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Involvement of nitric oxide-cyclic guanosine monophosphate pathway in the antidepressant-like effect of tropisetron and ondansetron in mice forced swimming test and tail suspension test

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ABSTRACT

Antidepressant-like effects of 5-hydroxytryptamine subtype 3 (5-HT_3) antagonists including tropisetron and ondansetron have been previously demonstrated in the literature. It was reported that stimulation of 5-HT_3 receptors activate the nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway, which is involved in regulation of behavioral and emotional functions. In our study, treating animals with tropisetron (5, 10, and 30 mg/kg) and ondansetron (0.01 and 0.1 μg/kg) significantly decreased the immobility time in forced swimming test (FST) and tail-suspension test (TST). Co-administration of subeffective doses of tropisetron (1 mg/kg) and ondansetron (0.001 μg/kg) with subeffective dose of L-NAME (10 mg/kg, nonselective NO synthase (NOS) inhibitor) and 7-nitroindazole (25 mg/kg, neural NOS inhibitor) exerted antidepressant-like effect in FST and TST, while aminoguanidine (50 mg/kg, inducible NOS inhibitor) did not enhance the antidepressant-like effect of 5-HT_3 antagonists. Besides, L-arginine (750 mg/kg, NO precursor) and sildenafil (5 mg/kg, phosphodiesterase inhibitor) suppressed the antiimmobility effect of 5-HT_3 antagonists. None of the treatments altered the locomotor behavior of mice in open-field test. Also, hippocampal (but not cortical) nitrite level was significantly lower in tropisetron and ondansetron-treated mice compared with saline-injected mice. Also, co-administration of 7-nitroindazole with tropisetron or ondansetron caused a significant decrease in hippocampal nitrite levels. In conclusion, we suggest that antidepressant-like effect of tropisetron and ondansetron are partially mediated by modulation of NO-cGMP pathway.

1. Introduction

Depression, one of the most common psychiatric disorders, has become a public health concern (Silva et al., 2014). In view of the fact that the prevalence of depression is progressively high (Compton et al., 2006), increasing attention is paid to treatment of patients with depression. Amongst the several routine prescribed antidepressant medications, which are mainly based on monoamine regulation, few are highly expected to show the desired outcomes (Arroll et al., 2005; Berton and Nestler, 2006). Thus, finding new antidepressants with favorable pharmacological properties is advantageous.

5-hydroxytryptamine 3 (5-HT_3) receptors are the only ligand-gated ion channel subtype among the 5-HT receptor family (Hoyer and Schoeffter, 1991). Tropisetron and ondansetron are highly selective competitive inhibitors of 5-HT_3 receptors (Broocks, 1992). These receptors are widely distributed in the central nervous system and play a pivotal role in regulation of a variety of processes in different brain structures including hippocampus. Evidence indicates that tropisetron and ondansetron exert antidepressant-like properties in rodent behavioral tests such as forced swimming test (FST), tail suspension test (TST), and learned helplessness (Bravo and Maswood, 2006; Martin et al., 1992; Nakagawa et al., 1998; Ramamoorthy et al., 2008). Also, several clinical investigations have assessed the beneficial effects of

Abbreviations: 5-HT, 5-hydroxytryptamine; NO, nitric oxide; NOS, nitric oxide synthase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; cGMP, cyclic guanosine monophosphate; FST, forced swimming test; TST, tail suspension test; OFT, open-field test; i.p., intraperitoneal; NIH, National Institutes of Health; L-NAME, N^G-L-arginine methyl ester; 7-NI, 7-nitroindazole; L-arg, L-arginine; PDE, phosphodiesterase; ANOVA, analysis of variance; PFC, prefrontal cortex

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ondansetron and tropisetron in psychiatric conditions including anxiety and depression (Haus, 2000; Hewlett et al., 2003; Lecrubier et al., 1993). These 5-HT_3 antagonists have desirable pharmacological profile and cause few adverse effects, and are consequently well-tolerated in patients (Greenshaw and Silverstone, 1997; Rajkumar and Mahesh, 2010).

It has been reported that nitric oxide (NO) is involved in many physiological functions of the 5-HT_3 receptors. In this context, activation of 5-HT_3 receptors has been reported to stimulate nitric oxide synthase (NOS) as well as cyclic guanosine monophosphate (cGMP) pathways (Giordano and Schultea, 2004; Wetzel et al., 1998). The NO–cGMP pathway is known to be implicated in regulation of various (patho)physiologic behavioral and emotional functions (Denninger and Marletta, 1999), and has been suggested as a therapeutic target for depression (Harkin et al., 1999). In this regard, many studies demonstrated that NOS and cGMP inhibition produce antidepressant-like effects in FST and TST (Heiberg et al., 2002; Zomkowski et al., 2010).

Although the antidepressant-like effect of tropisetron and ondansetron in the FST and TST has been reported in rodents, underlying mechanisms through which these drugs exert these antidepressant-like effects are not well defined. In this study, we investigated the possible participation of NO–cGMP pathway in the antidepressant effect of ondansetron and tropisetron in mice.

2. Materials and methods

2.1. Animals

The animals used for this study consisted of male NMRI mice (20–30 g) brought from the Pasteur Institute, Tehran, Iran. All animals were housed in groups of four or five, and were kept at the temperature of 21–23 °C under 12 h regular light/dark cycle. Mice were given access to food and water ad lib. All experiments were carried out between 10:00 and 14:00. All procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1978) and the institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS, No. 91-01-159-18022) (ethical permit number: 3186). All experimental groups included 6–8 animals and full efforts were made to minimize the use of animals and to optimize their comfort (number of animals in each experimental group were shown in Fig. captions).

2.2. Drugs

The following drugs were used in this study: tropisetron, ondansetron, fluoxetine, N^G-L-arginine methyl ester (L-NAME), aminoguanidine, 7-Nitroindazole (7-NI), L-arginine (L-arg), and sildenafil (All were purchased from Sigma, St Louis, MO, USA). Except 7-NI which was dissolved in Tween80 1% solution, all drugs were freshly dissolved in physiological saline and were prepared immediately before the experiments. All drugs were injected through intraperitoneal (i.p.) route and with a volume of 5 ml/kg body weight (Turner et al., 2011).

2.3. Open field test (OFT)

The OFT was used to evaluate the locomotor behavior of animals (Haj-Mirzaian et al., 2015a; Kulesskaya and Voikar, 2014). The open-field apparatus was made of white opaque Plexiglas (50 cm × 50 cm × 30 cm) which was dimly illuminated. Each mouse was placed gently on the center square (30 cm × 30 cm), and behaviors were recorded by a camera for 5 min and were analyzed by Ethovision software version 8 (Noldus, Netherlands). The total 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 the alterations in locomotor activity.

2.4. Forced swimming test (FST)

For conducting the FST, the animals were placed in an open cylinder-shaped flask (diameter 10 cm, height 25 cm) filled with 19 cm water at 24 ± 1 °C. The FST was used to assess behavioral immobility of mice as a selective standard animal test for antidepressant treatment (Haj-Mirzaian et al., 2015b; Porsolt et al., 1977). Being allowed to swim for 6 min, each mouse was assumed immobile when stopped struggling and floated motionless in the water, making only the movements for keeping its head above water. The time of remaining immobile within the last 4 min of the test was recorded.

2.5. Tail suspension test (TST)

In brief, each mouse was suspended on 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 manually measured for a 6 min observation period. Mice were considered immobile only when they hung down passively and were completely motionless (Cryan et al., 2005; Steru et al., 1985). Compounds that decrease immobility and increase active behaviors in FST and TST are considered to suppress indices of depression.

2.6. Treatments

First, we studied the effects of tropisetron and ondansetron on the FST, TST, and OFT. Intraperitoneal injection of ondansetron (0.001, 0.005, 0.01, and 0.1 μg/kg) and tropisetron (1, 5, 10, and 30 mg/kg) 30 min before the behavioral tests was performed to evaluate the effective and subeffective doses of these drugs. These doses and times of administration were based upon our pilot studies and previous reports (Bravo and Maswood, 2006; Ramamoorthy et al., 2008). Fluoxetine (20 mg/kg), a selective serotonin reuptake inhibitor, was used as a standard antidepressant drug. Fluoxetine was administered intraperitoneally 30 min before the tests and the results were considered as the positive control group (Haj-Mirzaian et al., 2014; 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.

In the same way, we assessed the effects of L-NAME (a non-selective NOS inhibitor, 10 mg/kg, 45 min prior to behavioral tests), aminoguanidine (a selective inducible NOS or iNOS inhibitor, 50 mg/kg, 45 min prior to behavioral tests), 7-NI (a selective neuronal NOS or nNOS inhibitor, 25 mg/kg, 30 min prior to behavioral tests), L-arg (an NO precursor, 750 mg/kg, 45 min prior to behavioral tests), and sildenafil (a selective phosphodiesterase (PDE)-5 inhibitor) (5 mg/kg, 30 min prior to behavioral tests) on behaviors of mice in the FST, TST, and OFT. The doses were based on a pilot study and also previous studies (Amiri et al., 2015a; da Silva et al., 2000; Ghasemi et al., 2008; Harkin et al., 2004; Kordjazy et al., 2015; Sadaghiani et al., 2011). To exclude the effect of vehicle administration on behavioral assessments, saline or Tween80 1% (5 ml/kg) were injected 30/45 min before the tests into control groups.

Further, the probable involvement of nitrergic system on the antidepressant-like activity of these 5-HT_3 antagonists was

examined through separately co-administering the subeffective doses of L-NAME (10 mg/kg), aminoguanidine (50 mg/kg), and 7-NI (25 mg/kg) along with the subeffective doses of tropisetron and ondansetron. Also, the effective doses of tropisetron and ondansetron along with subeffective L-arg (750 mg/kg) and sildenafil (5 mg/kg) were co-administered to the animals. After the treatments, animals were subjected to the OFT, and afterwards, to either FST or TST. To exclude the effect of vehicle administration on behavioral assessments, saline or Tween80 1% (5 ml/kg) were injected along with tropisetron and ondansetron into control groups.

2.7. Nitrite assay

To determine the NO level in hippocampus and prefrontal cortex (PFC), we measured the nitrite level as the stable NO end product in another group of animals after injection of different doses of tropisetron and ondansetron in the presence and absence of selective NOS inhibitor (Granger et al., 1996; Kordjazy et al., 2015). Each animal was decapitated 30 min after drug injection under halothane (2%) anesthesia and the hippocampus and PFC were dissected on ice-cold surface and immediately immersed into liquid nitrogen. Tissue homogenates were prepared and nitrite levels were determined by a colorimetric assay based on the Griess reaction. First, 100 µL of each sample was mixed with 100 µL Griess reagent (each hippocampus/PFC of experimental mice was lysed separately). Following 10 min incubation in 20 °C, the absorbance was quantified at 540 nm in an automated plate reader. Concentration of nitrite was determined by reference to a standard curve plotted for sodium nitrite (Sigma, USA) and normalized to the weight of each sample.

2.8. Statistical analysis

Statistical analyses and interpretations 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 multiple comparison test, were used for comparing the immobility times, locomotor activities, and hippocampal nitrite levels between the experimental groups. The analysis method used in each part of the study is mentioned in the Section 3 and "Fig. captions". Finally, $P<0.05$ was considered significant.

3. Results

3.1. Effect of 5-HT_3 antagonists on the immobility time in the FST, TST, and on the locomotor activity in the OFT

One-way ANOVA analysis showed significant effect of tropisetron treatment on the FST ($F(5, 42)=6.891$, $P<0.001$, Fig. 1A) and TST ($F(5, 36)=6.290$, $P<0.001$, Fig. 1B); however, no significant effect was observed in the OFT ($F(5, 42)=0.2830$, $P>0.05$, Fig. 1C). Tukey's post hoc analysis showed that treating mice with tropisetron (5, 10, and 30 mg/kg) significantly decreased the immobility time both in the FST ($P<0.05$, $P<0.01$, and $P<0.001$ respectively, Fig. 1A) and TST ($P<0.05$, $P<0.01$, and $P<0.01$ respectively, Fig. 1B) when compared with the saline-treated animals. However, tropisetron (1 mg/kg) did not show antidepressant-like effect in these tests ($P>0.05$). Also, tropisetron did not produce significant changes in the distance moved by mice in the OFT, as compared with saline-injected control group ($P>0.05$, Fig. 1C).

As shown in Fig. 2, one-way ANOVA analysis revealed that ondansetron treatment significantly affects animals' behavior in the FST ($F(5, 42)=4.752$, $P<0.01$, Fig. 2A) and TST ($F(5, 34)=4.719$, $P<0.01$, Fig. 2B), but not in the OFT ($F(5, 42)=0.1843$, $P>0.05$, Fig. 2C). Multiple comparisons using tukey's post hoc analysis demonstrated that although ondansetron (0.001 µg/kg) did not produce anti-immobility effect ($P>0.05$, Fig. 2A), at doses 0.005, 0.01, and 0.1 µg/kg significantly decreased the immobility time in the FST when compared to saline-injected group ($P<0.05$,

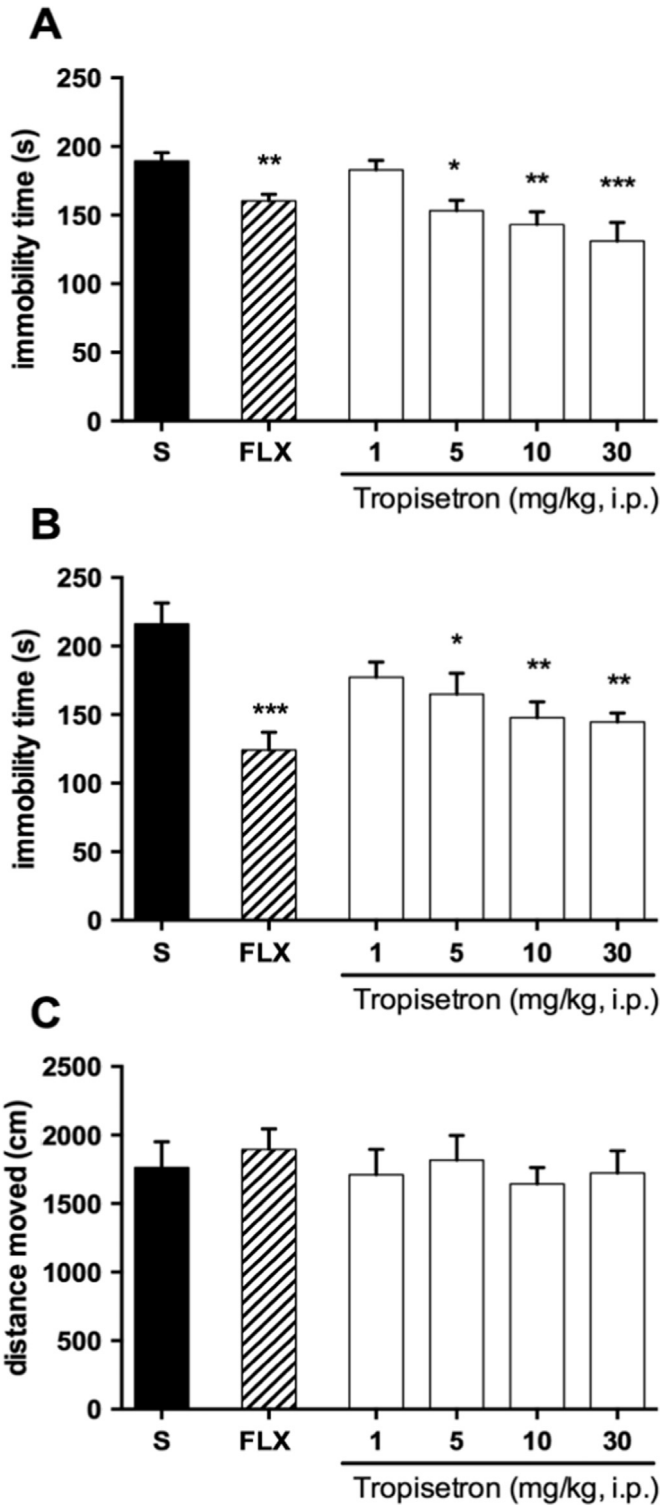

Fig. 1. Antidepressant effect of tropisetron: Effect of acute administration of tropisetron (1, 5, 10, and 30 mg/kg, i.p., 30 min before the tests): (A) Duration of immobility in FST (n=8). (B) Duration of immobility in TST (n=6–8). (C) Distance moved in OFT (n=8). Values are expressed as the mean ± S.E.M, and were analyzed using one-way ANOVA followed by tukey's post hoc test.'*$P<0.05$, **$P<0.01$, and ***$P<0.001$ compared with saline-treated (S) group. Fluoxetine (20 mg/kg, i.p., 30 min before the tests) (FLX) was used as the positive control group.

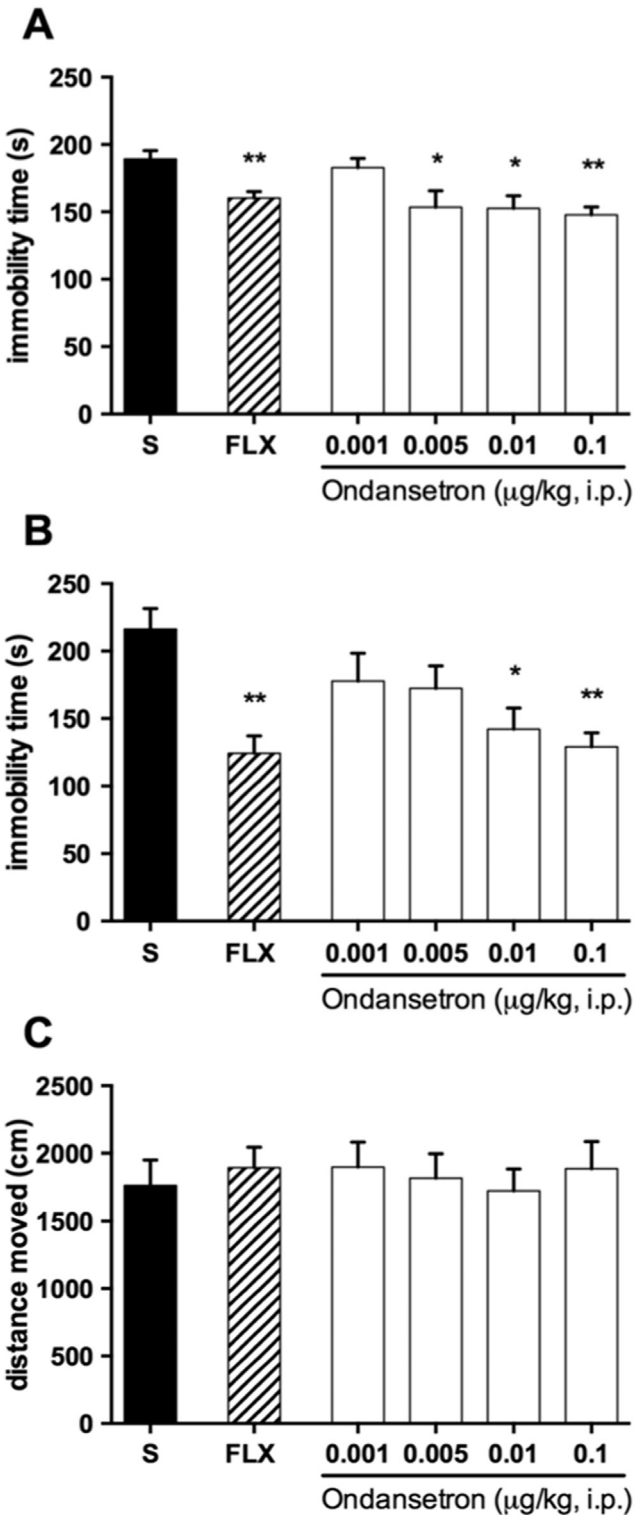

Fig. 2. Antidepressant effect of ondansetron: Effect of acute administration of ondansetron (0.001, 0.005, 0.01, and 0.1 μg/kg, i.p., 30 min before the tests): (A) Duration of immobility in FST (n=8). (B) Duration of immobility in TST (n=6–7). (C) Distance moved in OFT (n=8). Values are expressed as the mean ± S.E.M, and were analyzed using one-way ANOVA followed by tukey's post hoc test. $^{*}P<0.05$ and $^{**}P<0.01$ compared with the saline-treated (S) group. Fluoxetine (20 mg/kg, i.p., 30 min before the tests) (FLX) was used as the positive control group.

$P<0.05$, and $P<0.01$ respectively, Fig. 2A). In the TST, administration of ondansetron (0.01 and 0.1 μg/kg) induced an anti-immobility effect when compared with saline-injected group ($P<0.05$ and $P<0.01$ respectively, Fig. 2B). However, lower doses of this drug (0.005 and 0.001 μg/kg) did not alter the duration of immobility in the TST ($P>0.05$, Fig. 2B). None of the applied doses of ondansetron affected the total distance moved in the OFT ($P>0.05$, Fig. 2C). On the other hand, our data show that the anti-immobility effect of tropisetron/ondansetron was similar to the fluoxetine's, which significantly reduced the immobility time in both FST and TST, but did not alter the locomotor activity of animals in the OFT.

3.2. Effect of NOS inhibitors on the effect of subeffective doses of 5-HT$_3$ antagonists in the FST and TST and on the distance moved in OFT

Figs. 3 and 4 show the effects of subeffective doses of NOS inhibitors on the behavioral tests including FST, TST, and OFT. Tukey's analysis showed that administration of L-NAME (10 mg/kg) as a non-specific NOS inhibitor, 7-NI (25 mg/kg) as a selective nNOS inhibitor, and aminoguanidine (50 mg/kg) as a selective iNOS inhibitor had no effect on the behavioral tests in mice when compared with saline/Tween80-treated groups ($P>0.05$).

On the next step, the effects of co-administration of subeffective doses of 5-HT$_3$ antagonists with subeffective doses of NOS inhibitors on behavioral tests were determined. Tukey's post hoc analysis demonstrated that co-treatment of mice with tropisetron (1 mg/kg) and L-NAME (10 mg/kg) produced antidepressant-like effect in FST ($P<0.01$, Fig. 3A) and TST ($P<0.05$, Fig. 3B) when compared with saline-treated group. Two-way ANOVA showed significant or non-significant effect for tropisetron treatment (F (1, 28)=6.781, $P<0.05$) and (F (1, 20)=8.586, $P<0.01$), L-NAME treatment (F (1, 28)=6.945, $P<0.05$) and (F (1, 20)=0.8823, $P>0.05$), and L-NAME × tropisetron interaction (F (1, 28)=3.263, $P>0.05$) and (F (1, 20)=0.0024, $P>0.05$) in FST and TST, respectively.

Using tukey's multiple comparison, 7-NI (25 mg/kg) and tropisetron (1 mg/kg) co-administration exerted significant anti-immobility effect in FST ($P<0.01$, Fig. 3D) and TST ($P<0.001$, Fig. 3E) when compared with Tween80-treated group. Two-way ANOVA showed significant or non-significant effect for tropisetron treatment (F (1, 27)=10.46, $P<0.01$) (F (1, 22)=19.69, $P<0.001$), 7-NI treatment (F (1, 27)=5.873, $P<0.05$) (F (1, 22)=4.564, $P<0.05$), and their interaction (F (1, 27)=4.953, $P<0.05$) (F (1, 22)=1.27, $P>0.05$) in FST and TST, respectively.

However, aminoguanidine (50 mg/kg) did not potentiate the effect of subeffective dose of tropisetron in both FST ($P>0.05$, Fig. 3G) and TST ($P>0.05$, Fig. 3H). Likewise, no significant effect was observed in two-way ANOVA analysis for tropisetron treatment (F (1, 28)=1.282, $P>0.05$) (F (1, 22)=3.980, $P>0.05$), aminoguanidine treatment (F (1, 28)=0.2302, $P>0.05$) (F (1, 22)=0.0015, $P>0.05$), and their interaction (F (1, 28)=0.2358, $P>0.05$) (F (1, 22)=0.0055, $P>0.05$) in FST and TST, respectively.

Also, two-way ANOVA analysis followed by tukey's post hoc test showed that none of these treatments affected the locomotor activity of mice in the OFT ($P>0.05$, Fig. 3C, F, and I).

Fig. 4 shows that co-administration of the subeffective dose of L-NAME (10 mg/kg) with ondansetron (0.001 μg/kg) induced a significant antidepressant-like effect in the mouse FST ($P<0.05$, Fig. 4A) and TST ($P<0.05$, Fig. 4B), in comparison with saline-treated mice when analyzed by tukey's post hoc test. In two-way ANOVA we also detected significant or non-significant effect for ondansetron treatment (F (1, 28)=4.804, $P<0.05$) (F (1, 23)=8.183, $P<0.01$), L-NAME treatment (F (1, 28)=4.982, $P<0.05$) (F (1, 23)=1.498, $P>0.05$), and their interaction (F (1, 28)=1.360, $P>0.05$) (F (1, 23)=0.1512, $P>0.05$) in FST and TST, respectively.

The subeffective dose of 7-NI (25 mg/kg) administered in combination with subeffective ondansetron (0.001 μg/kg)

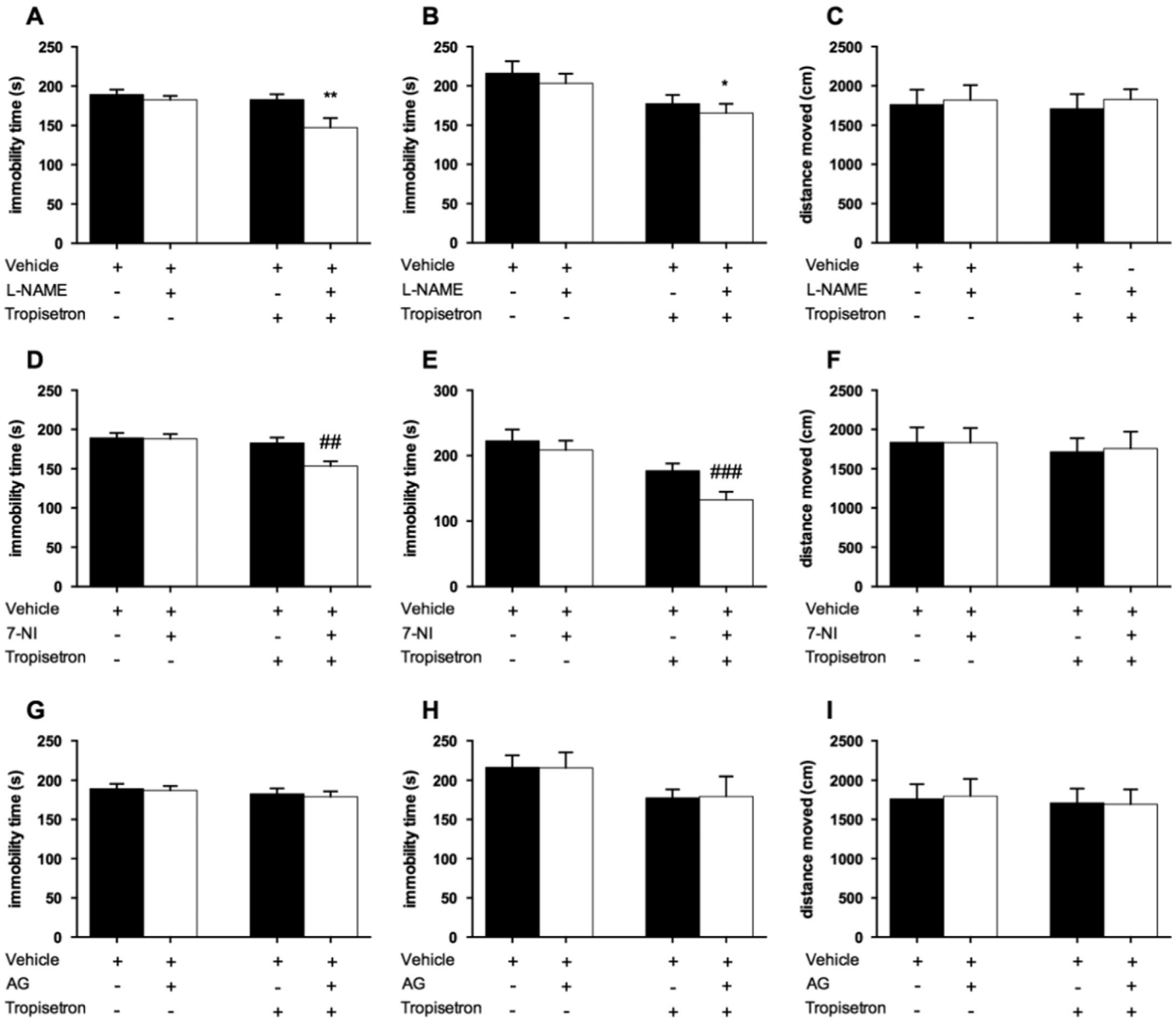

Fig. 3. Role of NO in the antidepressant effect of tropisetron: Effect of L-NAME (10 mg/kg) and tropisetron (1 mg/kg) co-administration on the (A) FST (n=8), (B) TST (n=6–7), and (C) OFT (n=8). Effect of 7-nitroindazole (7-NI, 25 mg/kg) and tropisetron (1 mg/kg) co-administration on the (D) FST (n=7–8), (E) TST (n=6–7), and (F) OFT (n=8). Effect of aminoguanidine (AG, 50 mg/kg) and tropisetron (1 mg/kg) co-administration on the (G) FST (n=8), (H) TST (n=6–7), and (I) OFT (n=8). Values are expressed as the mean ± S.E.M, and were analyzed using two-way ANOVA followed by tukey's post hoc test. $^{*}P<0.05$, $^{**}P<0.01$, $\#\#P<0.01$, and $\#\#\#P<0.001$ compared with vehicle-treated group (saline or Tween80 1%).

demonstrated a significant antidepressant-like effect in the FST ($P<0.001$, Fig. 4D) and TST ($P<0.01$, Fig. 4E) compared with Tween80-administered group using Tukey's post hoc test. Two-way ANOVA revealed significant or non-significant effect for ondansetron treatment (F (1, 28)=13.04, $P<0.01$) (F (1, 22)=15.15, $P<0.01$), 7-NI treatment (F (1, 28)=6.428, $P<0.05$) (F (1, 22)=4.091, $p=0.05$), and their interaction (F (1, 28)=5.591, $P<0.05$) (F (1, 22)=1.401, $P>0.05$) in FST and TST, respectively.

Tukey's analysis revealed that concurrent administration of subeffective doses of aminoguanidine with ondansetron did not significantly alter the immobility duration in FST ($P>0.05$, Fig. 4G) and TST ($P>0.05$, Fig. 4H). Further analysis with two-way ANOVA excluded any significant effect for ondansetron treatment (F (1, 28)=1.243, $P>0.05$) (F (1, 22)=6.067, $P<0.05$), aminoguanidine treatment (F (1, 28)=0.2077, $P>0.05$) (F (1, 22)=0.069, $P>0.05$), and their interaction (F (1, 28)=0.0159, $P>0.05$) (F (1, 22)=0.051, $P>0.05$) in FST and TST, respectively.

Likewise the last section, two-way ANOVA analysis followed by multiple comparison tests showed that administration of none of the applied drugs caused ambulation in locomotion of mice ($P>0.05$, Fig. 4C, F, I).

3.3. Effect of L-arginine pre-treatment on the anti-immobility effect of 5-HT$_3$ antagonists in the FST and TST and on the distance moved in OFT

Although L-arg (750 mg/kg) individually did not alter the immobility time of mice, tukey's analysis showed that it significantly reversed the antidepressant-like effect of tropisetron (10 mg/kg) in FST ($P<0.01$, Fig. 5A) and TST ($P<0.05$, Fig. 5B). Two-way ANOVA showed significant or non-significant effect for tropisetron treatment (F (1, 28)=13.99, $P<0.001$) (F (1, 24)=15.94, $P<0.001$), L-arg treatment (F (1, 28)=6.238, $P<0.05$) (F (1, 24)=3.680,

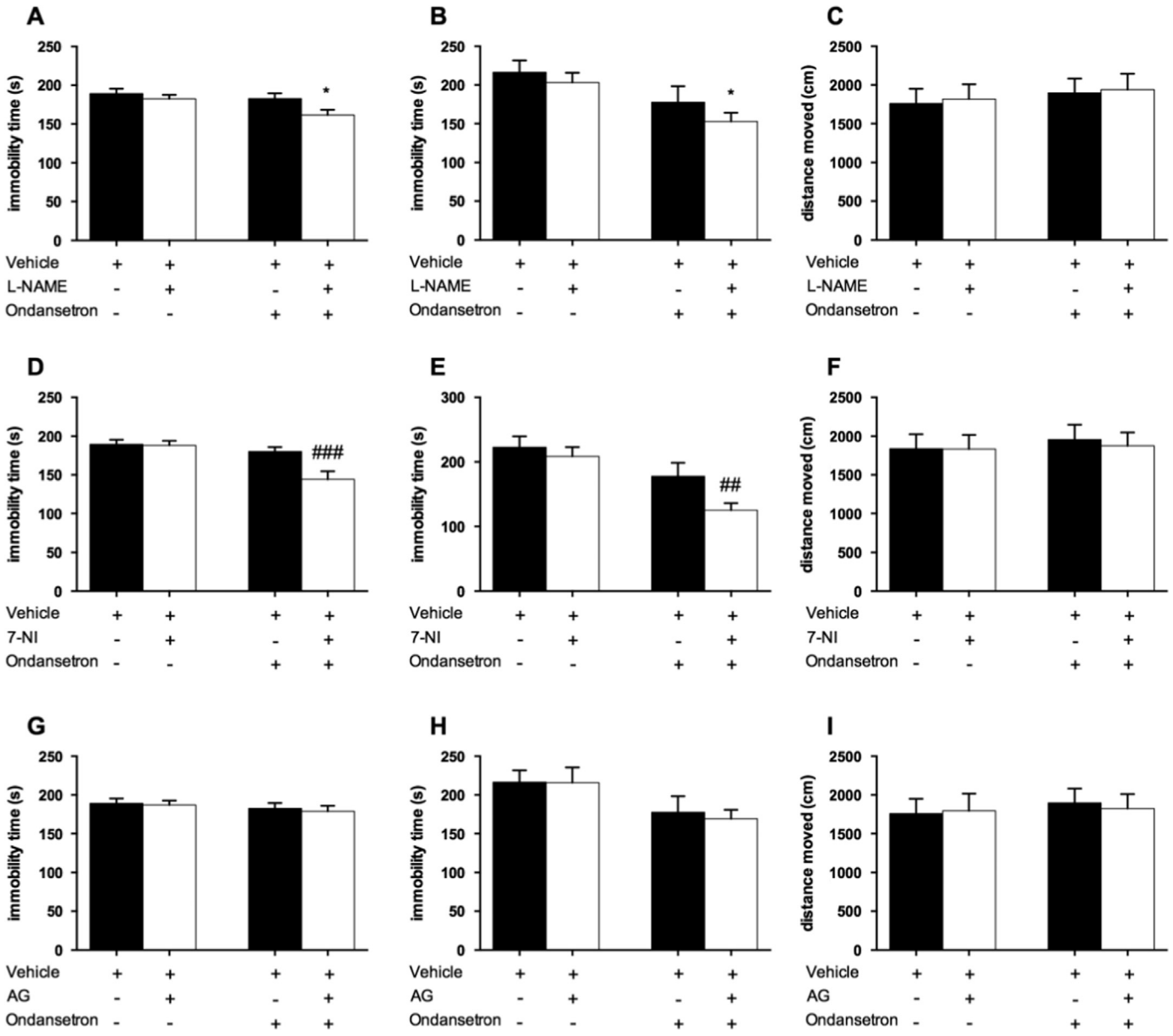

Fig. 4. Role of NO in the antidepressant effect of ondansetron: Effect of L-NAME (10 mg/kg) and ondansetron (0.001 μg/kg) co-administration on the (A) FST (n=8), (B) TST (n=6–8), and (C) OFT (n=8). Effect of 7-nitroindazole (7-NI, 25 mg/kg) and ondansetron (0.001 μg/kg) co-administration on the (D) FST (n=7–8), (E) TST (n=6–7), and (F) OFT (n=8). Effect of aminoguanidine (AG, 50 mg/kg) and ondansetron (0.001 μg/kg) co-administration on the (G) FST (n=8), (H) TST (n=6–7), and (I) OFT (n=8). Values are expressed as the mean ± S.E.M, and were analyzed using two-way ANOVA followed by tukey's post hoc test. *$P<0.05$, ##$P<0.01$, and ###$P<0.001$ compared with vehicle-treated group (saline or Tween80 1%).

$P>0.05$), and their interaction (F (1, 28)=6.238, $P<0.05$) (F (1, 24)=4.001, $p=0.05$) in FST and TST, respectively.

Our results show that pre-treatment with L-arg prevented the antidepressant-like effect of ondansetron (0.01 μg/kg) in the FST ($P<0.01$, Fig. 5D) and also the TST ($P<0.01$, Fig. 5E). Also, in two-way ANOVA we found a significant effect for ondansetron treatment (F (1, 28)=12.17, $P<0.01$) (F (1, 22)=16.16, $P<0.001$), L-arg treatment (F (1, 28)=7.052, $P<0.05$) (F (1, 22)=5.836, $P<0.05$), and their interaction (F (1, 28)=7.052, $P<0.05$) (F (1, 22)=6.265, $P<0.05$) in FST and TST, respectively.

Administration of L-arg alone or in combination with tropisetron/ondansetron did not affect the ambulatory behavior in the OFT when evaluated by two-way analysis followed by tukey's test ($P>0.05$, Fig. 5C and F).

3.4. Effect of sildenafil pre-treatment on the anti-immobility effect of 5-HT$_3$ antagonists in the FST and TST and on the distance moved in OFT

Fig. 6 shows that administration of sildenafil (5 mg/kg) did not alter the immobility time of mice individually in the FST and TST compared with saline-treated group ($P>0.05$). However, tukey's analysis showed that sildenafil significantly reversed the anti-immobility effect of tropisetron (10 mg/kg) in the FST ($P<0.001$, Fig. 6A) and TST ($P<0.05$, Fig. 6B). Two-way ANOVA showed significant or non-significant effect for tropisetron treatment (F (1, 28)=9.949, $P<0.01$) (F (1, 23)=26.04, $P<0.001$), sildenafil treatment (F (1, 28)=7.883, $P<0.01$) (F (1, 23)=3.642, $P>0.05$), and their interaction (F (1, 28)=12.87, $P<0.01$) (F (1, 23)=7.627, $P<0.05$) in FST and TST, respectively.

Tukey's analysis revealed that administration of sildenafil also attenuated the antidepressant-like effect of ondansetron

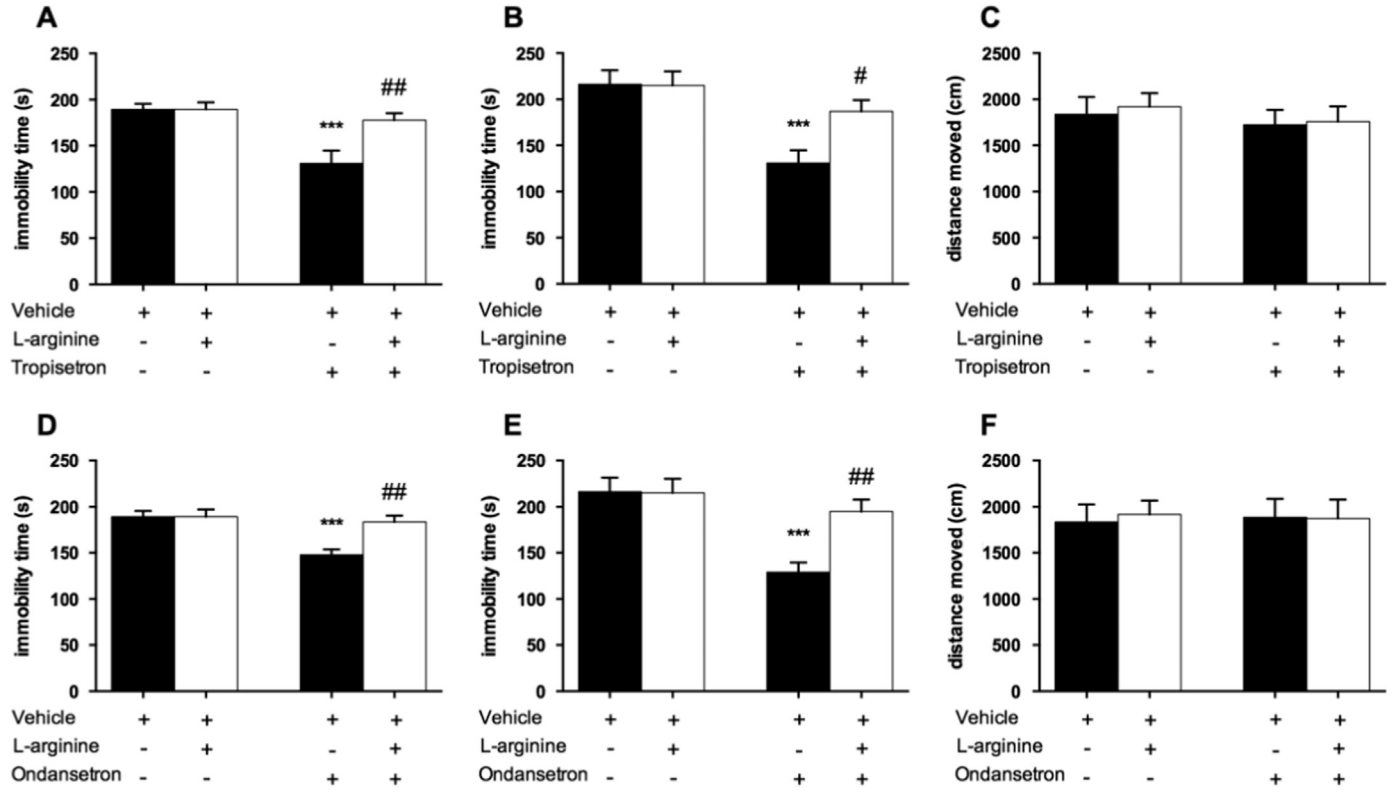

Fig. 5. Effect of NO precursor on the antidepressant effect of 5-HT_3 antagonists: Effect of subeffective dose of L-arginine (750 mg/kg) on the effects of tropisetron (10 mg/kg) in (A) FST (n=8), (B) TST (n=6-8), and (C) OFT (n=8). Effect of subeffective dose of L-arginine (750 mg/kg) on the effects of ondansetron (0.01 μg/kg) in (D) FST (n=8), (E) TST (n=6–7), and (F) OFT (n=8). Values are expressed as the mean ± S.E.M, and were analyzed using two-way ANOVA followed by tukey's post hoc test. $^{***}P < 0.001$ compared with the control vehicle treated group. $\#P < 0.05$ and $\#\#P < 0.01$ compared with the ondansetron/tropisetron-treated group.

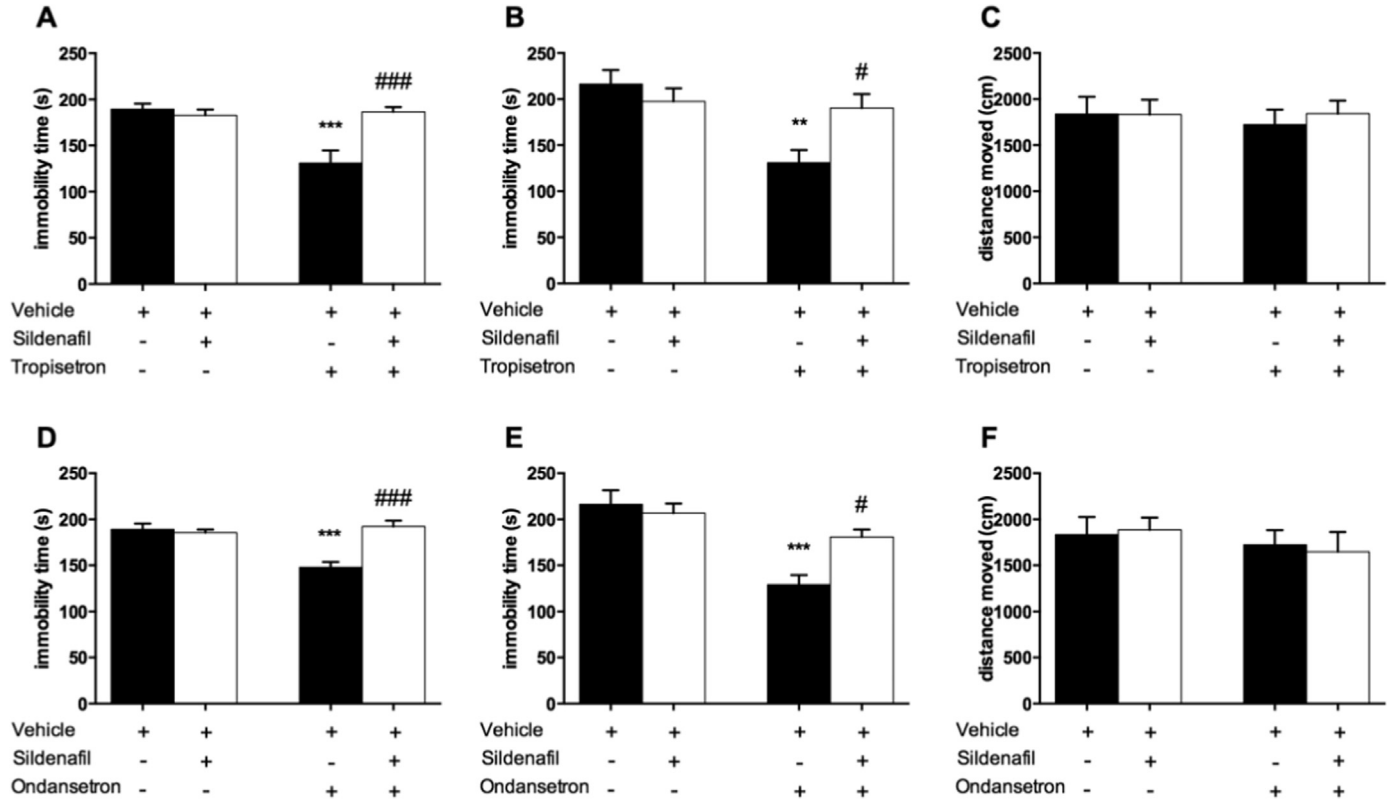

Fig. 6. Effect of PDE inhibitor on the antidepressant effect of 5-HT_3 antagonists: Effect of subeffective dose of sildenafil (5 mg/kg) on the effects of tropisetron (10 mg/kg) in (A) FST (n=8), (B) TST (n=6–8), and (C) OFT (n=8). Effect of subeffective dose of sildenafil (5 mg/kg) on the effects of ondansetron (0.01 μg/kg) in (D) FST (n=8), (E) TST (n=6–7), and (F) OFT (n=8). Values are expressed as the mean ± S.E.M, and were analyzed using two-way ANOVA followed by tukey's post hoc test. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the control vehicle treated group. $\#P < 0.05$ and $\#\#\#P < 0.001$ compared with the ondansetron/tropisetron-treated group.

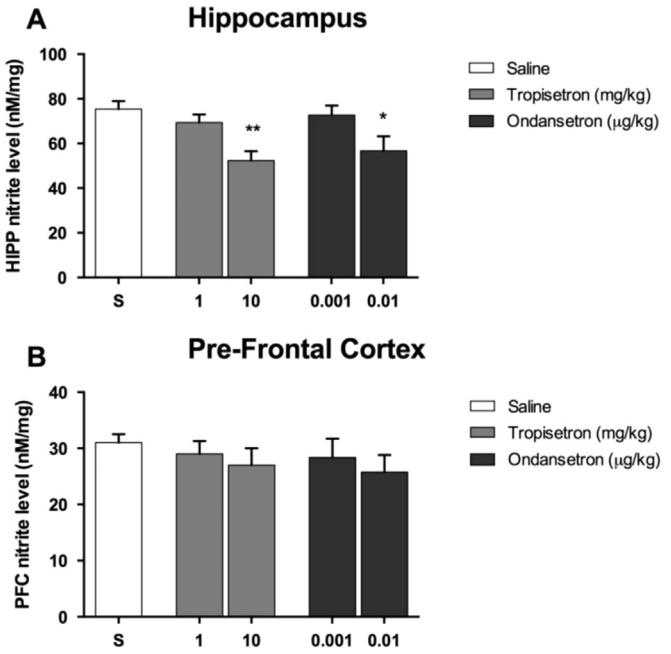

Fig. 7. Hippocampal and cortical nitrite assay: Effect of tropisetron 1 mg/kg (n=7–8) and 10 mg/kg (n=7–8), ondansetron 0.001 μg/kg (n=7) and 0.01 μg/kg (n=7) administration on the level of nitrite in the hippocampus (A) and PFC (B) of experimental mice. Values are expressed as the mean ± S.E.M., and were analyzed using one-way ANOVA followed by tukey's post hoc test. $^{*}P<0.05$ and $^{**}P<0.01$ compared with the saline-treated (S) group (n=7–8).

(0.01 μg/kg) in both FST ($P<0.001$, Fig. 6D) and TST ($P<0.05$, Fig. 6E). In two-way ANOVA also we detected significant or non-significant effect for ondansetron treatment (F (1, 28)=9.836, $P<0.01$) (F (1, 26)=9.746, $P<0.01$), sildenafil treatment (F (1, 28)=13.68, $P<0.001$) (F (1, 26)=1.847, $P>0.05$), and their interaction (F (1, 28)=18.73, $P<0.001$) (F (1, 26)=6.895, $P<0.05$) in FST and TST, respectively.

Sildenafil, concurrent with saline or 5-HT_3 antagonists, did not affect the locomotor activity of mice when evaluated by two-way analysis followed by tukey's multiple comparison tests ($P>0.05$, Fig. 6C and F).

3.5. Nitrite assay

One-way ANOVA revealed significant effects of tropisetron and ondansetron treatments on hippocampal nitrite levels (F (4, 33)=5.320, $P<0.01$, Fig. 7A). Tukey's post hoc showed that tropisetron (10 mg/kg) and ondansetron (0.01 μg/kg) induced a significant decline in the hippocampal nitrite levels compared to saline-administered animals ($P<0.01$ and $P<0.05$, respectively), while the subeffective doses of these drugs, which were obtained from the behavioral tests, did not decrease the nitrite level in hippocampus ($P>0.05$, Fig. 7). Obtained result was different in the PFC area; one-way ANOVA analysis revealed that there was no significant difference in the cortical nitrite level between treated groups (F (4, 30)=0.5340, $P>0.05$, Fig. 7B). Also, tukey's post-test did not show any significant difference between tropisetron/ondansetron-treated groups and saline-administered mice ($P>0.05$, Fig. 7B).

In the next part, the possible effect of 5-HT_3 antagonists and nNOS inhibitor co-administration on hippocampal (F (5, 37)=4.607, $P<0.01$, Fig. 8A) and cortical (F (5, 36)=0.6467, $P>0.05$, Fig. 8B) nitrite level was evaluated. In hippocampal area, results obtained from tukey's post hoc revealed that administration of 7-NI (25 mg/kg, subeffective dose) alone failed to alter the nitrite content, however co-administration of subeffective 7-NI with the subeffective doses of tropisetron (1 mg/kg) or ondansetron

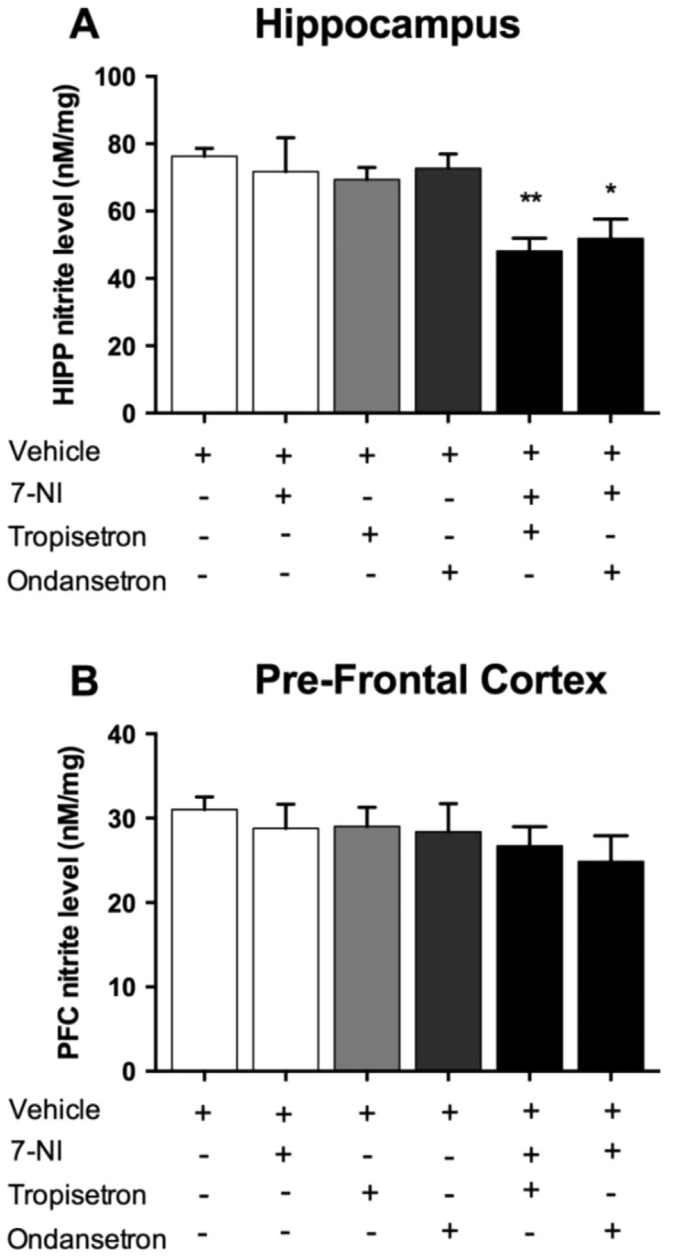

Fig. 8. Hippocampal and cortical nitrite assay: Effect of 7-NI (25 mg/kg, n=7) administration on the level of nitrite in the hippocampus (A) and PFC (B) of tropisetron (1 mg/kg, n=7–8) and ondansetron (0.001 μg/kg, n=7)-treated mice. Values are expressed as the mean ± S.E.M., and were analyzed using one-way ANOVA followed by tukey's post hoc test. $^{*}P<0.05$ and $^{**}P<0.01$ compared with the vehicle (Tween80/saline)-injected group (n=7–8).

(0.001 μg/kg) caused a significant decrease in hippocampal nitrite levels compared with the animals receiving saline/Tween80 ($P<0.01$ and $P<0.05$ respectively, Fig. 8A). However, all treatments as well as 5-HT_3 antagonists and nNOS inhibitor co-administration did not significantly change the nitrite level of PFC in animals ($P>0.05$, Fig. 8B).

4. Discussion

In this study, we have shown the antidepressant effect of tropisetron and ondansetron in behavioral tests of despair; and statistically, this effect is almost equal to the standard antidepressant fluoxetine (Cryan et al., 2002). Our results revealed that

administration of NOS inhibitors amplified the antidepressant effect of tropisetron and ondansetron. Also, administration of NO precursor and PDE inhibitor mitigated the antidepressant effect of these 5-HT_3 antagonists. Considering these, we have determined that the antidepressant effect of tropisetron and ondansetron is partly mediated through NO-cGMP pathway. Furthermore, we have found that these behavioral alterations are correlated with decreased hippocampal (but not cortical) nitrite level. Therefore, the hippocampal nitrergic system (especially nNOS) seems to be partly involved in the antidepressant-like effects of tropisetron and ondansetron. Although few earlier reports have challenged the possible effective value of 5-HT_3 antagonists in depression, to our knowledge, no studies have been conducted to investigate their exact mechanisms of action.

Antidepressant-like effect of ondansetron in the FST of male mice had been investigated in past studies with which our results are in accord (Ramamoorthy et al., 2008); however, our study for the first time reveals the dose-response effect of tropisetron in models of depression in male mice. The antidepressant-like behavior of ondansetron and tropisetron in our experiments was not related to the changes of locomotion and motoric behavior in the OFT. Previous clinical investigations have reported that selective antagonist of 5-HT_3 receptors might be effective in treatment of psychiatric disorders, including depression (Greenshaw and Silverstone, 1997; Haus, 2000; Hewlett et al., 2003; Lecrubier et al., 1993). Several experimental studies on animals provided evidence of antidepressant effect of ondansetron in different animal models of depression (Gupta et al., 2014; Martin et al., 1992; Ramamoorthy et al., 2008); and few studies have investigated the effect of tropisetron on behaviors related to depression in animal models, such as the learned-helplessness paradigm (Bravo and Maswood, 2006; Martin et al., 1992). In addition, recent studies have demonstrated the antidepressant-like effect of novel serotonin type 3 antagonists in FST without altering the baseline locomotor activity (Bhatt et al., 2013; Kurhe et al., 2014; Mahesh et al., 2014).

Our results rules out the involvement of iNOS, since aminoguanidine was not able to alter the effect of ondansetron and tropisetron in behavioral tests. Our data are consistent with the variety of evidence illustrating the role of NO in major depression and mechanism of action of antidepressants (Joca and Guimarães, 2006). Numerous studies have indicated that NOS inhibition results in antidepressant-like behavioral effect in a variety of animal tests of depression (Harkin et al., 1999). Interestingly, large body of evidence from previous studies have focused on the involvement of nNOS in the pathophysiology of depression (Heiberg et al., 2002; Joca and Guimarães, 2006; Zhou et al., 2007). Since nNOS is a more abundant isoenzyme in the CNS, especially hippocampus, antidepressant effects of non-selective NOS inhibitors have been mostly ascribed to nNOS (Guix et al., 2005; Steinert et al., 2010), since it is known that over-expression of iNOS is mostly observed during conditions such as inflammation and chronic stress (Harvey et al., 2004; Olivenza et al., 2000); that might be the explanation of our data regarding the experiments with aminoguanidine. On the other hand, a decline in the NO level within the hippocampus was shown to induce antidepressant-like effect, proposing the involvement of endogenous hippocampal NO in neurobiology of depression (Joca and Guimarães, 2006). Also, it has been shown that endogenous NO exerts a negative control over the levels of 5-HT and dopamine and NOS inhibitors increase the 5-HT and dopamine levels in the hippocampus (Wegener et al., 2000). Thus, possibly the antidepressant effect of NOS inhibitors is mediated through serotonergic and dopaminergic pathways. Nitric oxide has been proposed to modulate synaptic transmission in several ways; the most common is through activation of guanylate cyclase which is responsible for an increase in cGMP (Garthwaite and Boulton, 1995). Animal studies have demonstrated that by regulating the effect of cGMP (Denninger and Marletta, 1999), NO produces depression-like state in an animal model (Spiacci Jr. et al., 2008). Both the inhibition of soluble guanylate cyclase and weakening NOS activity may decrease the level of cGMP and may produce antidepressant-like behaviors (Ghasemi et al., 2008; Heiberg et al., 2002). The cGMP is degraded into GMP by the action of phosphodiesterase enzyme (Eckeli et al., 2000). Sildenafil, a 5-PDE inhibitor that causes an increase in level of cGMP in target tissues, is able to prevent the antidepressant-like effect elicited by some antidepressants (Dhir and Kulkarni, 2007; Ghasemi et al., 2008; Zomkowski et al., 2010).

Detecting a lower level of nitrite in the hippocampus (but not in the PFC) of ondansetron and tropisetron-treated mice plus the amplification of this nitrite reduction by 7-NI treatment is consistent with our behavioral results. We have investigated the hippocampal and cortical nitrite level because these region are of the most susceptible structures in the brain to the negative effects of stress due to presence of neural progenitor cells that conduct neurogenesis, a pivotal phenomenon necessary for cognitive and behavioral functions (Kempermann and Kronenberg, 2003; Sahay and Hen, 2007; Warner-Schmidt and Duman, 2006). It has been shown that nNOS expression density (under normal condition) is significantly higher in the hippocampus when compared with other brain regions such as cortical area (Blackshaw et al., 2003; Lourenço et al., 2014). Lourenço et al. studied the effect of L-glutamate (a N-Methyl-D-aspartic acid receptor agonist which causes intracellular calcium influx and activates nNOS enzyme) on nNOS activity in different brain structures including hippocampus and cortex (Lourenço et al., 2014). They found that the hippocampus has one of the highest NO content produced by local application of L-glutamate as compared with the other brain regions such as cortex. On the other hand, in our recent published study we have shown that nNOS activity might be more prominent in the hippocampus rather than cortical area (Amiri et al., 2015a).

5-HT_3 receptors are ion channels and activation of these receptors results in considerable increase in intracellular calcium level of hippocampus (Nayak et al., 1999). Calcium influx triggers multiple intracellular signaling cascades including nitrergic pathway and activates NOS enzymes. Since 5-HT_3 receptors are basically ion-channels, it is possible that blockade of these receptors results in the reduction of calcium influx and this effect inhibits the nNOS enzyme, which is calcium dependent. Reduction of the nNOS activity causes antidepressant effects through decreasing NO production (Joca and Guimarães, 2006; Zhou et al., 2007). The functional connection between the serotonergic and NO pathways has been also demonstrated previously, when it was reported that inhibiting the NOS enzyme could be used as a strategy to strengthen the clinical effect of serotonergic antidepressants (Harkin et al., 1999). In addition, contribution of NO to some peripheral (Sévoz-Couche et al., 2002) and central (Gholipour et al., 2010) effects of 5-HT_3 receptors have been understood. As examples, an observation by Javadi-Paydar et al. with application of the scopolamine-induced memory impairment in mice, suggested the involvement of nitric oxide in the enhancing effect of 5-HT_3 antagonist on memory (Javadi-Paydar et al., 2012). Also, in a recent study, QCM-4, a novel 5-HT_3 antagonist, attenuated the brain nitrite level elevation due to chronic mild stress in mice (Kurhe et al., 2014). In our recent published study, we have also shown that tropisetron induces its anxiolytic effect in social isolation stress paradigm through inhibition of hippocampal NOS (especially iNOS) activity (Amiri et al., 2015b). However, therewith we found the prominent role of iNOS, but not nNOS enzyme; because, chronic stress induces neuroinflammation in brain regions such as cortex and hippocampus and causes an increase in expression of iNOS enzyme which is not expressed in normal conditions such as that in the current study.

5. Conclusions

In conclusion, our results demonstrate that tropisetron and ondansetron, the 5-HT_3 receptor antagonists exert antidepressant-like effect in mouse, when administered through the i.p route. The data from our current study also suggest that the antidepressant-like effect of tropisetron and ondansetron is mediated by inhibition of NO-cGMP pathway. But, undeniably, it remains to be explored whether the contribution of nitrergic system in acute antidepressant effect of these 5-HT_3 antagonists is of value in clinical setting.

Conflict of interest

The authors declare that there are no conflicts of interest. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ejphar.2016.03.034.

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