

Involvement of serotonin receptor subtypes in the antidepressant-like effect of trim in the rat forced swimming test

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ARTICLE INFO

Article history:

Received 7 August 2009

Received in revised form 26 January 2010

Accepted 10 February 2010

Available online 18 February 2010

Keywords:

Antidepressant-like effect

Forced swimming test

TRIM

5-HT receptor

ABSTRACT

Depression is a common illness with severe morbidity and mortality. Nitric oxide synthase (NOS) inhibitors are shown to elicit antidepressant-like effect in various animal models. It is widely known that serotonin plays an important role in the antidepressant-like effect of drugs. The aim of this study is to investigate the involvement of 5-HT₁ and 5-HT₂ receptor subtypes in the antidepressant-like effect of TRIM, a nNOS inhibitor, in the rat forced swimming test (FST). TRIM displays an antidepressant-like activity in FST which is blocked by pretreatment with the NOS substrate L-arginine. Depletion of endogenous serotonin using parachlorophenylalanine (pCPA; 3 × 150 mg/kg, i.p.) partially attenuated TRIM (50 mg/kg)-induced reductions in immobility time in FST. Pretreatment with methiothepin (0.1 mg/kg, i.p., a non-selective 5-HT receptor antagonist), cyproheptadine (3 mg/kg i.p., a 5-HT₂ receptor antagonist) or ketanserin (5 mg/kg i.p., a 5HT_{2A/2C} receptor antagonist) prevented the effect of TRIM (50 mg/kg) in the FST. WAY 100635 (0.1 mg/kg i.p., a selective 5-HT_{1A} receptor antagonist) and GR 127935 (3 mg/kg i.p., a selective 5-HT_{1B/1D} receptor antagonist) slightly reversed the immobility-reducing effect of TRIM in the FST, but this failed to reach a statistically significant level. The results of this study demonstrate that antidepressant-like effect of TRIM in the FST seems to be mediated, at least in part, by an interaction with 5-HT₂ receptors while non-significant effects were obtained with 5-HT₁ receptors.

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1. Introduction

Depression is a frequently seen psychiatric illness resulting with loss of psychosocial ability. It is a serious public health problem with high morbidity and mortality and it also increases the risk of comorbidity. The prevalence of depression during life is 17–19% and suicide during depression is 15% (Kessler et al., 1994).

An important theory for the formation of depression is the monoamine hypothesis which suggests that there is a decreasing effect of biological amines like serotonin (5-HT), noradrenaline and dopamine in depression (Schildkraut, 1965). It is well known that serotonin system plays an important role in the neural regulation of mood (Duman et al., 1997) and enhancement of 5-HT neurotransmission underlies in the therapeutic response to different class of antidepressant treatment. The studies using drugs affecting the serotonergic system did not only include the inhibition of serotonin

reuptake in the synaptic terminal or inhibiting its metabolism (monoaminoxidase inhibitors); there were also antidepressants affecting 5-HT receptor subtypes and this class of antidepressants were also frequently used in the therapy of depression (Blier and Ward, 2003).

Various inhibitors of nitric oxide synthase (NOS) have been shown to exert antidepressant-like behavioural effect in a variety of animal models (Harkin et al., 1999; Volke et al., 2003; Yıldız et al., 2000). Nitric oxide (NO) plays an important role in the brain, and pharmacological manipulations of the NO pathway will constitute a novel approach for therapeutic applications in the future. In the brain, NO is synthesized from L-arginine by NOS, as a response to activation of N-methyl-D-aspartate (NMDA) receptors by excitatory amino acids (Garthwaite, 1991; Moncada et al., 1991). A number of studies have demonstrated that NOS can modulate the release of central noradrenaline (Satoh et al., 1996), dopamine (Segieth et al., 2000; Wegener et al., 2000) and 5-HT (Smith and Whitton, 2000; Wegener et al., 2000).

TRIM exerted an antidepressant-like effect in the forced swimming test (FST), a pre-clinical behavioural method used for studying the antidepressant activity of drugs (Borsini, 1995; Cryan et al., 2002; Trullas and Skolnick, 1990; Ulak et al., 2008; Volke et al., 2003) and in the chronic mild stress model (Mutlu et al., 2009) in animals. Since TRIM has been shown to be a relatively selective inhibitor of nNOS and failed to influence mean arterial blood pressure (Handy et al., 1995,

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1996); it can be an appropriate agent in investigating the biological roles of nNOS in the central nervous system. Thus far, no studies have been reported investigating the possible involvement of 5-HT system in the antidepressant-like effect of TRIM in FST.

The present study was undertaken to investigate the role of serotonin receptor subtypes, particularly the 5-HT_{1A} and 5-HT₂ receptors in the antidepressant-like behavioural effect of TRIM in the rat FST. Thus depletion of endogenous serotonin was assessed with the tryptophan hydroxylase inhibitor para-chlorophenylalanine to test to what extent depletion of 5-HT affected the antidepressant-like effect of TRIM and methiothepin (a non-selective 5-HT receptor antagonist), WAY 100635 (a selective 5-HT_{1A} receptor antagonist), GR 127935 (a selective 5-HT_{1B/1D} receptor antagonist), cyproheptadine (a 5-HT₂ receptor antagonist) or ketanserin (a 5HT_{2A/2C} receptor antagonist) were used to investigate the contribution of serotonin receptor subtypes to these effects.

2. Materials/methods

2.1. Animals

Adult male Wistar rats (Istanbul University Medical Sciences Research Center, DETAM, Turkey) weighing 220–300 g were housed five to six per cage in an animal colony facility for 2 weeks before the start of the experiment. The animals were maintained in constant room temperature (22 ± 2 °C) under a 12-h light/dark cycle (light onset at 08:00 h). Tap water and food pellets were provided ad libitum. All animals used for the experiments were naive to the swimming and the locomotor activity test. Each rat was tested only once.

All procedures for the treatment of animals were in compliance with the European Community Council Directive of 24 November 1986 and ethical approval was granted by the Ethics Committee of Kocaeli University (Number: AEK 121 / 6, Kocaeli, Turkey).

2.2. Drugs and treatments

The following drugs were used: Imipramine (30 mg/kg), TRIM (15, 30, 50 mg/kg), L-arginine (300 mg/kg), D-arginine (300 mg/kg), para-chlorophenylalanine (pCPA, 3×150 mg/kg), methiothepin (0.1 mg/kg), WAY 100635 [(N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N(2-pyridinyl) cyclohexane carboxamide trihydrochloride] (0.1 mg/kg), GR 127935 (3 mg/kg), cyproheptadine (3 mg/kg), ketanserin (5 mg/kg), fluoxetine (20 mg/kg); all provided from Sigma Chemicals (St. Louis, MO, USA). All drugs were dissolved in 0.9% physiological saline, freshly prepared and administered by the intraperitoneal (i.p) route in a volume of 0.2 ml per 100 g body weight of rats.

The doses of drugs used were selected on the basis of literature data which were previously reported not to affect the locomotor activity (O'Neill and Conway, 2001; Redrobe et al., 1996; Redrobe and Bourin, 1997; Rojas-Corrales et al., 1998; Zomkowski et al., 2004).

2.3. Forced swimming test

The forced swimming test (FST), a currently used behavioural test for the detection of antidepressants, was performed following the procedure described by Porsolt et al. (1977, 1978). The rats were placed individually in plexiglas cylinders (40 cm in height, 18 cm in diameter) filled with water (25 °C) up to 15 cm. A 15-min preswimming period was followed 24 h later by a 5-min test period during which the total immobility time was recorded. Rats were considered immobile when they made no further attempts to escape, except for necessary movements to keep their heads above the water. The absence of hind leg movement was recorded as immobility by stopwatch cumulation by a single observer during the exposures. The water in the cylinders was changed before every trial. Each

experimental group consisted of 6–16 rats. All experiments were performed between 10:00 and 12:00 a.m. The animals were used only once in this test. Drugs were administered just before the trial in the second day according to the previous studies (Porsolt et al., 1977, 1978; Volke et al., 2003; Yildiz et al., 2000; Ulak et al., 2008).

The antidepressant drug imipramine (30 mg/kg) or TRIM (15, 30, 50 mg/kg) was injected 30 and 50 min respectively before being tested in the FST. In a separate experiment, effects of L-arginine or D-arginine (300 mg/kg) given alone or 10 min before TRIM (50 mg/kg) in the rat FST were evaluated.

In order to investigate the possible contribution of serotonergic system to the anti-immobility effect of TRIM in the FST, animals were pretreated with pCPA (150 mg/kg, an inhibitor of serotonin synthesis) or saline, once a day, for 3 consecutive days. In previous studies, this treatment regimen of pCPA produced a greater than 90% depletion of brain serotonin concentration in the rat (Connor et al., 2001; Harkin et al., 2003). The animals received an injection of TRIM (50 mg/kg) or saline 72 h after the last pCPA or saline injection and were tested in the FST 30 min later. In another experiment, rats were pretreated with methiothepin (0.1 mg/kg, a non-selective 5-HT receptor antagonist) or saline and after 20 min they received TRIM (50 mg/kg) or saline injection before being tested in the FST 30 min later.

To investigate the possible involvement of 5-HT₁ receptors in the effect of TRIM in the FST, rats were pretreated with WAY 100635 (0.1 mg/kg, a selective 5-HT_{1A} receptor antagonist), GR127935 (3 mg/kg, a selective 5-HT_{1B/1D} receptor antagonist) or saline and after 10 min they received TRIM (50 mg/kg) or saline injection before being tested in the FST after 50 min.

In another set of experiments, in order to investigate the involvement of 5-HT₂ receptor subtype in the effect of TRIM, rats were pretreated with cyproheptadine (3 mg/kg, a 5-HT₂ receptor antagonist), ketanserin (5 mg/kg, a 5HT_{2A/2C} receptor antagonist) or saline, and after 10 min, they received TRIM (50 mg/kg) or saline injection before being tested in the FST after 50 min.

We also investigated the ability of TRIM to potentiate the antidepressant-like effect of fluoxetine, a selective serotonin reuptake inhibitor. Fluoxetine (10, 20, 40 mg/kg) was injected to the rats 30 min before being tested in the FST. In a separate series of experiments the animals received an injection of fluoxetine (40 mg/kg), or saline 72 h after the last pCPA or saline injection and were tested in the FST 30 min later. Then rats were pretreated with a subeffective dose of TRIM (15 mg/kg) or saline, and 20 min later the animals were treated with a subeffective dose of fluoxetine (20 mg/kg). The FST test was carried 30 min later.

2.4. Locomotor activity test

The changes in locomotor activity may lead to false negative/positive results in the FST test. The spontaneous locomotor activity of the animals was therefore assessed by monitoring their activity in a locomotor activity cage (May 9803 Activity Monitoring System, Commat Iletisim Ltd. May Pentium Computer, Ankara, Turkey). Rats were individually placed in a plexiglas cage (42 × 42 × 30 cm) and the total distance travelled and the number of movements were evaluated for a 5 min period. Immediately after the measurement of the locomotor activity, the rats were assessed in the FST.

2.5. Statistical analysis

In evaluating dose dependent effects of drugs one-way analysis of variance (ANOVA) was used. Post-hoc comparisons between individual groups were carried out by means of Tukey's HSD test. Two-way analysis of variance (ANOVA) (group × immobility time) post-hoc Tukey's HSD test was used to evaluate the drug interaction data. Data are expressed as the mean ± SEM with $p < 0.05$ being considered statistically significant.

3. Results

3.1. Imipramine or TRIM reduces immobility time in the rat FST

One-way ANOVA showed a significant effect of drug treatment upon immobility time in FST [$F(4,62) = 25.47, p < 0.001$; Fig. 1]. Post-hoc comparisons revealed that imipramine (30 mg/kg) ($p < 0.001$, Tukey's test) and TRIM (30, 50 mg/kg) ($p < 0.05, p < 0.001$ respectively, Tukey's test) significantly shortened the immobility time compared to vehicle-treated group (Fig. 1).

3.2. Effects of L-arginine or D-arginine on immobility time given alone or before TRIM in the rat FST

There was a significant difference between groups when the effects of L-arginine or D-arginine given alone or together with TRIM were compared [$F(5,53) = 84.800, p < 0.001$, two-way ANOVA test; Fig. 2]. While L-arginine or D-arginine (300 mg/kg) given 60 min before testing, had no effect on immobility time in the FST, pretreatment with L-arginine, but not with D-isomer, antagonized the effect of TRIM (50 mg/kg) [$p < 0.01$, Tukey's test (Fig. 2)].

3.3. Pretreatment with pCPA or methiothepin attenuates the antidepressant-like effect of TRIM in the rat FST

Fig. 3a shows the effect of pretreatment with pCPA (150 mg/kg, i.p., for 3 consecutive days, an inhibitor of serotonin synthesis) on the reduction in immobility time elicited by TRIM in the FST. There was a significant difference between the groups on the immobility time in the FST when pCPA and TRIM given alone or pCPA pretreated TRIM groups were compared [$F(3,33) = 27.292, p < 0.001$, two-way ANOVA test; Fig. 3a]. Post-hoc analyses showed that while pCPA alone did not modify the immobility time, the pretreatment of rats with pCPA partially attenuated the decrease in immobility time in the FST induced by TRIM ($p < 0.05$, Tukey's test).

The effect of pretreatment with methiothepin (0.1 mg/kg, a 5-HT antagonist) on the reduction in immobility time induced by TRIM (50 mg/kg) is shown in Fig. 3b. There was a significant difference between the groups when the effect of methiothepin given alone or together with TRIM groups were compared ($F(3,24) = 62.900, p < 0.001$, two-way ANOVA test; Fig. 3b). While methiothepin had no effect on immobility time, pretreatment with methiothepin reversed the immobility time decreased by TRIM (50 mg/kg) ($p < 0.001$, Tukey's test; Fig. 3b).

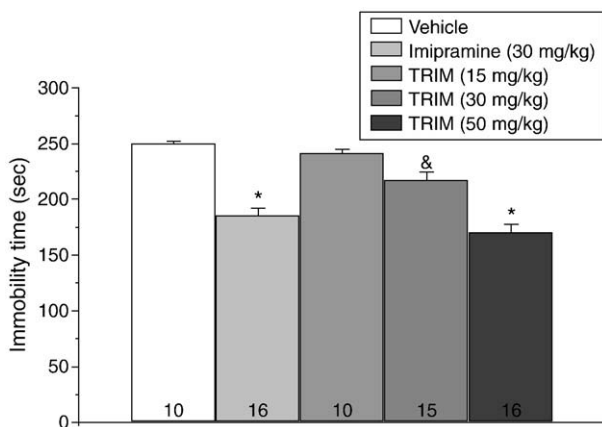


Fig. 1. Effects of imipramine or TRIM on the immobility time in the rat FST. The number of animals per group is shown in the columns. Results are expressed as mean \pm SEM. * $p < 0.001$, [&] $p < 0.05$ compared to vehicle control (Tukey's test).

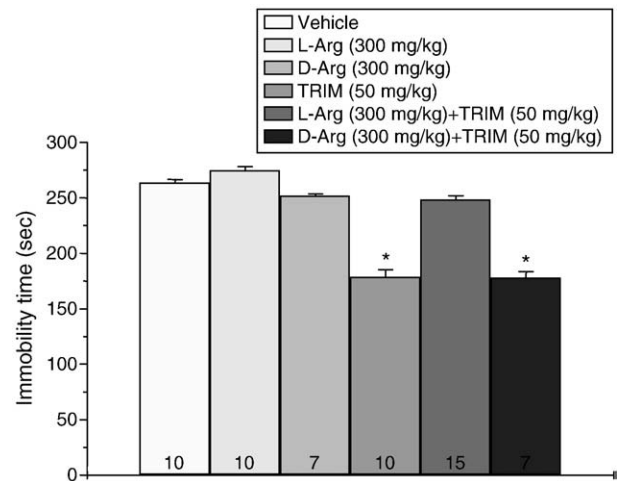


Fig. 2. Effects of pretreatment with L-arginine or D-arginine on TRIM-induced reductions on immobility time in the rat FST. L-arginine or D-arginine and TRIM were injected respectively 60 and 50 min prior to testing. The number of animals per group is shown in the columns. Results are expressed as mean \pm SEM. * $p < 0.001$ compared to vehicle control (Tukey's test).

3.4. Interaction of TRIM with 5-HT₁ receptor antagonists on immobility time in the rat FST

The results of pretreatment of rats with selective 5-HT_{1A} receptor antagonist WAY-100635 (0.1 mg/kg) or 5-HT_{1B/1D} receptor antagonist

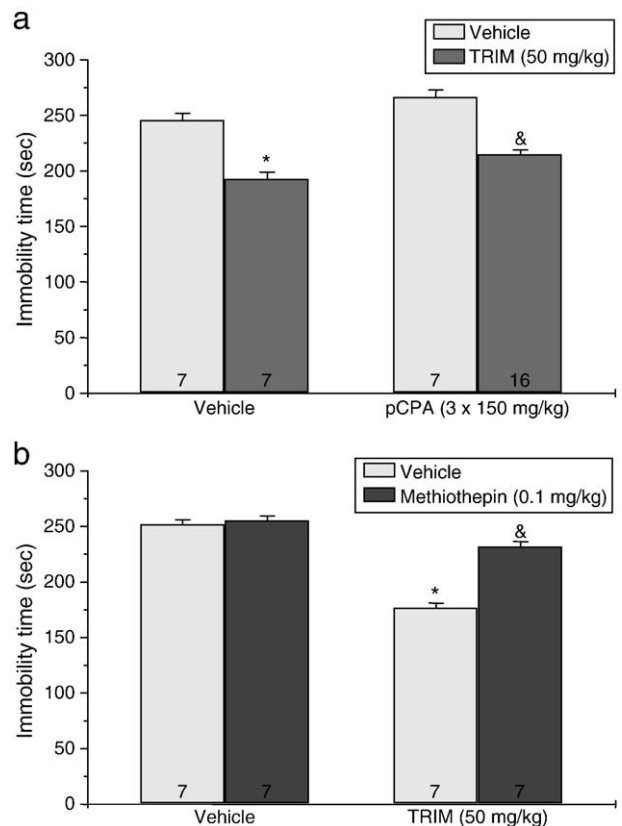


Fig. 3. a: Effects of the pretreatment of animals with pCPA on TRIM-induced reductions on immobility time in the rat FST. The number of animals per group is shown in the columns. Results are expressed as mean \pm SEM * $p < 0.001$ compared to control (Tukey's test), [&] $p < 0.05$ compared to pCPA untreated TRIM group (Tukey's test). b: Effects of pretreatment of animals with methiothepin on TRIM-induced reductions on immobility time in the rat FST. The number of animals per group is shown in the columns. Results are expressed as mean \pm SEM * $p < 0.001$ compared to control (Tukey's test), [&] $p < 0.001$ compared to methiothepine untreated TRIM group (Tukey's test).

GR 127935 (3 mg/kg) on the reduction in immobility time induced by TRIM in the FST is shown in Fig. 4(a and b). Two-way ANOVA revealed significant differences of pretreatment [respectively, $F(3,23) = 24.60$ and $F(3,22) = 17.43, p < 0.001$]. While WAY-100635 or GR 127935 did not affect the immobility time when tested alone, pretreatment of rats with these drugs slightly reversed the the immobility-reducing effect of TRIM in the FST but it failed to reach a statistically significant level (Tukey's test).

3.5. Interaction of TRIM with 5-HT₂ receptor antagonists on immobility time in the rat FST

Fig. 5 shows the effects of pretreatment of rats with cyproheptadine (3 mg/kg, a 5-HT₂ receptor antagonist) or ketanserin (5 mg/kg, a 5-HT_{2A/2C} receptor antagonist) on the reduction in immobility time elicited by TRIM(50 mg/kg) in the FST. One-way ANOVA revealed significant differences with pretreatment interactions [$F(5,35) = 23,892, p < 0.001$; Fig. 5]. While cyproheptadine or ketanserin alone did not modify the immobility time, pretreatment of rats with these drugs reversed the decrease in immobility time induced by TRIM in the FST ($p < 0.05$, Tukey's test; Fig. 5).

3.6. Effects of fluoxetine on immobility time in the rat FST

As shown in Fig. 6a, a selective serotonin reuptake inhibitor fluoxetine (10, 20, 40 mg/kg) given 30 min before testing, reduced the immobility time compared to vehicle-treated controls in the FST in rats. Drug treatment had a significant effect upon immobility time in FST as shown by one-way ANOVA [$F(3,24) = 28,032, p < 0.001$; Fig. 6a]. Post-hoc comparisons revealed that fluoxetine (40 mg/kg) significantly shortened the immobility time compared to vehicle-treated group ($p < 0.001$, Tukey's test; Fig. 6a).

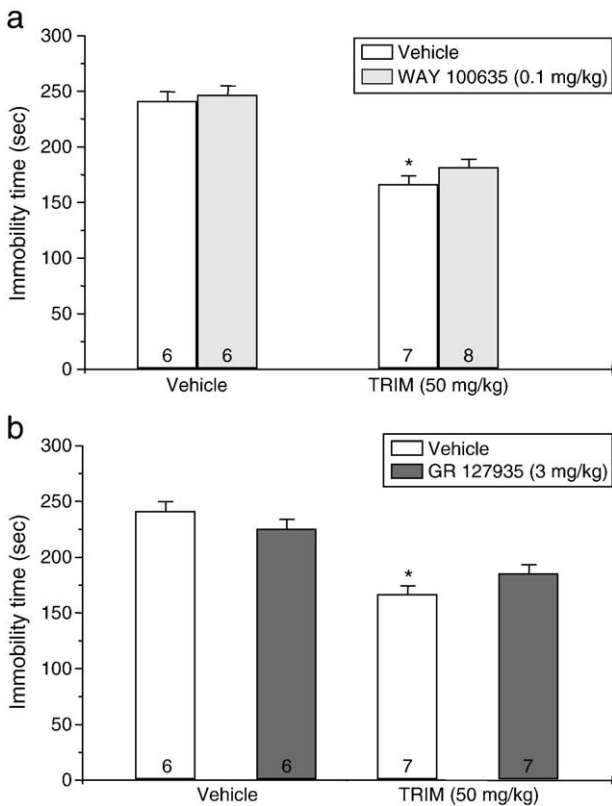


Fig. 4. Effects of pretreatment of animals with: (a) WAY 100635, (b) GR 127935 on TRIM-induced reductions in immobility time in the rat FST. The number of animals per group is shown in the columns. Results are expressed as mean ± SEM. * $p < 0.001$ compared to control (Tukey's test).

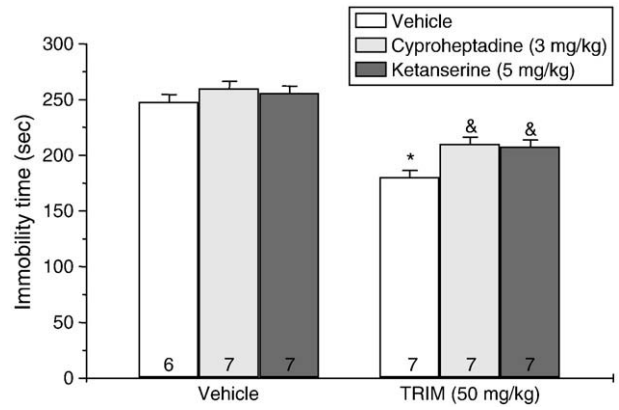


Fig. 5. The effect of pretreatment of animals with cyproheptadine or ketanserin on TRIM-induced reductions in immobility time in the rat FST. The number of animals per group is shown in the columns. Results are expressed as mean ± SEM. * $p < 0.001$ compared to control (Tukey's test), [&] $p < 0.05$ compared to TRIM + vehicle administered group (Tukey's test).

Fig. 6b shows the effect of pretreatment with pCPA (150 mg/kg, i.p., for 3 consecutive days, an inhibitor of serotonin synthesis) on the reduction in immobility time elicited by fluoxetine in the FST. There was a significant difference between the groups on the immobility time in the FST when pCPA and fluoxetine given alone or pCPA pretreated fluoxetine groups were compared [$F(3,27) = 79,198$,

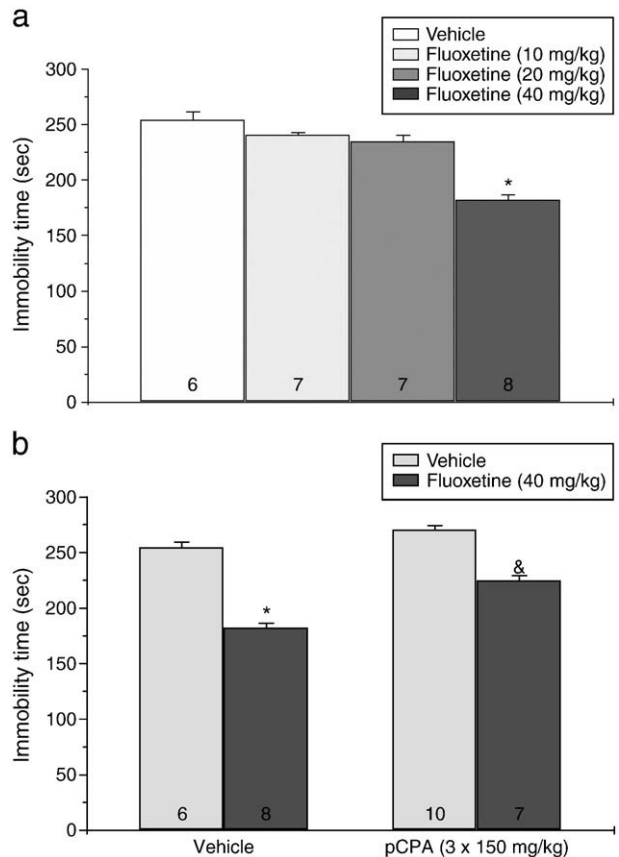


Fig. 6. a: Effects of fluoxetine on immobilization time in FST in rats. The number of animals per group is shown in the columns. Results are expressed as mean ± SEM. * $p < 0.001$ compared to control (Tukey's test). b: Effects of the pretreatment of animals with pCPA on fluoxetine-induced reductions on immobility time in the rat FST. The number of animals per group is shown in the columns. Results are expressed as mean ± SEM * $p < 0.001$ compared to control (Tukey's test), [&] $p < 0.05$ compared to pCPA untreated fluoxetine group (Tukey's test).

$p < 0.001$, two-way ANOVA; Fig. 6b). Post-hoc analyses showed that while pCPA alone did not modify the immobility time, pretreatment of rats with pCPA attenuated the decrease in immobility time in the FST induced by fluoxetine. ($p < 0.05$, Tukey's test; Fig. 6b).

3.7. Interaction of TRIM with fluoxetine in the rat FST

Fig. 7 shows the effect of pretreatment with a subeffective dose of TRIM (15 mg/kg) given in combination with a subeffective dose of fluoxetine (20 mg/kg) on the immobility time in the FST. Two-way ANOVA revealed significant differences between the groups [$F(3,27) = 18.735$, $p < 0.001$; Fig. 7]. Post-hoc analysis indicated that while subeffective doses of TRIM and fluoxetine had no effect on immobility time in FST, the antidepressant-like effect of a subeffective dose of fluoxetine was significantly enhanced by pretreatment with TRIM ($p < 0.001$, Tukey's test; Fig. 7).

3.8. Effects of drugs on locomotor activity

Since antidepressant-like effects in the FST can be also evoked by drugs which induce hyperactivity (Maj et al., 1992), the effect of all the above treatments on the locomotor activity was concurrently evaluated. ANOVA showed a significant effect when all the groups were compared [$F(14,135) = 2.72$; $p < 0.05$] although neither TRIM (15, 30, 50 mg/kg) nor above treatments modified the total distance travelled by the rats compared to control group ($p > 0.05$; Tukey's test, Table 1).

4. Discussion

It is well known that serotonin (5-hydroxytryptamine, 5-HT) plays an important role in depression and in the mechanism of action of antidepressant drugs. In this study, the involvement of the serotonergic system in the antidepressant-like effect of TRIM in the FST in rats was studied by depleting endogenous 5-HT with the tryptophan hydroxylase inhibitor pCPA (Connor et al., 2001; Page et al., 1999) and by using 5-HT₁ and 5-HT₂ receptor antagonists to investigate the behavioural responses to TRIM in the FST since it is well known that that these receptors play an important role in mood disorders (Clenet et al., 2001; Gardier et al., 1996; O'Neill and Conway, 2001; Redrobe et al., 1996; Redrobe and Bourin, 1998). This study extended the previous data of our group, which had shown that TRIM augmented the effect of antidepressants acting via serotonergic system in the FST in rats (Ulak et al., 2008).

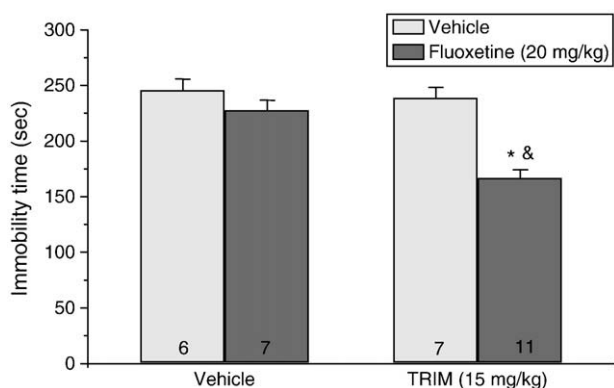


Fig. 7. Effect of coadministration of the subeffective doses of TRIM (15 mg/kg) and fluoxetine (20 mg/kg) on immobilization time in FST in rats. The number of animals per group is shown in the columns. Results are expressed as mean \pm SEM. * $p < 0.001$ compared to control (Tukey's test), $\&p < 0.001$ compared to fluoxetine or TRIM administered group (Tukey's test).

Table 1

Effects of drugs on total distance travelled in the locomotor activity test. The number of animals in each group is 10. Results are expressed as mean \pm SEM. * $p < 0.05$ compared to D-Arg 300 mg/kg group (Tukey's test).

Locomotor activity test	
Treatment (mg/kg)	Total distance travelled
Vehicle	719.11 \pm 164.44
Imip 30	503.57 \pm 139.32
TRIM 30	458.67 \pm 56.95
TRIM 50	*355.5 \pm 57.43
L-Arg 300	*379.4 \pm 46.86
D-Arg 300	1062.42 \pm 249.86
pCPA	771.41 \pm 181.79
Methiothepin 0,1	681.75 \pm 62.82
Fluoxetine 20	*368.7 \pm 66.99
Fluoxetine 40	*363 \pm 87.6
WAY 100635 0,1	*342.83 \pm 52.84
GR 127935 3	447.17 \pm 68.68
Cyproheptadine 3	690.86 \pm 177.00
Ketanserine 5	734.33 \pm 132.05
TRIM 15 + Flu 20	*401.2 \pm 80.14

In the present study TRIM (30, 50 mg/kg) exerted antidepressant-like activity comparable to that of the tricyclic antidepressant imipramine (30 mg/kg). Together with other investigators, we had (Ghasemi et al., 2008; Ulak et al., 2008; Volke et al., 2003; Yildiz et al., 2000) previously shown that various inhibitors of NOS, such as N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-L-arginine (L-NA), 7-nitroindazole (7-NI), N^W-propyl-L-arginine (L-NPA) and TRIM possess antidepressant-like properties in animal models. It had been claimed that NOS was an important target in FST, a pre-clinical behavioural method widely used for studying the antidepressant activity of drugs (Porsolt et al., 1977, 1978). NO modulated the extracellular levels of various neurotransmitters such as serotonin, noradrenaline, dopamine and glutamate in the central nervous system (Wegener et al., 2003) while serotonin reuptake may be influenced by the NO pathway. It was postulated that the antidepressant-like behaviour of L-NA and 7-NI is endogenous serotonin dependent and besides other mechanisms, antidepressant-like effect of NOS inhibitors may result from a change of 5-HT level in the brain (Harkin et al., 2003; Volke et al., 2003; Wegener et al., 2000). Since TRIM, a potent inhibitor for the neuronal isoform of NOS enzyme, failed to influence mean arterial blood pressure (Handy et al., 1995, 1996), it can be used as an appropriate agent in investigating the biological roles of nitric oxide with the central nervous system.

It was postulated that antidepressant-like effect of NOS inhibitors can be reversed by pretreatment with NO synthase substrate L-arginine (Harkin et al., 1999; Yildiz et al., 2000). In our study, the antidepressant-like effect of TRIM was counteracted by pretreatment with L-arginine, supporting the idea that antidepressant-like effect of TRIM is dependent on NOS enzyme inhibition. In order to test while this effect is NO mediated or due to the nonspecific effect of arginine amino acid, effect of D-arginine on immobility time was also investigated. As it is known, L-isoform of arginine produces NO formation while D form lacks this effect. D-arginine had no effect on immobility time and pretreatment with D-arginine failed to reverse TRIM-induced effect on immobility time in FST. So, the reversing effect of TRIM by NO synthase substrate L-arginine, showed that NO played a role in this effect.

The results of our study reveal that TRIM-induced reduction of immobility time in the FST was partially attenuated by pretreatment with the 5-HT depleting agent pCPA in rats. The treatment regimen of pCPA used in this study produces a greater than 90% depletion of cortical 5-HT concentration in the rat but had no effect on cortical dopamine and noradrenaline concentrations (Connor et al., 2001; Harkin et al., 2003). Pretreatment with pCPA did not alter the immobility time of control animals but attenuated the anti-immobility effect of TRIM. Thus the results of our study suggest that endogenous 5-HT is involved in the

antidepressant-like effect of TRIM. In line with our findings, serotonin depletion with pCPA prevented the antidepressant-like effect of the NOS inhibitors 7-NI and NG-nitro-L-arginine in FST (Harkin et al., 2003; Yildiz et al., 2000).

Antidepressant-like effect of NOS inhibitors in the FST is dependent on NOS and NMDA receptor inhibition (Wiley et al., 1995; Mutlu et al., 2009). Recent experimental evidence indicates an important role for NO and NMDA receptors in the modulation of serotonergic system. Moreover it is reported that NOS inhibitors as well as NMDA receptor antagonists cause the release of 5-HT and stimulate its turnover in some brain regions (Callado et al., 2000; Kaehler et al., 1999; Smith and Whitton, 2000; Wegener et al., 2000). So reversal of the TRIM-induced antidepressant-like effect by pCPA treatment might be due to activation of 5-HT resulting from NOS inhibition and blockade of NMDA receptors (Zomkowski et al., 2002).

While methiothepin, a non-selective 5-HT receptor antagonist had no effect on immobility time in FST as in our study and others (Buckley et al., 2004; Zomkowski et al., 2004), the reversal of antidepressant-like effect of TRIM by pretreatment of rats with methiothepin, reinforce the idea that 5-HT is involved in the action of TRIM in the FST.

Recent studies have focused on the involvement of 5-HT_{1A} receptors in the mechanism of action of antidepressant drugs (Blier and Ward, 2003). 5-HT_{1A} receptor is the best known in 14 serotonergic receptor groups (Pucadyil et al., 2005) and it is important in psychiatric disorders like schizophrenia (Millan, 2000; Yasuno et al., 2004) and depression (Celada et al., 2004). The discovery of WAY 100635, the first highly selective, potent and silent 5-HT₁ receptor antagonist (Forster et al., 1995), enabled further clarification on the role of 5-HT_{1A} receptors in the antidepressant-like effects of drugs. There are few studies investigating the interaction between the antidepressants and 5-HT receptor subtype ligands in animal depression models. Tatarczynska et al., 2004 suggested that pretreatment of rats with 5-HT_{1A} receptor antagonist and with 5-HT_{1B/1D} receptor antagonist reversed the immobility-reducing effect of TRIM, in the rat FST. 5-HT_{1B} receptor antagonists were also reported to reverse the antidepressant-like effect of paroxetine and imipramine (Gardier et al., 2003; O'Neill and Conway, 2001). The results of our study revealed that pretreatment of rats with WAY 100635, a selective 5-HT_{1A} receptor antagonist or with GR127935, a selective 5-HT_{1B/1D} receptor antagonist) slightly reversed the immobility-reducing effect of TRIM, but this failed to reach a statistically significant level; while alone they had no effect in the rat FST. So further experiments are needed to clarify the involvement of 5-HT₁ receptors in the antidepressant-like effect of TRIM.

Inhibition of 5-HT_{2A} receptor expression exerts antidepressant-like effect in FST (Sibille et al., 1997). Suicide attempt in severe depressive patients due to the polymorphism in the 5-HT₂ receptor gene is established (Du et al., 2000) and this supports the role of 5-HT_{2A} receptors in depression. Inhibition of 5-HT_{2A/2C} receptors play an important role in the antidepressant-like effect of conventional antidepressants in FST (Van Oekelen et al., 2003; Redrobe and Bourin, 1997). Although the role of 5-HT₂ receptors on the effect of antidepressants was investigated before (Middlemiss et al., 2002; Redrobe and Bourin, 1998), there is no study about the interaction of 5-HT₂ receptor antagonists and nNOS inhibitors in animal models of depression. So another important finding of our study was that, the antidepressant-like effect of TRIM in the FST was prevented by pretreatment with cyproheptadine, a 5-HT₂ receptor antagonist and with ketanserin, a 5HT_{2A/2C} receptor antagonist having higher affinity for 5HT_{2A} receptors than for 5HT_{2C} receptors (Van Oekelen et al., 2003). TRIM may interact with both 5HT_{2A} and 5HT_{2C} receptors which are also reported to play role in the action of antidepressants (Clenet et al., 2001; Oekelen, 2003). Thus 5-HT_{2A} receptors, at least partially, have a role on the antidepressant-like effect of TRIM. It is reported that NOS inhibitors increased the release of 5-HT in prefrontal cortex (Smith and Whitton, 2000). Therefore, it could be postulated that TRIM affects 5-HT_{2A} receptors by increasing 5-HT level in the synaptic terminal.

In the current study, at the doses used neither TRIM nor other drugs affected the locomotor activity of the animals compared to control group while it has been reported that TRIM suppresses locomotor activity at 50 mg/kg (Volke et al., 2003) and 120 mg/kg doses (Dzoljic et al., 1997). So the ability of TRIM and other drugs in the FST can't be attributed to a nonspecific locomotor stimulant effect of these drugs. The locomotor activity of the D-arginine group was increased compared to some other groups. This can be related to some nonspecific effects like experimental conditions except the effect of D-arginine on locomotion because in recent studies both D-arginine and L-arginine didn't change locomotion compared to control group (Yildiz et al., 2000) as in this study.

Another finding of our study, as in line with other studies (Harkin et al., 2003; Ulak et al., 2008; Yildiz et al., 2000) was that a SSRI drug fluoxetine shortened the immobility time in FST. SSRI drugs are believed to exert their clinical antidepressant effect by blocking the reuptake of serotonin at the synapse, resulting in an elevation of extracellular serotonin concentrations in brain. Fluoxetine is one of the most currently used antidepressant among this group of drugs. In our study, preadministration of pCPA to fluoxetine treated rats, significantly prolonged the immobilization time as in line with the previous studies showing that the behavioural effect of the SSRI fluoxetine in FST could be blocked by serotonin depletion (Connor et al., 2001; Page et al., 1999; Zomkowski et al., 2004).

Another important finding of our study was the synergistic antidepressant-like augmenting effect of coadministration behaviourally subeffective doses of fluoxetine and TRIM. The reason for this effect in FST can be explained by similar activities of fluoxetine and TRIM on serotonergic and nitrergic system. It has been postulated that the inhibition of 5-HT_{1A} receptors by WAY 100635 cause the synergic potentialization of the increased extracellular 5-HT levels by serotonergic antidepressants (Romero et al., 1996). Besides, when WAY 100635 is combined with the subactive dose of fluoxetine in FST, it significantly decreased the immobility time in FST (Rocha et al., 1997). The antidepressant augmenting effects TRIM may be attributed to the modulation of serotonin release since it has been demonstrated that inhibition of NOS can modulate the release of central serotonin (Kiss, 2000; Wegener et al., 2000), this may be the point of view.

In conclusion, in our study the antidepressant-like effect of TRIM in the FST seems to be mediated, at least in part, by an interaction with 5-HT₂ receptors while non-significant effects were obtained with 5-HT₁ receptors. Thus further studies are needed to enlighten whether the antidepressant-like effect of NOS inhibitors are dependent on endogen serotonin and these serotonin receptors are playing role in this effect. Besides, the potentiation of the antidepressant-like effect of fluoxetine by TRIM might have a therapeutic value for NOS inhibitors being a new treatment strategy to increase the clinical effect of antidepressants.

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