming test (FST) and tail suspension test (TST) (Bravo and Maswood, 2006; Martin et al., 1992; Nakagawa et al., 1998; Ramamoorthy et al., 2008). Also, some clinical experiments have assessed the beneficial effect of tropisetron and ondansetron in anxiety and depression states which were comorbidities of other conditions such as fibromyalgia and obsession (Haus et al., 2000; Hewlett et al., 2003; Lecrubier et al., 1993), even though this effect has not been widely experimented in major depressive individuals. These 5-HT3 antagonists have desirable pharmacological profile and cause few adverse effects and thus they are well-tolerated in patients (Greenshaw and Silverstone, 1997; Rajkumar and Mahesh, 2010). A number of reports focused on the interaction of 5-HT3 and N-methyl-D-aspartate (NMDA) receptors. Some evidence suggests that NMDA antagonists exert inhibitory effect on 5-HT3 receptors besides acting on NMDA receptors (Kos et al., 2006; Rammes et al., 2001).

Discovery of the antidepressant effect of NMDA receptor antagonists has linked the glutamatergic pathway to the pathophysiology of depression (Manji et al., 2001) and the mechanism of action of some antidepressants (Berman et al., 2000; Szasz et al., 2007). Antidepressant-like behavior following acute NMDA receptor antagonist treatment have been previously observed in many rodents tests of depression, including the TST (Mantovani et al., 2003) and FST (Autry et al., 2011; Maj et al.,

* Corresponding author at: Experimental Medicine Research Center, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran.
E-mail addresses: dehpoura@sina.tums.ac.ir, dehpour@yahoo.com (A.R. Dehpour).
[1] Please note that the first two authors are considered as the first authors.

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1992; Yildiz et al., 2000). It is now well recognized that NMDA receptors blockade by certain drugs can exhibit antidepressant activity.

Although the antidepressant effect of 5-HT3 antagonists in clinical and animal studies is established, the underlying mechanism of this effect has not been completely discovered. Therefore, we aimed to evaluate the possible role of NMDA receptors in the antidepressant-like effect of tropisetron and ondansetron, selective 5-HT3 receptor antagonists, through the mouse FST and TST, which are extensively used methods for behavioral changes measurement.

2. Methods and materials

2.1. Animals

In this study, we used 20–30 g male NMRI mice (from Pasteur Institute, Tehran, Iran). Animals were housed in groups (4–5 mice per cage) and were kept under standard laboratory condition (temperature: 21–23 °C, 12-h light/dark cycle) and with free access to food and water. All experiments were carried out between 12:00 and 16:00. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications #80-23) and the institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS). Each experimental group consisted of 6 to 9 animals.

2.2. Drugs

The following drugs were used in this study: tropisetron, ondansetron, fluoxetine, ketamine, MK-801 (dizocilpine), magnesium sulfate, and NMDA (All were purchased from Sigma, St. Louis, MO, USA). All drugs were dissolved in saline and administered through intraperitoneal (i.p.) route in a constant volume of 5 ml/kg body weight.

2.3. Open-field test (OFT)

The OFT was used to evaluate the locomotor behavior of animals (Amiri et al., 2015b; Kulesskaya and Voikar, 2014). The open-field apparatus was made of white opaque Plexiglas (50 cm × 50 cm × 30 cm). Each mouse was placed gently on the center square (30 cm × 30 cm) and its behaviors were recorded by a camera for 5 min and then data were analyzed by Ethovision software version 8 (Noldus, Netherlands). The distance moved (horizontal activity) in the OFT was evaluated to ensure that the decrease or increase in animal's motionlessness in FST/TST is not due to alterations in locomotor activity.

2.4. Forced swimming test (FST)

The FST was used to assess behavioral despair in mice as a standard rodent test for screening antidepressant activity of drugs (Ghasemi et al., 2009b; Porsolt et al., 1977). Animals were placed in an open cylinder-shaped flask (diameter 10 cm, height 25 cm) filled with water (19 cm, 24 ± 1 °C). Each mouse was regarded as immobile when stopped struggling and floated motionless on the water, making only the movements for keeping its head above water. The behaviors were assessed in a 6 min period and the duration of immobility within the last 4 min of the test was recorded.

2.5. Tail suspension test (TST)

In order to perform TST, each mouse was suspended from the edge of a rod (50 cm above a table top) using adhesive Scotch tape placed approximately 1 cm from the tip of the tail. Tail climbing was prevented by passing the mouse's tail through a small plastic cylinder prior to suspension. The duration of immobility was scored in a 6 min period. Mice were considered immobile when they hung down passively and remained completely motionless (Cryan et al., 2005; Steru et al., 1985).

2.6. Treatments

In order to investigate the behavioral effects of tropisetron and ondansetron on mice, we used FST, TST, and OFT. Ondansetron (0.001, 0.005, 0.01, 0.1 μg/kg, i.p.) and tropisetron (1, 5, 10, and 30 mg/kg, i.p.) were injected to mice 30 min before the behavioral tests in order for determination of the effective and subeffective doses of the drugs. Time of administration and doses of drugs were chosen based on our pilot studies and previous reports (Bravo and Maswood, 2006; Ramamoorthy et al., 2008). Fluoxetine (5 mg/kg) was administered 30 min before the tests and the results were considered as the positive control group (Kordjazy et al., 2015; Moretti et al., 2012). Also, in order to exclude the effect of saline administration on behavioral tests, saline (5 ml/kg) was injected 30 min before the tests into control group.

After defining the effective and subeffective doses of aforementioned drugs, in order to assess the behavioral measures in animals, Ketamine (1 and 3 mg/kg, 60 min prior to the tests) (Ghasemi et al., 2009a), MK-801 (0.05 and 0.1 mg/kg, 45 min prior to the tests) (Ghasemi et al., 2009a), magnesium sulfate (10 and 30 mg/kg, 30 min prior to the tests) (Poleszak et al., 2006, 2007), and NMDA (75 and 150 mg/kg, 30 min prior to the tests) (Ghasemi et al., 2009a) were injected to animals. The doses were chosen based on our pilot study and previous reports (Haj-Mirzaian et al., 2015a, 2015b). The corresponding control group received saline prior to the test. Investigating the possible involvement of NMDA receptors in antidepressant-like activity of 5-HT3 antagonists, we co-administered subeffective doses of tropisetron or ondansetron with subeffective doses of NMDA antagonists. Further, we co-administered the subeffective dose of NMDA with the effective doses of tropisetron or ondansetron.

2.7. Statistical analysis

All data are expressed as mean ± S.E.M and the statistical analyses and data interpretations and also preparing the illustrations were performed using the SPSS and graph-pad Prism software package. The one-way and two-way analyses of variance (ANOVAs), followed by Tukey's *post hoc*, were used for comparison of the immobility times and locomotor activities between the experimental groups and controls. Finally, $p < 0.05$ was considered as the significance level.

3. Results

3.1. Effects of 5-HT3 antagonists on the behavioral profile of mice in the FST, TST and OFT

Fig. 1 shows that treatment of mice with tropisetron (5, 10, and 30 mg/kg) significantly decreased the immobility time both in the FST ($F(5, 42) = 6.891$, $P < 0.05$, $P < 0.01$, and $P < 0.001$ respectively, Fig. 1A) and TST ($F(5, 36) = 6.519$, $P < 0.05$, $P < 0.01$, and $P < 0.01$ respectively, Fig. 1B) compared with saline-treated animals. However, tropisetron (1 mg/kg) did not show any antidepressant-like effect in these tests ($P > 0.05$). Also, tropisetron did not cause significant changes in the distance moved in OFT compared with saline-injected control group ($F(5, 42) = 0.2830$, $P > 0.05$, Fig. 1C).

Although ondansetron (0.001 μg/kg) did not produce anti-immobility effect ($P > 0.05$), at doses 0.005, 0.01, and 0.1 μg/kg it significantly decreased the immobility time in the FST when compared with saline-injected group ($F(5, 42) = 4.752$, $P < 0.05$, $P < 0.05$, and $P < 0.01$ respectively, Fig. 1D). In the TST, administration of ondansetron (0.01 and 0.1 μg/kg) induced anti-immobility effect compared with saline-injected group ($F(5, 34) = 4.719$, $P < 0.05$ and $P < 0.01$ respectively, Fig. 1E). However, lower doses of this drug (0.005 and 0.001 μg/kg) did not alter the duration of immobility in the TST ($P > 0.05$). None of the applied doses of ondansetron affected the total distance moved in the OFT ($F(5, 42) = 0.1843$, $P > 0.05$, Fig. 1F).

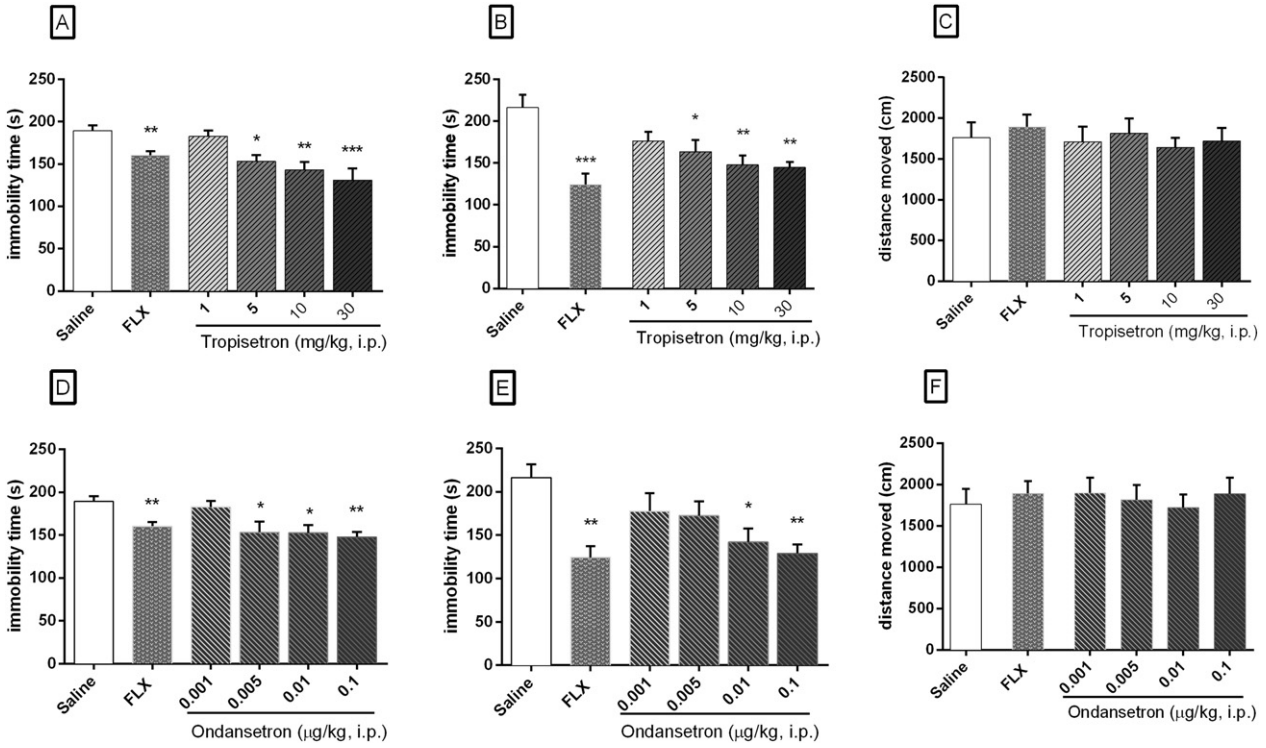

Fig. 1. Effect of acute administration of tropisetron (1, 5, 10, and 30 mg/kg, i.p.) and ondansetron (0.001, 0.005, 0.01, and 0.1 μg/kg, i.p.) on: **(A, D)** Duration of immobility in FST. **(B, E)** Duration of immobility in TST. **(C, F)** Distance moved in OFT. Values are expressed as the mean ± S.E.M (n = 6–9) using one-way ANOVA followed by Tukey's post hoc. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with saline-treated group.

Data showed that the anti-immobility effect of tropisetron/ondansetron was similar to fluoxetine, which significantly reduced the immobility time in both FST and TST.

3.2. Effect of different doses of NMDA antagonists and agonist on FST, TST and OFT

Fig. 2 presents effects of treatment with different doses of NMDA receptor antagonists in FST, TST, and OFT. Ketamine (1 mg/kg) did not significantly reduce the immobility time of mice ($P > 0.05$). However, a significant decrease in the immobility time was observed in FST and TST when ketamine (3 mg/kg) was administered to animals ($F(2, 21) = 2.79$, $P < 0.05$, Fig. 2A and $F(2, 21) = 7.009$, $P < 0.01$, Fig. 2B, respectively). Also, a significant anti-immobility effect was observed in MK-801-treated animals (0.1 mg/kg) in FST ($F(2, 21) = 8.381$, $P < 0.01$, Fig. 2A) and TST ($F(2, 21) = 8.992$, $P < 0.01$, Fig. 2B). However, MK-801 at the dose 0.05 mg/kg did not induce significant decrease in the immobility time in FST and TST ($P > 0.05$). Magnesium sulfate (30 mg/kg) showed anti-immobility effect in FST and TST (($F(2, 21) = 5.539$, $P < 0.05$, Fig. 2A) and ($F(2, 21) = 7.521$, $P < 0.05$, Fig. 2B)), while, treating mice with 10 mg/kg magnesium sulfate did not produce the same effects ($P > 0.05$). While administration of NMDA (150 mg/kg) significantly increased the immobility time in FST ($F(2, 18) = 3.399$, $P < 0.05$, Fig. 2D) and TST ($F(2, 21) = 3.971$, $P < 0.05$, Fig. 2E), NMDA (75 mg/kg) did not affect the behavior of mice in FST and TST ($P > 0.05$). Moreover, treating mice with NMDA receptor agonist/antagonists with doses mentioned above did not make significant changes in the distance moved in the OFT ($P > 0.05$, Fig. 2C and F).

3.3. Effect of NMDA antagonists on the behavioral effects of tropisetron in the FST, TST and OFT

As shown in Fig. 3, administration of the subeffective dose of ketamine (1 mg/kg) in combination with subeffective dose of tropisetron (1 mg/kg) produced anti-immobility effect as compared with the administration of either drug alone. Two-way ANOVA proved significant differences in FST and TST for tropisetron treatment (($F(1, 14) = 10.62$, $P < 0.01$) and ($F(1, 14) = 17.39$, $P < 0.001$)), ketamine treatment (($F(1, 14) = 6.682$, $P < 0.05$) and ($F(1, 14) = 4.254$, $P < 0.05$)), and tropisetron × ketamine interaction (($F(1, 14) = 4.915$, $P < 0.05$) and ($F(1, 14) = 5.64$, $P < 0.05$)). Also, administration of the subeffective dose of MK-801 (0.05 mg/kg) to tropisetron-treated (1 mg/kg) animals caused an obvious anti-immobility effect in FST and TST. Two-way ANOVA revealed significant differences in FST and TST for tropisetron treatment (($F(1, 14) = 19.23$, $P < 0.001$) and ($F(1, 14) = 11.34$, $P < 0.01$)), MK-801 treatment (($F(1, 14) = 12.66$, $P < 0.01$) and ($F(1, 14) = 9.357$, $P < 0.01$)), and tropisetron × MK-801 interaction (($F(1, 14) = 7.797$, $P < 0.01$) and ($F(1, 14) = 4.625$, $P < 0.05$)). Moreover, combination of the subeffective doses of tropisetron (1 mg/kg) and magnesium sulfate (10 mg/kg) significantly reduced the immobility time of mice in FST and TST and in two-way ANOVA, we observed significant effect for tropisetron treatment (($F(1, 14) = 13.48$, $P < 0.01$) and ($F(1, 14) = 11.40$, $P < 0.01$)), magnesium treatment (($F(1, 14) = 8.69$, $P < 0.01$) and ($F(1, 14) = 6.567$, $P < 0.05$)), and tropisetron × magnesium interaction (($F(1, 14) = 4.667$, $P < 0.05$) and ($F(1, 14) = 11.24$, $P < 0.01$)). None of the treatments made changes in the ambulatory behavior of mice in the OFT when compared to control animals ($P > 0.05$, Fig. 3).

3.4. Effect of NMDA antagonists on the behavioral effects of ondansetron in the FST, TST and OFT

Data illustrated in Fig. 4 shows that co-administration of the subeffective dose of ondansetron (0.001 μg/kg) with subeffective doses of ketamine (1 mg/kg), MK-801 (0.05 mg/kg), and magnesium sulfate (10 mg/kg) resulted in a significant decrease in immobility time of mice both in FST ($P < 0.001$, $P < 0.01$, and $P < 0.01$) and TST ($P < 0.001$, $P < 0.01$, and $P < 0.001$). Two-way ANOVA verified significant effects in FST and TST for ondansetron treatment (($F(1, 14) =$

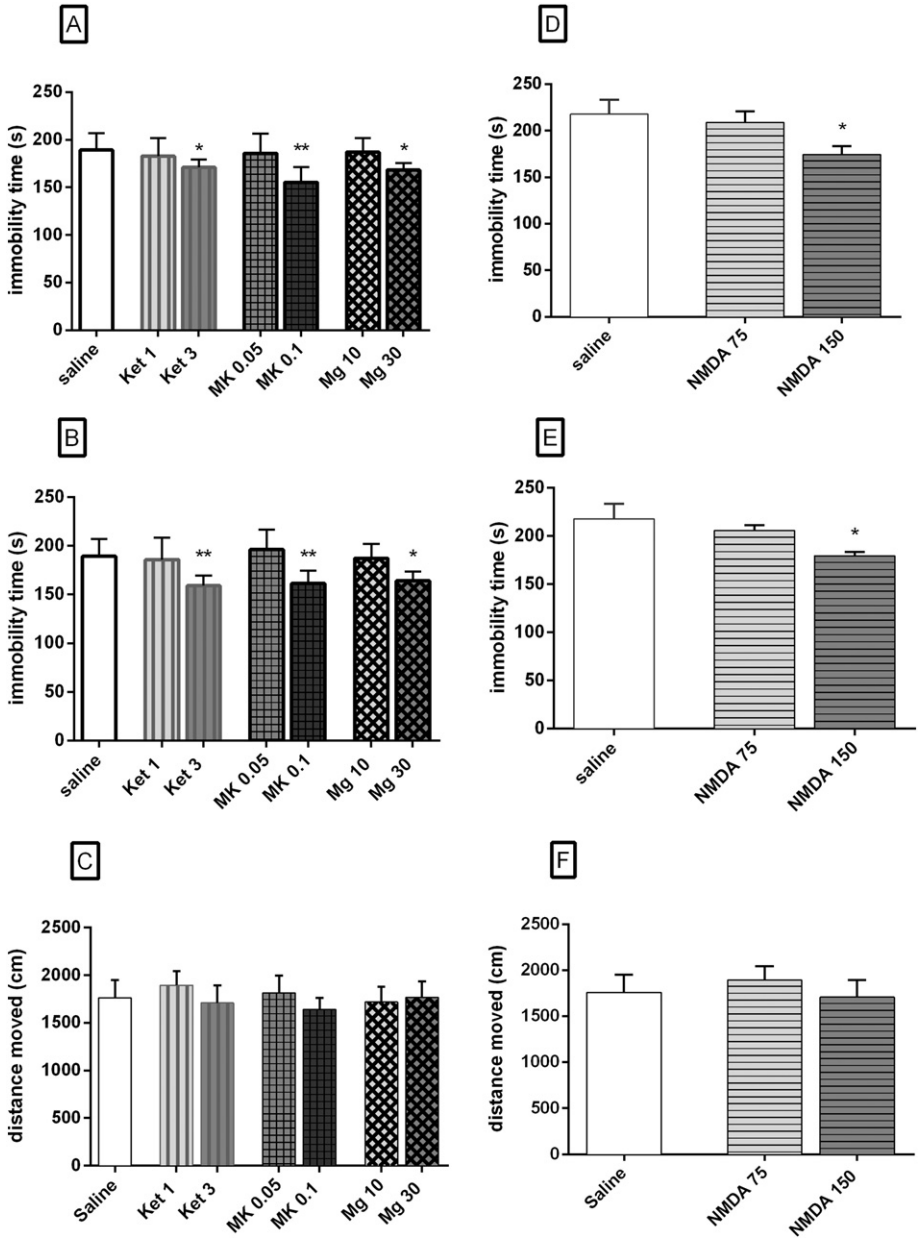

Fig. 2. Effect of acute administration of NMDA antagonists (ketamine, MK-801, and magnesium sulfate) and NMDA agonist (NMDA) on: **(A, D)** Duration of immobility in FST. **(B, E)** Duration of immobility in TST. **(C, F)** Distance moved in OFT. Values are expressed as the mean ± S.E.M (n = 8) using one-way ANOVA followed by Tukey's post hoc. *P < 0.05, **P < 0.01 compared with saline-treated group.

15.8, $P < 0.001$) and ($F(1, 14) = 23.38$, $P < 0.001$)), ketamine treatment (($F(1, 14) = 11.12$, $P < 0.01$) and ($F(1, 14) = 12.86$, $P < 0.01$)), and ondansetron × ketamine interaction (($F(1, 14) = 9.694$, $P < 0.01$) and ($F(1, 14) = 6.52$, $P < 0.05$)). Also, two way ANOVA revealed significant differences in FST and TST for ondansetron treatment (($F(1, 14) = 27.25$, $P < 0.001$) and ($F(1, 14) = 10.24$, $P < 0.01$)), MK-801 treatment (($F(1, 14) = 8.352$, $P < 0.01$) and ($F(1, 14) = 9.413$, $P < 0.01$)), and ondansetron × MK-801 interaction (($F(1, 14) = 8.889$, $P < 0.01$) and ($F(1, 14) = 4.865$, $P < 0.05$)). Moreover, data analysis showed significant differences for ondansetron effect (($F(1, 14) = 9.356$, $P < 0.01$) and ($F(1, 14) = 21.44$, $P < 0.001$)), magnesium effect (($F(1, 14) = 8.017$, $P < 0.01$) and ($F(1, 14) = 12.13$, $P < 0.01$)), and ondansetron × magnesium interaction (($F(1, 14) = 4.816$, $P < 0.05$) and ($F(1, 14) = 7.204$, $P < 0.05$)). The aforementioned treatments did not result in significant changes in the ambulation of mice in the OFT when compared to control group ($P > 0.05$, Fig. 4).

3.5. Effect of NMDA agonist on the behavioral effects of 5-HT3 antagonists in the FST, TST and OFT

Fig. 5 shows that the anti-immobility effect of tropisetron (10 mg/kg) was significantly abolished by pretreatment of mice with NMDA (75 mg/kg). A two-way ANOVA proved significant differences in immobility time in FST and TST for the tropisetron-treated group (($F(1, 14) = 15.02$, $P < 0.001$) and ($F(1, 14) = 13.54$, $P < 0.001$)), NMDA-treated group (($F(1, 14) = 6.524$, $P < 0.05$) and ($F(1, 14) = 16.29$, $P < 0.001$)), and NMDA × tropisetron interaction (($F(1, 14) = 13.28$, $P < 0.01$) and ($F(1, 14) = 11.65$, $P < 0.01$)).

Also, treating mice with NMDA (75 mg/kg) reversed the anti-immobility effect of ondansetron (0.01 μg/kg). Two-way ANOVA showed significant differences for ondansetron treatment, NMDA-treatment, and ondansetron × NMDA interaction in FST (($F(1, 14) = 10.19$, $P < 0.01$), ($F(1, 14) = 20.04$, $P < 0.001$), and ($F(1, 14) = 24.76$,

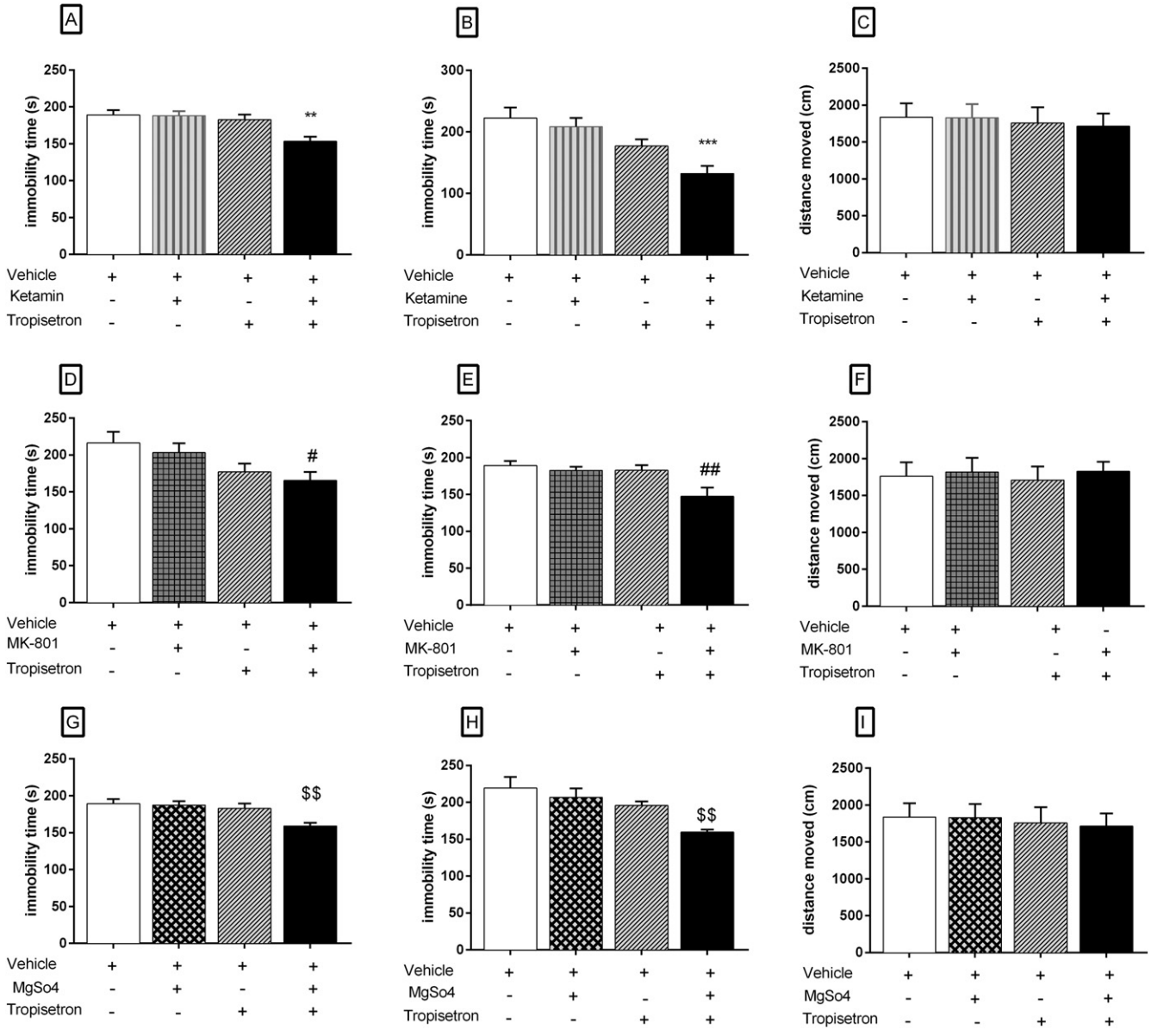

Fig. 3. Effect of pretreatment with subeffective doses of NMDA antagonists on subeffective tropisetron-treated animals in FST, TST, and OFT. Values are expressed as the mean ± S.E.M (n = 8) using two-way ANOVA and **P < 0.01, ***P < 0.001, #P < 0.05, ##P < 0.01, $$P < 0.01 compared with saline-treated group.

$P < 0.001$) respectively), and TST (($F(1, 14) = 6.989$, $P < 0.05$), ($F(1, 14) = 16.05$, $P < 0.001$), and ($F(1, 14) = 33.176$, $P < 0.001$) respectively).

Administration of NMDA alone or in combination with tropisetron/ondansetron did not affect the total distance moved in the OFT (P > 0.05, Fig. 5).

4. Discussion

Our current experiments demonstrated that tropisetron and ondansetron, 5-HT3 receptor antagonists, exhibit antidepressant-like effect in TST and FST. We investigated the possible involvement of glutamatergic NMDA receptors in the antidepressant-like effect of these 5-HT3 antagonists. Results showed that NMDA receptor antagonists enhanced the anti-immobility effect of tropisetron and ondansetron; whereas, NMDA agonist treatment weakened the antidepressant-like effect of these 5-HT3 antagonists.

Amongst the 5-HT receptor subtypes, 5-HT3 receptors are the only ligand-gated ion channels (Rajkumar and Mahesh, 2010). Tropisetron and ondansetron are highly selective, competitive inhibitors of 5-HT3 receptors (Broocks, 1992, Färber et al., 2000) and are known as the main drug of choice in management of chemotherapy-induced nausea and vomiting (Rajkumar and Mahesh, 2010). 5-HT3 receptors are not only available at a high density in the area postrema (the nausea and vomiting regulatory center in brain) but also in the hippocampal and amygdala region of the limbic system; as a result, it is speculated that 5-HT3 selective agents exhibit psychotropic effects (Broocks, 1992; Ostadhadi et al., 2015). In this regard, some researches suggest that 5-HT3 antagonists exhibit protective and therapeutic effects in variety of psychiatric disorders, such as depression (Greenshaw and Silverstone, 1997; Lecrubier et al., 1993). Using FST and TST as valid behavioral tests for screening the antidepressant effect of novel drugs (Cryan et al., 2002), we showed that both tropisetron and ondansetron (similar to fluoxetine) reduce the immobility time in these tests, indicating the ability of these drugs to attenuate the behavioral despair in mice. The antidepressant-like behavior due to ondansetron and tropisetron treatment in our experiments was not related to the changes of locomotion, since the mice treated with ondansetron and

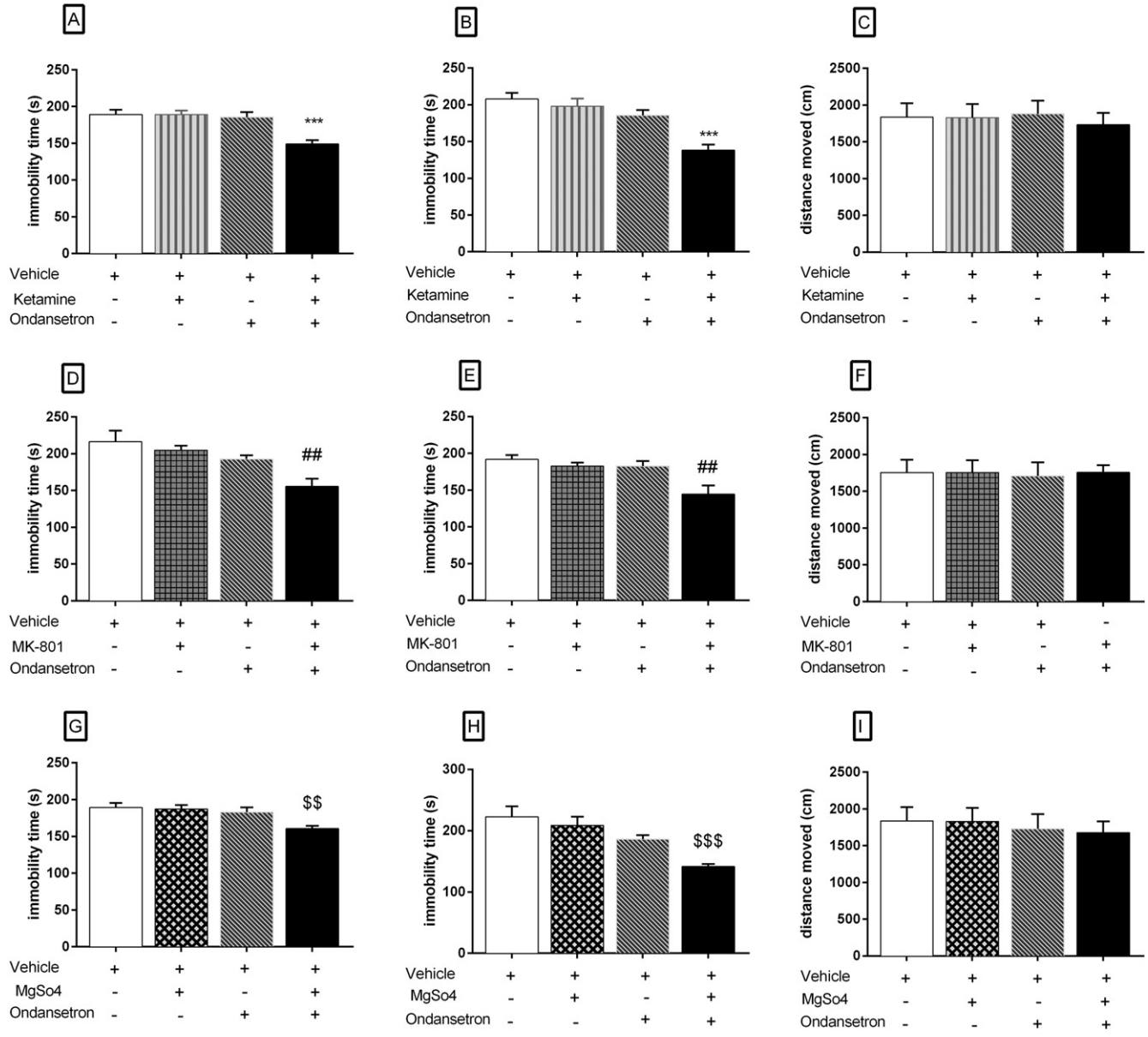

Fig. 4. Effect of pretreatment with subeffective doses of NMDA antagonists on subeffective ondansetron-treated animals in FST, TST, and OFT. Values are expressed as the mean ± S.E.M. (n = 8) using two-way ANOVA and ***P < 0.001, ##P < 0.01, $$P < 0.01 and $$$P < 0.001 compared with saline-treated group.

tropisetron did not show enhanced ambulation in the OFT. Our results are in agreement with previous studies that reported selective antagonists of 5-HT3 receptors exert antidepressant-like properties in animal models of depression such as learned helplessness, olfactory bulbectomy and chronic unpredictable mild stress (Gupta et al., 2014; Martin et al., 1992; Ramamoorthy et al., 2008). Antidepressant-like effect of ondansetron in FST in male mice had been discovered in past studies; however, our study for the first time reveals the dose–response effect of tropisetron in models of depression in male mice. A few studies have investigated the effect of tropisetron on behaviors related to depression in animal models, such as the learned-helplessness paradigm (Martin et al., 1992). Likewise, Bravo and Maswood observed that treatment with tropisetron reduced immobility of female rats in FST (Bravo and Maswood, 2006). In addition, many studies detected the antidepressant-like effect of novel serotonin subtype 3 antagonists in rodent behavioral tests such as FST (Bhatt et al., 2013; Kurhe et al., 2014; Mahesh et al., 2014). Focusing on the clinical investigations, we realize that ondansetron and tropisetron have been shown to improve depression scores in obsessive-compulsive patients (Hewlett et al., 2003) as well as patients with fibromyalgia, respectively (Haus et al., 2000).

Vast majority of evidence showed the rapid antidepressant effect of NMDA receptor antagonists both in humans and animals (Berman et al., 2000; Haj-Mirzaian et al., 2015a; Rosa et al., 2003; Tokita et al., 2012). In our study, considering the anti-immobility effects of NMDA receptor antagonists, we determined the effective and subeffective doses of ketamine, MK-801, and magnesium sulfate in FST and TST. We showed that ketamine (3 mg/kg) significantly decreased the immobility duration, but at the dose 1 mg/kg, it did not affect the immobility time. In previous studies, these doses of ketamine were also recognized as effective and sub-effective doses, respectively (Owolabi et al., 2014). MK-801 (0.1 mg/kg) reduced the immobility time of mice significantly, but at the dose 0.05 mg/kg, it did not cause significant reduction in the immobility time. These results are in accordance with the previous findings which determined 0.05 mg/kg MK-801 as the sub-effective dose and 0.1 mg/kg as the effective dose in FST (Haj-Mirzaian et al., 2014). It was observed that the immobility time of mice and rats in FST was significantly reduced by magnesium ions (Poleszak et al., 2005a, 2005b).

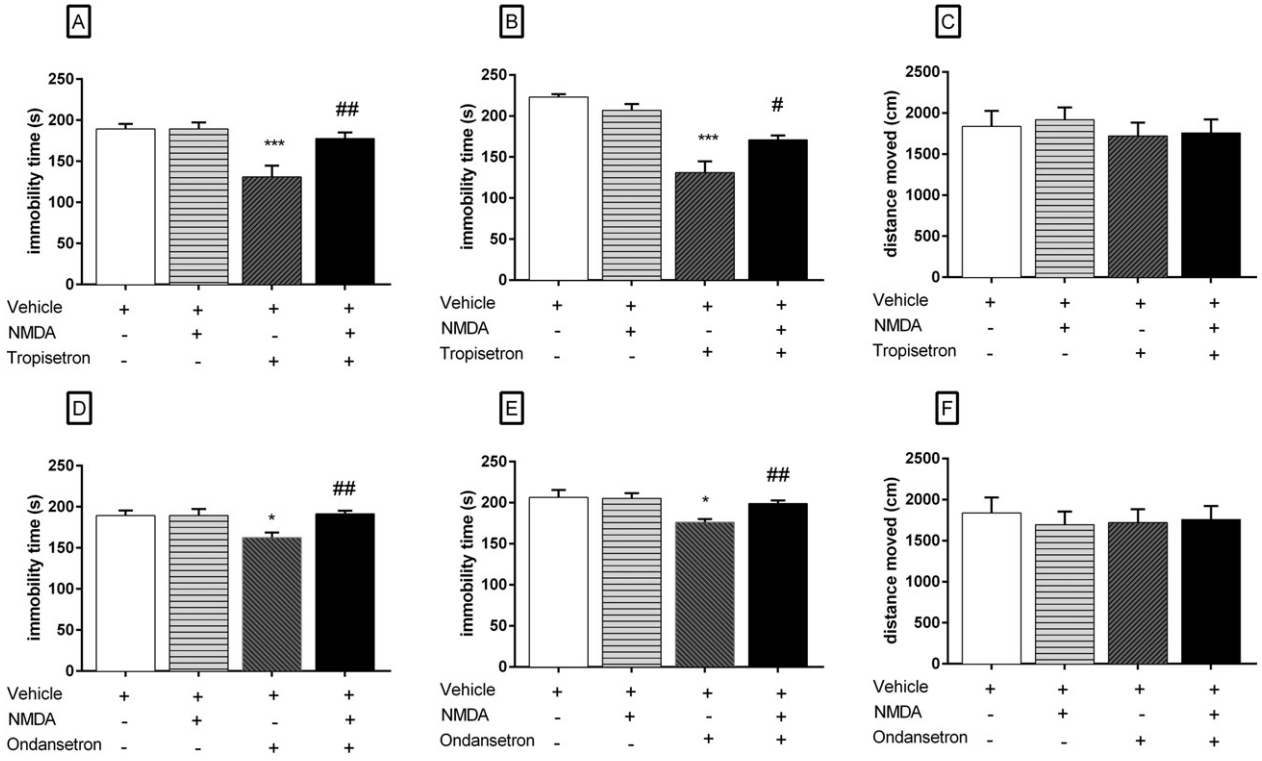

Fig. 5. Effect of pretreatment with subeffective dose of NMDA (75 mg/kg) on the effective doses of 5-HT3 antagonists in FST, TST, and OFT. Values are expressed as the mean ± S.E.M (n = 8) using two-way ANOVA and *P < 0.05 and ***P < 0.001 compared with the saline-treated group. #P < 0.05 and ##P < 0.01 compared with the ondansetron/tropisetron-treated group with saline pretreatment.

Also, administration of magnesium sulfate to rats subjected to traumatic brain injury significantly decreased the incidence and severity of post-traumatic depression (Fromm et al., 2004). In our current study, magnesium sulfate exerted antidepressant-like effect in behavioral tests at the dose 30 mg/kg, but it did not significantly alter the immobility time of mice at the dose 10 mg/kg (Poleszak et al., 2006, 2007). Thus, our data are consistent with the variety of evidence illustrating the role of NMDA receptors in major depression and mechanism of action of antidepressants.

Surprisingly, few studies have focused on the interaction of 5-HT3 and NMDA receptors in psychiatric disorders. It has been reported that inhibition of 5-HT3 receptors by their selective antagonists could be used as a strategy to enhance the positive effects of NMDA antagonists on depressive-like behaviors. In a study by Kos et al., authors showed that MDL 72222, a 5-HT3 antagonist, potentiated the anti-immobility effect of ketamine in TST (Kos et al., 2006). Another study also demonstrated that uncompetitive NMDA receptor antagonists might antagonize 5-HT3 receptors in vitro. Ketamine has been shown to have a weak 5-HT3 antagonist-like property in addition to its NMDA antagonistic effects (Rammes et al., 2001). On the other hand, 5-HT3 receptors have been reported to directly interact with NMDA receptor-mediated response in pyramidal cells of the rat cortex (Liang et al., 1998). Interestingly, it was shown that pretreatment with ondansetron prevents some undesirable side effects of ketamine and MK-801 (Suzuki et al., 1999).

Although the antidepressant effect of some 5-HT3 receptor antagonists had been demonstrated in the past studies, the exact mechanism of action through which these drugs exert this effect was not fully understood. Investigating the underlying mechanisms involved in these effects improves our knowledge in order to introduce and design more efficient pharmacological agents for the treatment of mental disorders. Based on the results of the current study and previous reports, it could be speculated that the effect of 5-HT3 antagonists is possibly mediated through NMDA receptors, or these drugs act through the same signaling pathway and induce synergic effects. Both 5-HT3 and NMDA receptors are ion channels and activation of these receptors results in a considerable increase in intracellular calcium level. Calcium influx triggers multiple intracellular signaling cascades including nitric oxide (NO) pathway, thereby activating the nitric oxide synthase enzyme which has been revealed to play a role in pathobiology of depressive disorders (Esplugues, 2002). In order to explain the synergistic effect of NMDA antagonists with 5-HT3 antagonists regarding their antidepressant-like properties, it has been reported that a decrease in NO content in limbic regions such as the hippocampus is associated with the antidepressant activity of various medications such as selective serotonin reuptake inhibitors and imipramine (Dhir and Kulkarni, 2007; Krass et al., 2011; Zomkowski et al., 2010). Since 5-HT3 and NMDA receptors are basically ion-channels, it is plausible that blocking these receptors through these co-treatments results in the reduction of calcium influx, and finally leads to antidepressant effect through NO production diminution (Joca and Guimarães, 2006). This hypothesis is in line with our recent published study which presented that tropisetron attenuated the anxiety-like behaviors of socially isolated animals through moderating the nitrergic system and mitochondrial dysfunction in the hippocampus (Amiri et al., 2015a).

In conclusion, the results of our current study revealed that applying 5-HT3 antagonists, provokes antidepressant-like effects through reducing the passive behavior of animals in FST and TST. Also, we suggested a possible connection between 5-HT3 receptors and NMDA receptors, meaning that co-administration of subeffective doses of their antagonists synergistically produced antidepressant effects.

Compliance with ethical standards

The authors declare that they have no conflict of interest. Also, all applicable international and institutional guidelines for the care and use of animals were followed.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

Amiri, S., Amini-Khoei, H., Haj-Mirzaian, A., Rahimi-Balaei, M., Naserzadeh, P., Dehpour, A., et al., 2015a. Tropisetron attenuated the anxiogenic effects of social isolation by modulating nitrergic system and mitochondrial function. Biochim. Biophys. Acta Gen. Subj. 1850, 2464–2475.

Amiri, S., Haj-Mirzaian, A., Rahimi-Balaei, M., Razmi, A., Kordjazy, N., Shirzadian, A., et al., 2015b. Co-occurrence of anxiety and depressive-like behaviors following adolescent social isolation in male mice; possible role of nitrergic system. Physiol. Behav. 145, 38–44.

Autry, A.E., Adachi, M., Nosyreva, E., Na, E.S., Los, M.F., Cheng, P.-f., et al., 2011. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature 475, 91–95.

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., et al., 2000. Antidepressant effects of ketamine in depressed patients. Biol. Psychiatry 47, 351–354.

Bhatt, S., Mahesh, R., Devadoss, T., Jindal, A.K., 2013. Antidepressant-like effect of novel 5-HT3 receptor antagonist Nn-butyl-3-ethoxyquinoxalin-2-carboxamide (6p): an approach using rodent behavioral antidepressant tests. Indian J. Pharmacol. 45, 348.

Bravo, G., Maswood, S., 2006. Acute treatment with 5-HT3 receptor antagonist, tropisetron, reduces immobility in intact female rats exposed to the forced swim test. Pharmacol. Biochem. Behav. 85, 362–368.

Broocks, A., 1992. Ondansetron—the first highly selective 5-HT3 antagonist in therapy of psychiatric diseases. Fortschr. Neurol. Psychiatr. 60, 227–236.

Cakmakci, E., Caliskan, K.C., Tabakci, O.N., Tahtabasi, M., Karpat, Z., 2013. Percutaneous liver biopsies guided with ultrasonography: a case series. Iranian J. Radiol. 10, 182.

Compton, W., Conway, K., Stinson, F., Grant, B., 2006. Changes in the prevalence of major depression and comorbid substance use disorders in the United States between 1991–1992 and 2001–2002. Am. J. Psychiatry 163, 2141–2147.

Cryan, J.F., Markou, A., Lucki, I., 2002. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol. Sci. 23, 238–245.

Cryan, J.F., Mombereau, C., Vassout, A., 2005. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci. Biobehav. Rev. 29, 571–625.

Dhir, A., Kulkarni, S.K., 2007. Involvement of l-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect of venlafaxine in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 31, 921–925.

Esplugues, J.V., 2002. NO as a signalling molecule in the nervous system. Br. J. Pharmacol. 135, 1079–1095.

Färber, T.S., Brückle, W., Späth, M., Pongratz, D., Lautenschläger, J., Kötter, I., Zöller, B., Peter, H.-H., Neeck, G., Alten, R., Müller, W.L., 2000. Efficacy and tolerability of tropisetron in primary fibromyalgia-a highly selective and competitive 5-HT3 receptor antagonist. Scand. J. Rheumatol. 29, 49–54.

Fromm, L., Heath, D.L., Vink, R., Nimmo, A.J., 2004. Magnesium attenuates post-traumatic depression/anxiety following diffuse traumatic brain injury in rats. J. Am. Coll. Nutr. 23, 529S–533S.

Ghasemi, M., Raza, M., Dehpour, A.R., 2009a. NMDA receptor antagonists augment antidepressant-like effects of lithium in the mouse forced swimming test. J. Psychopharmacol.

Ghasemi, M., Sadeghipour, H., Poorheidari, G., Dehpour, A.R., 2009b. A role for nitrergic system in the antidepressant-like effects of chronic lithium treatment in the mouse forced swimming test. Behav. Brain Res. 200, 76–82.

Greenshaw, A.J., Silverstone, P.H., 1997. The non-antiemetic uses of serotonin 5-HT3 receptor antagonists. Drugs 53, 20–39.

Gupta, D., Radhakrishnan, M., Kurhe, Y., 2014. 5HT3 receptor antagonist (ondansetron) reverses depressive behavior evoked by chronic unpredictable stress in mice: modulation of hypothalamic–pituitary–adrenocortical and brain serotonergic system. Pharmacol. Biochem. Behav.

Haj-Mirzaian, A., Amiri, S., Kordjazy, N., Rahimi-Balaei, M., Haj-Mirzaian, A., Marzban, H., et al., 2015a. Blockade of NMDA receptors reverses the depressant, but not anxiogenic effect of adolescence social isolation in mice. Eur. J. Pharmacol. 750, 160–166.

Haj-Mirzaian, A., Kordjazy, N., Haj-Mirzaian, A., Ostadhadi, S., Ghasemi, M., Amiri, S., et al., 2015b. Evidence for the involvement of NMDA receptors in the antidepressant-like effect of nicotine in mouse forced swimming and tail suspension tests. Psychopharmacology 1-11.

Haj-Mirzaian, A., Ostadhadi, S., Kordjazy, N., Dehpour, A.R., Mehr, S.E., 2014. Opioid/NMDA receptors blockade reverses the depressant-like behavior of foot shock stress in the mouse forced swimming test. Eur. J. Pharmacol.

Haus, B.V., Stratz, T., Späth, M., Müller, W., Ulrike, 2000. Oral treatment of fibromyalgia with tropisetron given over 28 days: influence on functional and vegetative symptoms, psychometric parameters and pain. Scand. J. Rheumatol. 29, 55–58.

Hewlett, W.A., Schmid, S.P., Salomon, R.M., 2003. Pilot trial of ondansetron in the treatment of 8 patients with obsessive–compulsive disorder. J. Clin. Psychiatry 64, 1025–1030.

Joca, S.R.L., Guimarães, F.S., 2006. Inhibition of neuronal nitric oxide synthase in the rat hippocampus induces antidepressant-like effects. Psychopharmacology 185, 298–305.

Kessler, R.C., Aguilar-Gaxiola, S., Alonso, J., Chatterji, S., Lee, S., Ormel, J., et al., 2009. The global burden of mental disorders: an update from the WHO World Mental Health (WMH) surveys. Epidemiol. Psichiatr. Soc. 18, 23–33.

Kordjazy, N., Haj-Mirzaian, A., Amiri, S., Ostadhadi, S., Kordjazy, M., Sharifzadeh, M., et al., 2015. Elevated level of nitric oxide mediates the anti-depressant effect of rubidium chloride in mice. Eur. J. Pharmacol. 762, 411–418.

Kos, T., Popik, P., Pietraszek, M., Schäfer, D., Danysz, W., Dravolina, O., et al., 2006. Effect of 5-HT3 receptor antagonist MDL 72222 on behaviors induced by ketamine in rats and mice. Eur. Neuropsychopharmacol. 16, 297–310.

Krass, M., Wegener, G., Vasar, E., Volke, V., 2011. The antidepressant action of imipramine and venlafaxine involves suppression of nitric oxide synthesis. Behav. Brain Res. 218, 57–63.

Kulesskaya, N., Voikar, V., 2014. Assessment of mouse anxiety-like behavior in the light–dark box and open-field arena: role of equipment and procedure. Physiol. Behav. 133, 30–38.

Kurhe, Y., Radhakrishnan, M., Gupta, D., Devadoss, T., 2014. QCM-4 a novel 5-HT3 antagonist attenuates the behavioral and biochemical alterations on chronic unpredictable mild stress model of depression in Swiss albino mice. J. Pharm. Pharmacol. 66, 122–132.

Lecrubier, Y., Puech, A., Azcona, A., Bailey, P., Lataste, X., 1993. A randomized double-blind placebo-controlled study of tropisetron in the treatment of outpatients with generalized anxiety disorder. Psychopharmacology 112, 129–133.

Liang, X., Arvanov, V.L., Wang, R.Y., 1998. Inhibition of NMDA-receptor mediated response in the rat medial prefrontal cortical pyramidal cells by the 5-HT3 receptor agonist SR 57227A and 5-HT: intracellular studies. Synapse 29, 257–268.

Mahesh, R., Dhar, A.K., Jindal, A., Bhatt, S., 2014. Design, synthesis and evaluation of antidepressant activity of novel 2-methoxy 1, 8 naphthyridine 3-carboxamides as 5-HT3 receptor antagonists. Chem. Biol. Drug Des. 83, 583–591.

Maj, J., Rogóż, Z., Skuza, G., Sowińska, H., 1992. Effects of MK-801 and antidepressant drugs in the forced swimming test in rats. Eur. Neuropsychopharmacol. 2, 37–41.

Manji, H.K., Drevets, W.C., Charney, D.S., 2001. The cellular neurobiology of depression. Nat. Med. 7.

Mantovani, M., Pértile, R., Calixto, J.B., Santos, A.R., Rodrigues, A.L.S., 2003. Melatonin exerts an antidepressant-like effect in the tail suspension test in mice: evidence for involvement of N-methyl-D-aspartate receptors and the L-arginine-nitric oxide pathway. Neurosci. Lett. 343, 1–4.

Martin, P., Gozlan, H., Puech, A.J., 1992. 5-HT3 receptor antagonists reverse helpless behaviour in rats. Eur. J. Pharmacol. 212, 73–78.

Moretti, M., Colla, A., de Oliveira, B.G., dos Santos, D.B., Budni, J., de Freitas, A.E., et al., 2012. Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. J. Psychiatr. Res. 46, 331–340.

Nakagawa, Y., Ishima, T., Takashima, T., 1998. The 5-HT3 receptor agonist attenuates the action of antidepressants in the forced swim test in rats. Brain Res. 786, 189–193.

Ostadhadi, S., Kordjazy, N., Haj-Mirzaian, A., Mansouri, P., Dehpour, A.R., 2015. 5-HT3 receptors antagonists reduce serotonin-induced scratching in mice. Fundam. Clin. Pharmacol. 29, 310–315.

Owolabi, R.A., Akanmu, M.A., Adeyemi, O.I., 2014. Effects of ketamine and N-methyl-D-aspartate on fluoxetine-induced antidepressant-related behavior using the forced swimming test. Neurosci. Lett. 566, 172–176.

Poleszak, E., Wlaź, P., Kędzierska, E., Nieoczym, D., Wróbel, A., Fidecka, S., et al., 2007. NMDA/glutamate mechanism of antidepressant-like action of magnesium in forced swim test in mice. Pharmacol. Biochem. Behav. 88, 158–164.

Poleszak, E., Wlaz, P., Kêdzierska, E., Nieoczym, D., Wyska, E., Szymura-Oleksiak, J., et al., 2006. Immobility stress induces depression-like behavior in the forced swim test in mice: effect of magnesium and imipramine. Pharmacol. Rep. 58, 746.

Poleszak, E., Wlaz, P., Kêdzierska, E., Radziwon-Zaleska, M., Pilc, A., Fidecka, S., et al., 2005a. Effects of acute and chronic treatment with magnesium in the forced swim test in rats. Pharmacol. Rep. 57, 654–658.

Poleszak, E., Wlaź, P., Szewczyk, B., Kędzierska, E., Wyska, E., Librowski, T., et al., 2005b. Enhancement of antidepressant-like activity by joint administration of imipramine and magnesium in the forced swim test: behavioral and pharmacokinetic studies in mice. Pharmacol. Biochem. Behav. 81, 524–529.

Porsolt, R., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327.

Rajkumar, R., Mahesh, R., 2010. Review: the auspicious role of the 5-HT3 receptor in depression: a probable neuronal target? J. Psychopharmacol. 24, 455–469.

Ramamoorthy, R., Radhakrishnan, M., Borah, M., 2008. Antidepressant-like effects of serotonin type-3 antagonist, ondansetron: an investigation in behaviour-based rodent models. Behav. Pharmacol. 19, 29–40.

Rammes, G., Rupprecht, R., Ferrari, U., Zieglgänsberger, W., Parsons, C., 2001. The N-methyl-D-aspartate receptor channel blockers memantine, MRZ 2/579 and other amino-alkyl-cyclohexanes antagonise 5-HT3 receptor currents in cultured HEK-293 and N1E-115 cell systems in a non-competitive manner. Neurosci. Lett. 306, 81–84.

Rosa, A.O., Lin, J., Calixto, J.B., Santos, A.R.S., Rodrigues, A.L.S., 2003. Involvement of NMDA receptors and L-arginine-nitric oxide pathway in the antidepressant-like effects of zinc in mice. Behav. Brain Res. 144, 87–93.

Silva, M.T., Galvao, T.F., Martins, S.S., Pereira, M.G., 2014. Prevalence of depression morbidity among Brazilian adults: a systematic review and meta-analysis. Rev. Bras. Psiquiatr. 36, 262–270.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.

Suzuki, T., Aoki, T., Kato, H., Yamazaki, M., Misawa, M., 1999. Effects of the 5-HT$_3$ receptor antagonist ondansetron on the ketamine-and dizocilpine-induced place preferences in mice. Eur. J. Pharmacol. 385, 99–102.

Szasz, B.K., Mike, A., Karoly, R., Gerevich, Z., Illes, P., Vizi, E.S., et al., 2007. Direct inhibitory effect of fluoxetine on N-methyl-D-aspartate receptors in the central nervous system. Biol. Psychiatry 62, 1303–1309.

Tokita, K., Yamaji, T., Hashimoto, K., 2012. Roles of glutamate signaling in preclinical and/or mechanistic models of depression. Pharmacol. Biochem. Behav. 100, 688–704.

Yildiz, F., Erden, B.F., Ulak, G., Utkan, T., Gacar, N., 2000. Antidepressant-like effect of 7-nitroindazole in the forced swimming test in rats. Psychopharmacology 149, 41–44.

Zomkowski, A.D.E., Engel, D., Gabilan, N.H., Rodrigues, A.L.S., 2010. Involvement of NMDA receptors and L-arginine-nitric oxide-cyclic guanosine monophosphate pathway in the antidepressant-like effects of escitalopram in the forced swimming test. Eur. Neuropsychopharmacol. 20, 793–801.