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Dual effects of nitric oxide in the mouse forced swimming test: possible contribution of nitric oxide mediated serotonin release and potassium channel modulation

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Abstract

Recent findings have indicated that nitric oxide (NO) may change the duration of immobility biphasically in the forced swimming test, which is a useful experimental model for screening antidepressant-like activity in rodents. In the present study, we have investigated the role of serotonin and of potassium (K^+) channels in the dual effects of NO in the mouse forced swimming test (MFST). For this purpose, we tested the effects of L-arginine, an NO precursor, the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), and of K^+-channel blockers tetraethylammonium (TEA) and 3,4-diaminopyridine (3,4-DAP). In addition, we used sertraline as a serotonin reuptake inhibitor and cyproheptadine as a serotonin antagonist. L-Arginine increased the duration of immobility in the MFST in low doses (25 mg/kg ip) but decreased it in higher doses (500 and 1000 mg/kg ip). Low doses of L-NAME (50 and 75 μg icv) decreased while higher dose of this drug (150 μg icv) increased the immobility time. TEA (5 μg icv) and 3,4-DAP (0.05 μg icv) significantly reduced the time, whereas K^+ channel opener pinacidil increased the duration of immobility. L-Arginine (100 mg/kg ip) significantly antagonised the effects of L-NAME (50 μg), 3,4-DAP and TEA. Higher dose of L-arginine (500 mg/kg ip) significantly potentiated the effects of 3,4-DAP and TEA, but reduced the effect of pinacidil. Low doses of L-arginine antagonized, but higher doses of L-arginine potentiated the antidepressant-like effect of sertraline. Sertraline potentiated the effects of 3,4-DAP and TEA, but reversed the effect of pinacidil. Cyproheptadine reduced the anti-immobility effect of L-arginine and 3,4-DAP. At the highest effective doses, drugs did not impair the motor functions. These data support the hypothesis that NO effects may involve the release of serotonin and/or modulation of K^+ channels.

Keywords: Nitric oxide; Potassium channels; Sertraline; Forced swimming test; Mice

1. Introduction

Nitric oxide (NO) is an important messenger in the central nervous system. It has been suggested that NO may be involved in the pathophysiology of Alzheimer's disease, cerebral ischemia, alcohol-induced brain damage, long-term potentiation, learning, memory, wakefulness, circadian rhythm, nociception, olfaction, food intake, drinking, regulation of neurotransmitter release, and anxiety (Dawson and Dawson, 1996). Some reports have indicated that NO affects many functions such as pain (Kawabata et al., 1993; Prado et al., 2002; Sousa and Prado, 2001), synaptic plasticity (Li and Wieraszko, 1994), and neuronal damage (Nara et al., 1999; Weissman et al., 1992) biphasically. Some contradictory results also have been shown for the effects of NO-modulating agents in the mouse forced swimming test (MFST), which provides a useful experimental model for screening preclinical antidepressant activity in rodents (Porsolt et al., 1977; David et al., 2003; Gavioli et al., 2003; Makino et al., 2000; Sortwell and Sagen, 1993). The NO precursor L-arginine was found to be ineffective on the immobility time in the MFST (Harkin et al., 1999). da Silva et al. (2000) showed that L-arginine significantly decreased the immobility time at the doses of 250 and 500 mg/kg. In the same study, at 1000 mg/kg, L-arginine increased the immobility time when compared to the dose of 500 mg/kg, this suggests a dual effect. NO synthase (NOS) inhibitors such as N^G-nitro-L-arginine (L-NNA), L-NAME and 7-nitroindazole (7-NI), and the NO-soluble guanylate cyclase inhibitor ODQ have been found to reduce the duration of immobility in a

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dose-dependent manner in the MFST (Jefferys and Funder, 1996; Harkin et al., 1999; da Silva et al., 2000; Yildiz et al., 2000; Heiberg et al., 2002). da Silva et al. (2000) have suggested that NO has a dual role in the modulation of depression. Harkin et al. (1999) have suggested that the biphasic effect of some NO synthase antagonists may be attributed to adverse effects of high doses of compounds. However, the detailed underlying mechanisms of the biphasic role of NO in depression has not been clarified yet.

NO donors and inhibitors have been shown to affect serotonin release in a dose-dependent manner in rodents (Lorrain and Hull, 1993; Kaehler et al., 1999; Smith and Whitton, 2000). 7-NI, a neuronal NOS inhibitor, given alone decreased raphe serotonin release with a concomitant increase being seen in the frontal cortex. Low concentrations of SNAP, which is a NO donor, infused in the raphe were found to decrease serotonin release locally but increase in the frontal cortex. In contrast the highest concentration of SNAP used was found to have the opposite effect in both brain regions (Smith and Whitton, 2000). The NO donors linsidomine, diethylamine/nitric oxide, SNAP, SNOG, and sodium nitroprusside influenced the release of serotonin in a biphasic way. Low concentrations of drugs diminished, while higher concentrations of these compounds enhanced the outflow of serotonin. High concentrations of L-NAME diminished, while low concentrations of this compound enhanced the outflow of serotonin (Kaehler et al., 1999). These data suggest that endogenous NO modulates the release of serotonin in a biphasic way.

Moreover, it has been reported that NO can activate different types of K^+ channels in several tissues (Bolotina et al., 1994; Armstead, 1996; Shin et al., 1997; Jeong et al., 2001). Several studies suggest that NO affects K^+ channels. Activation of NO–cyclic GMP pathway followed by the opening of ATP-sensitive K^+ channels results in antinociceptive action (Lazaro-Ibanez et al., 2001). NO donors have long-lasting anti-inflammatory effect in mouse paw edema, which involves guanylate cyclase and tetraethylammonium-sensitive K^+ channels (Fernandes et al., 2002). Large-conductance Ca^{2+}-activated K^+ channels are suggested as one of the physiological targets of NO in the brain (Jeong et al., 2001). It has been suggested that K^+ channels play a role in depression (Galeotti et al., 1999). Different K^+-channel blockers such as tetraethylammonium (TEA), apamin, charybdotoxin, and gliquidone may impair the duration of immobility in the MFST, whereas the K^+ channel openers such as minoxidil or cromakalim may increase the duration of immobility (Galeotti et al., 1999).

Taken together, the above data suggest that NO effects on the MFST could involve the modulation of serotonin release and/or activation of K^+ channels. To investigate this possibility, the effects of the NO precursor L-arginine, NOS inhibitor L-NAME, serotonin reuptake inhibitor sertraline, serotonin antagonist cyproheptadine, K^+-channel opener pinacidil, and K^+-channel blockers TEA and 3,4-diaminopyridine (3,4-DAP) in the MFST were used. Rotarod and activity cage tests were applied to separate groups of mice to examine the probability that the drugs used in the experiments elicited their modulatory effects in the MFST by changing either motor coordination or spontaneous locomotor activity.

2. Materials and methods

2.1. Animals

Male, albino, inbred BALB/c mice weighing 20–25 g obtained from Animal Care Division of Physiology Department in Cukurova University were used in the experiments. The mice were housed five per cage and kept in a regulated environment (24 ± 1 °C, light–dark cycle, with the light on between 0600 and 1800 h). They received food and water ad libitum. All mice used for the experiments were naive to the forced swimming test. The experiments were conducted between 0900 and 1100 h in a sound-attenuated laboratory. There were 10 mice in each group. Experiments were performed according to the guidelines of the principles of laboratory animal care published by NIH and all protocols were approved by the Animal Ethics Committee of Cukurova University.

2.2. Drugs

We purchased 3,4-diaminopyridine (D-7148), tetraethylammonium chloride (T-2265), N^G-nitro-L-arginine methyl ester dihydrochloride (A-5881), D-arginine hydrochloride (A-6757), and cyproheptadine hydrochloride (C-6022) from Sigma (Steinheim, Germany), L-arginine monohydrochloride from Merck (Darmstadt, Germany), and pinacidil (P-154) from RBI (Natick, MA, USA). Sertraline hydrochloride (LUSTRAL) was a gift from Pfizer (Turkey). All drugs except pinacidil were dissolved in 0.9% NaCl (saline) solution. Pinacidil was dissolved in DMSO (3:1). 3,4-DAP, TEA, pinacidil, and L-NAME were given intracerebroventricularly and administered 10 min before the test. Doses of 3,4-DAP, TEA, and pinacidil were chosen according to our preliminary studies and the literature (Galeotti et al., 1999). L-Arginine was given intraperitoneally. Sertraline was given orally by gavage in a volume of 0.3 ml/10 g body weight, 60 min before the test. Intraperitoneal injections were given in a volume of 0.1 ml/10 g body weight 30 min before the test. Control groups received saline or vehicle (DMSO + distilled water; 3:1). In sertraline + L-arginine groups, sertraline was administered 30 min before L-arginine. In sertraline + 3,4-DAP, TEA, or pinacidil groups, sertraline was given 60 min before these drugs.

2.3. Intracerebroventricular injection technique

Intracerebroventricular administration was performed under isoflurane anaesthesia, according to the method de-

scribed by Haley and McCormick (1957) and modified by Wang et al. (1994). Briefly, mice were exposed to isoflurane in an anaesthesia chamber by inhalation. Adequate anesthetic level was indicated by loss of the tail movements and eye reflex to determine the injection time. During anaesthesia, a 0.4-mm-external-diameter hypodermic needle attached to a 25-μl Hamilton syringe (# 802, Reno, NV, USA) was inserted perpendicularly through the skull no more than 2 mm into the brain of the mouse and 5 μl were then administered. The injection site was 1 mm to the right or left from the midpoint on a line drawn through to the anterior base of the ears. Injections were performed into the right or left ventricle randomly. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice (30%) were injected with 5 μl of diluted 1:10 methylene blue, and their brains were examined macroscopically after sectioning. The accuracy of the injection technique was evaluated, and the percentage of correct injections was 95%.

2.4. Mouse forced swimming test

The MFST was similar to that described by Porsolt et al. (1977). Briefly, mice were gently lowered individually into a glass cylinders (height 19 cm, diameter 15 cm) containing 9 cm of water maintained at 23–25 °C and left there for 6 min. A mouse was judged to be immobile when it floated in the water, in an upright position, and made only small movements to keep its head above water. Since little immobility was observed during the first 2 min the duration of immobility was recorded during the last 4 min of the 6 min test. A decrease in the duration of immobility was interpreted as indicating an antidepressant-like effect. In each test, fresh water was used. The test was performed 10 min after intracerebroventricular administration of the drugs.

2.5. Rotarod and activity cage tests

For rotarod testing, the mice receiving the highest effective doses of drugs or saline were placed on the rotating bar (20 rpm) of the rotarod apparatus (Rotarod treadmills, Ugo Basile, 7600, Italy) for 5 min, and time to fall was determined. To examine spontaneous locomotor activity, the mice receiving the drugs or saline were placed in the activity cage (Ugo Basile, 7400), and their activity (number of beam crossings) was evaluated for 15 min.

2.6. Statistics

Results were expressed as the means ± S.E.M. One-way analysis of variance (ANOVA) followed by a post hoc Student–Newman–Keuls test was used for the comparison of multiple groups, and unpaired Student *t* test was used for the comparison of two groups. Significance was set at $P < .05$.

3. Results

3.1. Effect of L-arginine on the duration of immobility

The dose–response curve for L-arginine (25, 100, 500, and 1000 mg/kg ip) in the MFST is shown in Fig. 1. At doses of 500 and 1000 mg/kg, L-arginine significantly reduced the duration of immobility when compared to the control group ($P < .05$, one-way ANOVA, post hoc Student–Newman–Keuls test). However, 25 mg/kg L-arginine showed a significant increase in immobility time ($P < .05$, one-way ANOVA, post hoc Student–Newman–Keuls test). 100 mg/kg L-arginine and 500 mg/kg D-arginine had no effect on the MFST.

3.2. Effect of L-NAME on the duration of immobility

Fig. 2 illustrates the dose–response curve for L-NAME (25, 50, 75, and 150 μg/per mouse icv) in the MFST. L-NAME significantly reduced the duration of immobility at the doses of 50 and 75 μg/per mouse ($P < .05$, one-way ANOVA, post hoc Student–Newman–Keuls test). At the dose of 150 μg, L-NAME showed a significant increase in immobility time when compared to the control group ($P < .05$, one-way ANOVA, post hoc Student–Newman–Keuls test). As shown in the figure, a U-shaped dose curve was observed with L-NAME.

3.3. Effect of sertraline on the duration of immobility

The dose–response curve for sertraline (50, 200, and 500 mg/kg po) in the MFST was reported in Fig. 3. Sertraline significantly reduced the duration of immobility in all doses ($P < .05$, one-way ANOVA, post hoc Student–Newman–Keuls test).

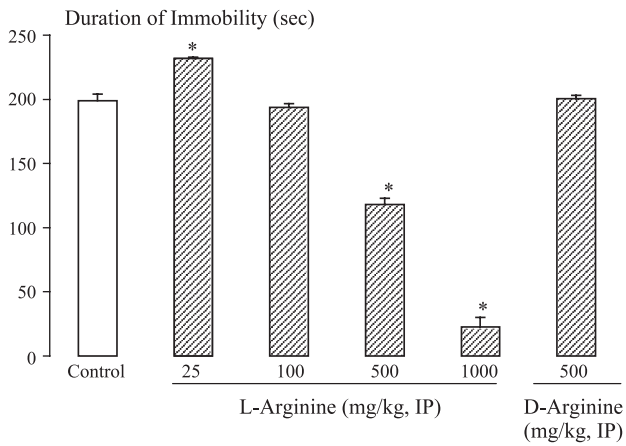

Fig. 1. Effect of L-arginine (mg/kg ip) and D-arginine (500 mg/kg ip) on the duration of immobility in the MFST. * $P < .05$, significantly different when compared to the control (0.9% saline po) group, using one-way ANOVA, post hoc Student–Newman–Keuls test. Ten mice were used in each group.

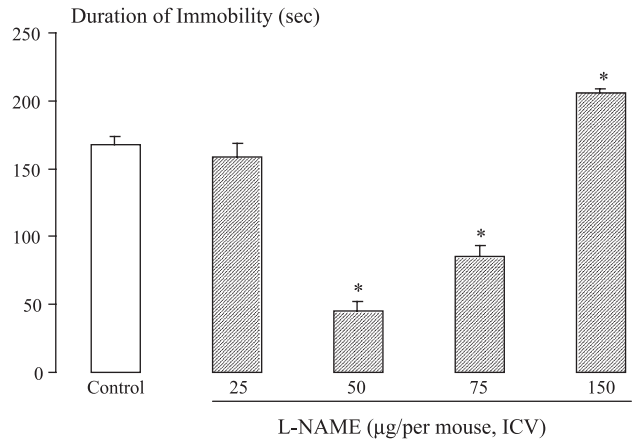

Fig. 2. Effect of L-NAME (μg/per mouse icv) on the duration of immobility in the MFST. * $P<.05$, significantly different when compared to the control (0.9% saline icv) group, using one-way ANOVA, post hoc Student–Newman–Keuls test. Ten mice were used in each group.

3.4. Effect of 3,4-DAP, TEA, and pinacidil on the duration of immobility

The effects of 3,4-DAP, TEA, and pinacidil on the duration of immobility are shown in Fig. 4. Both 3,4-DAP (0.05 μg/per mouse icv) and TEA (5 μg/per mouse icv) significantly reduced the duration of immobility in the MFST ($P<.05$, unpaired Student t test). However, pinacidil (10 μg/per mouse icv) significantly increased the duration of immobility in the MFST ($P<.05$, unpaired Student t test). Higher dose of 3,4-DAP (5 μg/per mouse icv) showed hyperexcitation, tremors, very fast rotations, jumping, hyperlocomotion, and clonic–tonic convulsion-induced death.

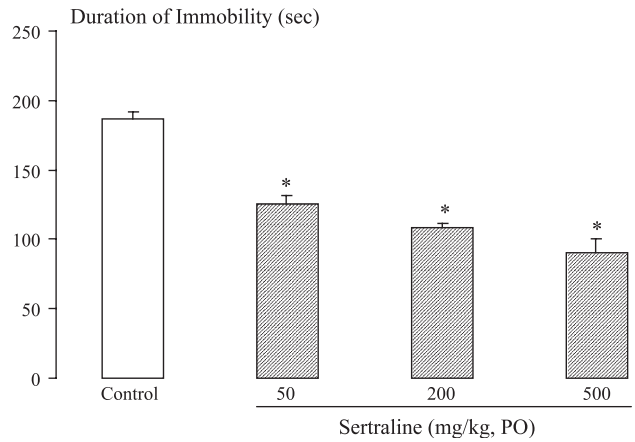

Fig. 3. Effect of sertraline (mg/kg po) on the duration of immobility in the MFST. * $P<.05$, significantly different when compared to the control (0.9% saline po) group, using one-way ANOVA, post hoc Student–Newman–Keuls test. Ten mice were used in each group.

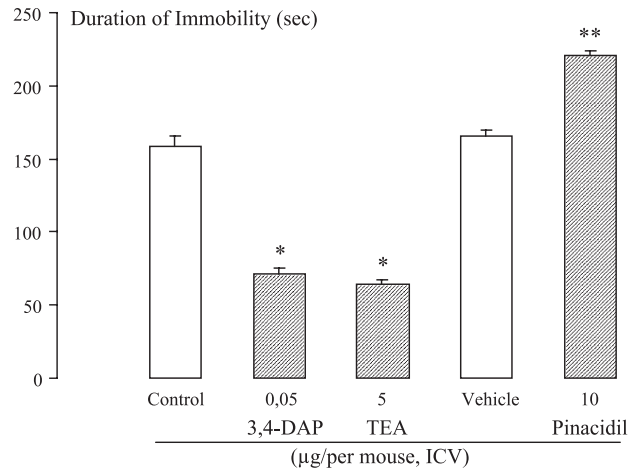

Fig. 4. Effect of 3,4-DAP (μg/per mouse icv), TEA (μg/per mouse icv) and pinacidil (μg/per mouse icv) on the duration of immobility in the MFST. * $P<.05$, significantly different when compared to the control (0.9% saline icv) group and ** $P<.05$, significantly different when compared to vehicle (DMSO 3:1 icv) group, using one-way ANOVA, post hoc Student–Newman–Keuls test. Ten mice were used in each group.

3.5. The antagonistic effect of L-arginine on the antidepressant-like effect of L-NAME, 3,4-DAP, and TEA

Preadministration (30 min before the intracerebroventricular administration) of L-arginine (100 mg/kg ip) significantly antagonized the antidepressant-like effects of L-NAME (50 μg/per mouse icv), 3,4-DAP (0.05 μg/per mouse icv) and TEA (5 μg/per mouse icv) (Fig. 5; $P<.05$, unpaired Student t test).

3.6. The effect of L-arginine on the antidepressant-like effect of 3,4-DAP and TEA or on the depressant-like effect of pinacidil

Preadministration of L-arginine (500 mg/kg ip) significantly potentiated the antidepressant-like effects of 3,4-DAP

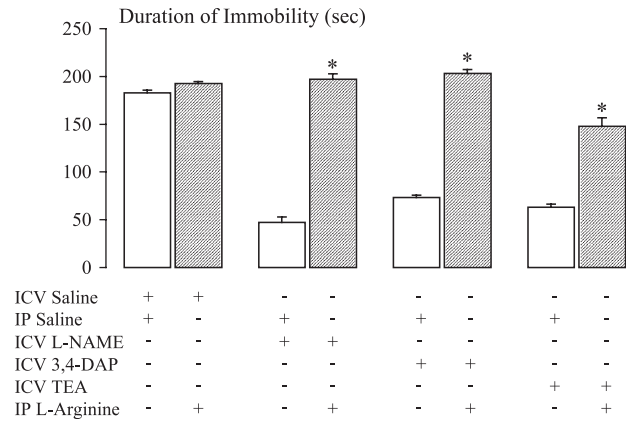

Fig. 5. The antagonistic effect of L-arginine (100 mg/kg ip) on the antidepressant-like effects of L-NAME (50 μg/per mouse icv), 3,4-DAP (0.05 μg/per mouse icv) and TEA (5 μg/per mouse icv) in the MFST. * $P<.05$; significantly different when compared to the saline + L-NAME, 3,4-DAP or TEA group, using unpaired Student t test. Ten mice were used in each group.

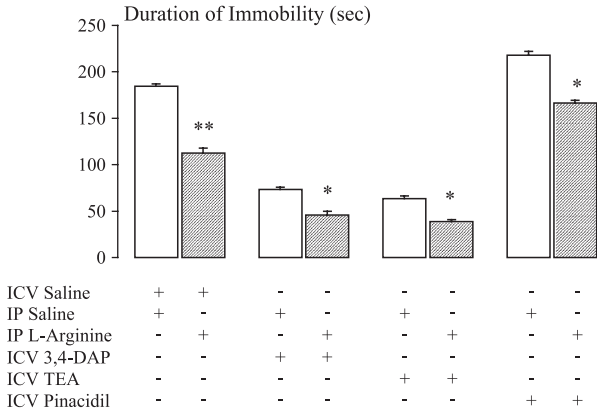

Fig. 6. The effect of L-arginine (500 mg/kg ip) on the antidepressant-like effect of 3,4-DAP (0.05 μg/per mouse icv) and TEA (5 μg/per mouse icv), or on the depressant-like effect of pinacidil (10 μg/per mouse icv) in the MFST. * $P<.05$, significantly different when compared to the saline + 3,4-DAP, TEA or pinacidil group, and ** $P<.05$, significantly different when compared to the saline (icv) + saline (ip) group, using unpaired Student t test. Ten mice were used in each group.

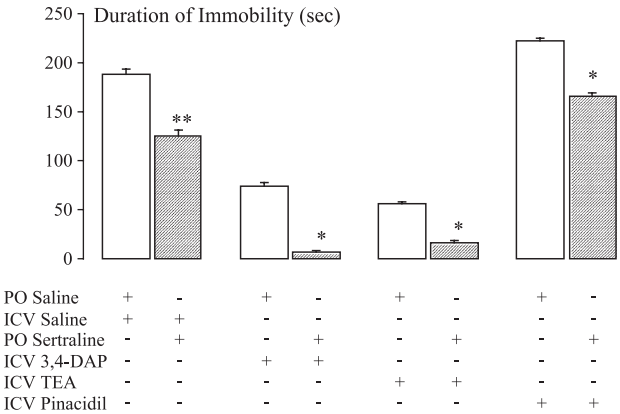

Fig. 8. The effect of sertraline (50 mg/kg po) on the antidepressant-like effect of 3,4-DAP (0.05 μg/per mouse icv) and TEA (5 μg/per mouse icv) or depressant-like effect of pinacidil (10 μg/per mouse icv) in the MFST. * $P<.05$, significantly different when compared to the saline + 3,4-DAP, TEA or pinacidil group. ** $P<.05$, significantly different when compared to the saline (po) + saline (icv) group, using unpaired Student t test. Ten mice were used in each group.

and TEA in the MFST (Fig. 6; $P<.05$, unpaired Student t test). L-Arginine also significantly reduced the depressant-like effect of pinacidil (10 μg/per mouse icv) in the MFST ($P<.05$, unpaired Student t test).

3.7. The effect of L-arginine on the antidepressant-like effect of sertraline

The interaction between sertraline (50 mg/kg po) and L-arginine (25, 100, and 500 mg/kg ip) in the duration of immobility is shown in Fig. 7. L-Arginine at the doses of 25 and 100 mg/kg antagonized the antidepressant-like effect of sertraline ($P<.05$, one-way ANOVA, post hoc Student–Newman–Keuls test). However, when combined with sertraline, 500 mg/kg L-arginine significantly potentiated the antidepressant-like effect of sertraline in the MFST ($P<.05$, one-way ANOVA, post hoc Student–Newman–Keuls test).

3.8. The effect of sertraline on the antidepressant-like effect of 3,4-DAP and TEA or on the depressant-like effect of pinacidil

The effects of sertraline (50 mg/kg po) on the effects of 3,4-DAP (0.05 μg/per mouse icv), TEA (5 μg/per mouse icv), and pinacidil (10 μg/per mouse icv) in the MFST are shown in Fig. 8. Sertraline significantly potentiated the anti-immobility effects of 3,4-DAP and TEA in the MFST, but it significantly reversed the depressant-like effect of pinacidil ($P<.05$, unpaired Student t test).

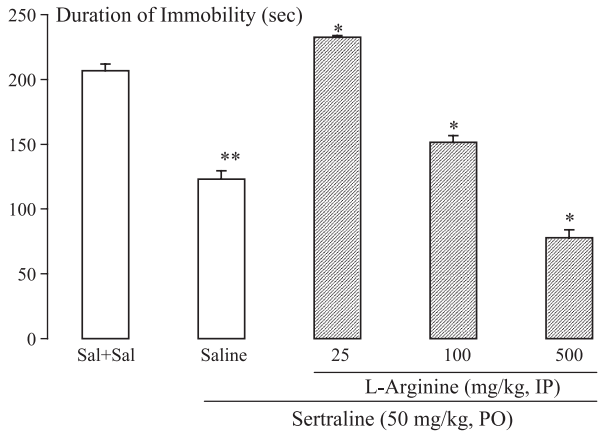

Fig. 7. The effect of L-arginine on the antidepressant-like effect of sertraline (50 mg/kg po) in the MFST. * $P<.05$, significantly different when compared to the sertraline + saline group and ** $P<.05$, significantly different when compared to the saline (po) + saline (ip) group, using one-way ANOVA, post hoc Student–Newman–Keuls test. Ten mice were used in each group.

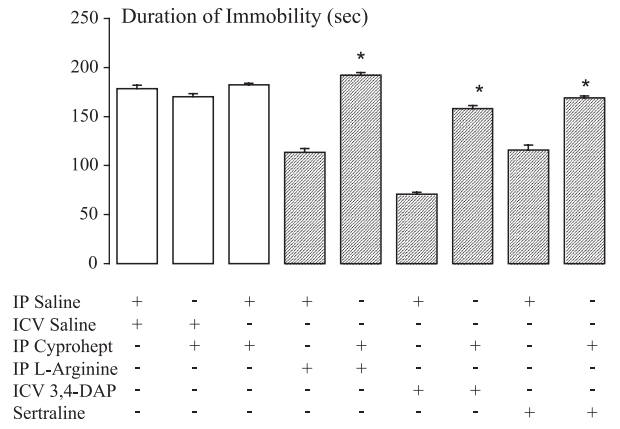

Fig. 9. The antagonism by cyproheptadine (25 mg/kg ip) of the antidepressant-like effect of L-arginine (500 mg/kg ip), 3,4-DAP (0.05 μg/per mouse icv) and sertraline (50 mg/kg po) in the MFST. * $P<.05$, significantly different when compared to the saline + L-arginine, 3,4-DAP or sertraline group, using unpaired Student t test. Ten mice were used in each group.

Table 1
The effects of the drugs on motor coordination as revealed by the rotarod test or on spontaneous locomotor activity as revealed by the activity cage test

Drug	Time to fall (s)	Number of beam crossings
NaCl, po	296 ± 2.8	2625 ± 175
Sertraline, 500 mg/kg po	294.9 ± 3.4	2470 ± 258
NaCl, ip	293.5 ± 3.4	2597.5 ± 252.5
L-Arginine, 500 mg/kg ip	297.9 ± 2.1	2392 ± 411
NaCl, icv	286.7 ± 4.8	2295 ± 283
L-NAME, 150 μg/per mouse icv	287.3 ± 4.6	2156.5 ± 158.5
3,4-DAP, 0.05 μg/per mouse icv	288.9 ± 4.9	2351 ± 254
TEA, 5 μg/per mouse icv	288.1 ± 4.4	2301.5 ± 113.5
Pinacidil, 10 μg/per mouse icv	285.1 ± 4.3	2185.5 ± 72.5

Neither of the drugs impaired motor coordination or changed spontaneous locomotor activity (one-way ANOVA, post hoc Student–Newman–Keuls test). Ten mice were used in each group.

3.9. The antagonism by cyproheptadine of the antidepressant-like effect of L-arginine, 3,4-DAP, and sertraline

The antagonism by cyproheptadine (25 mg/kg ip) of the antidepressant effects of L-arginine (500 mg/kg ip), 3,4-DAP (0.05 μg/per mouse icv), and sertraline (50 mg/kg po) are shown in Fig. 9. Preadministration of cyproheptadine significantly reduced the antidepressant-like effects of L-arginine, 3,4-DAP, and sertraline in the MFST ($P < .05$, unpaired Student t test).

3.10. Effects of drugs on the rotarod and activity cage performances

Neither of the drugs at their highest doses impaired motor coordination as revealed by the rotarod test or spontaneous locomotor activity as revealed by the activity cage (Table 1; one-way ANOVA, post hoc Student–Newman–Keuls test).

4. Discussion

In the present study, both L-arginine and L-NAME affected the duration of immobility in various doses in the MFST. While L-arginine increased the immobility time in its low dose (25 mg/kg ip), it decreased the immobility time in its high doses (500 and 1000 mg/kg ip) when compared to the control group. Furthermore, L-NAME decreased the immobility time in its low doses (50 and 75 μg/per mouse icv) and increased the immobility time in its high dose (150 μg/per mouse icv). These results are in accordance with previous studies that indicated the dose-dependent antidepressant-like effects of NOS inhibitors and the dual effects of the NO precursor L-arginine in the MFST (Jefferys and Funder, 1996; Harkin et al., 1999; Karolewicz et al., 1999; da Silva et al., 2000). Harkin et al. (1999) speculated that the biphasic effect of NO synthase antagonist may be attributed to impaired cerebrovascular circulation or loss of control over locomotion and coordination. L-NOARG induced catalepsy in a dose-dependent manner in mice (Marras et al., 1995). Similarly 7-NI produced loss of locomotion, coordination, and righting reflex at high doses (Volke et al., 2003). However, neither NOS inhibitors nor L-arginine produced significant change in locomotor activity in naive mice at the doses that produced change in immobility time (Harkin et al., 1999; da Silva et al., 2000). Similarly, L-arginine and L-NAME did not produce any significant effect at the highest doses in the rotarod and activity cage tests in our study.

The dual effect of NO may be related to serotonin release in the central nervous system, since NO modulatory agents have been shown to affect serotonin release in a dose-dependent manner in rodents (Hui and Chan, 1995; Kaehler et al., 1999; Smith and Whitton, 2000). Serotonin release is enhanced by NO precursors and NO donors in their high concentrations. However, low concentrations of NO precursors and NO donors decreased the release of serotonin. On the contrary, high concentrations of L-NAME diminished serotonin release, and low concentrations of L-NAME enhanced the release of serotonin (Kaehler et al., 1999). We suggest that there is a relationship between dose-dependent dual effects of NO on the mechanisms of depression and serotonin release. Our results indicating the effects of sertraline, a serotonin reuptake inhibitor, alone and combined with various doses of L-arginine on the MFST, support this view, since low doses of L-arginine reversed and high doses of L-arginine potentiated the antidepressant-like effects of sertraline. The antagonistic effect of cyproheptadine (a serotonin antagonist) on the antidepressant-like effects of L-arginine may confirm the involvement of serotonin in the NO-mediated changes in the mechanisms of MFST. On the other hand, cyproheptadine interacts not only with serotonin receptors, but also with histamine-H_1 and muscarinic receptors (Bush and Mayer, 2001). It has been shown that anticholinergic agents reduced the immobility time in the forced swimming test (Browne, 1979). Reversal of the effects of L-arginine by cyproheptadine in the present study may be attributed to cholinergic mechanisms. Therefore, the precise mechanism can be investigated using more specific serotonin antagonists.

Another possible mechanism for the dual effects of NO in the MFST may be related to the ability of NO to modify K^+ channel function. The ATP-sensitive K^+-channel blocker glyburide when combined with antidepressant drugs enhanced the antidepressant-like effects of these drugs (Guo et al., 1996). Another ATP-sensitive K^+-channel blocker, gliquidone, produced an antidepressant-like effect in mice when given alone (Galeotti et al., 1999). Ca^{2+}-activated K^+-channel blockers apamin and charybdotoxin reduced the time of immobility in the forced swimming test (Galeotti et al., 1999). Cromacalim, an ATP-sensitive K^+-channel activator, antagonized the antidepressant-like effects of antidepressant drugs (Redrobe et al., 1996). TEA, which blocks different types of K^+ channels including Ca^{2+}-activated and voltage-dependent K^+ channels and 3,4-

DAP, which blocks Ca^{2+}-activated K^+ channels, both reduced the immobility time. In contrast, the ATP-sensitive K^+-channel activator pinacidil increased the immobility time. These results are in accordance with the previous suggestion that K^+ channels play a role in the modulation of immobility time in the MFST and that more than one type of K^+ channel is involved (Galeotti et al., 1999). In our study, antidepressant-like effects of K^+-channel blockers were reversed by moderate dose of L-arginine (100 mg/kg ip), which is ineffective in the MFST, but high concentration of L-arginine (500 mg/kg ip) potentiated the antidepressant-like effects of K^+-channel blockers. A high dose of L-arginine (500 mg/kg ip) decreased the depressant-like effect of pinacidil as well. Thus, we suggest a mechanism that NO may activate K^+ channels, which results in changes in immobility time in the MFST. On the other hand, it has been suggested that the blockade of K^+ channels enhanced the basal release of serotonin in rat hippocampal slices (Schechter, 1997). The findings of this study support the suggestion above since sertraline (50 mg/kg po) potentiated the antidepressant-like effects of TEA and 3,4-DAP or reversed the depressant-like effect of pinacidil in the MFST. Also, the data obtained from cyproheptadine antagonizing the effect of 3,4-DAP may be supporting evidence for the involvement of K^+-channel blockade and serotonergic interaction in the MFST. Further experiments using specific serotonin antagonists are necessary to suggest more reasonable explanations.

In conclusion, we suggest that a possible dual role of NO in the MFST may involve serotonin and K^+ channels.

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