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Neuronal NOS inhibitor 1-(2-trifluoromethylphenyl)-imidazole augment the effects of antidepressants acting via serotonergic system in the forced swimming test in rats

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ABSTRACT

Treatment-resistant depression has necessitated new therapeutic strategies in augmenting the therapeutic actions of currently existing antidepressant drugs. The aim of this study was to investigate the possibility of synergistic interaction between 1-(2-trifluoromethylphenyl)-imidazole (TRIM), a novel neuronal nitric oxide synthase (nNOS) inhibitor and conventional antidepressants of different classes in the forced swimming test (FST) in rats. TRIM decreased the immobility time at 50 mg/kg doses in the FST in rats. Treatment with a behaviourally subeffective dose of TRIM (20 mg/kg) augmented the behavioural effect of tricyclic antidepressant imipramine, selective serotonin re-uptake inhibitor (SSRI) citalopram and fluoxetine or selective serotonin reuptake enhancer tianeptine but failed to augment the antidepressant effect of reboxetine, a noradrenaline re-uptake inhibitor, in this test. Therefore inhibition of NOS augments the effects of antidepressants acting on serotonergic system in the FST. Neither TRIM (10–50 mg/kg) nor other drug treatments affected the locomotor activity of animals. These findings are in agreement with the view that antidepressant effects or augmentation of these effects in the FST may be explained with inhibition of NOS activity and this may be a new approach in offering greater therapeutic efficacy of antidepressants acting via serotonergic system.

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1. Introduction

Depression is one of the most prevalent psychopathologies in the Western world. Conventional antidepressant treatment has many limitations such as some are slow to take effect, side effect profile limiting compliance and there are also a large number of treatment-resistant patients. Such a profile has necessitated new therapeutic strategies in offering faster onset of action and augmenting the therapeutic actions of currently existing antidepressants and thus getting a greater efficacy in a larger proportion of patients.

Various inhibitors of nitric oxide synthase (NOS) have been shown to exert anxiolytic and antidepressant-like behavioural effects in a variety of animal models. 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). NMDA receptor activity appears to play a role in some neurophysiological phenomena and administration of NMDA antagonists exerted antidepressant-like effects in the forced swimming test (FST) in animals, a pre-clinical behavioural method used for studying the antidepressant activity of drugs (Borsini, 1995; Cryan et al., 2002a; Trullas and Skolnick, 1990).

We and other investigators have previously shown that retention of the immobile response in the Porsolt swimming test was impaired in rats given NOS inhibitors and suggested that NOS is an important target in the FST (Harkin et al., 1999a; Volke et al., 2003; Yildiz et al., 2000). 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, 1996) it can be an appropriate agent in investigating the biological roles of nNOS in the central nervous system.

The classical antidepressant imipramine exerts its effect by inhibiting both 5-HT and noradrenaline (NA) reuptake (Carrodi and Fuxe, 1968). SSRIs have been approved for the treatment of depression and several studies have shown that a single intraperitoneal injection of SSRIs increased the extracellular concentration of 5-HT in rat brain (Boothman et al., 2006; Felton et al., 2003). Tianeptine, a selective 5-HT reuptake enhancer (Datla and Curzon, 1993; Fattacini et al., 1990; Mennini et al., 1987) exhibited an antidepressant-like effect in several animal models of depression similar to that of selective serotonin reuptake inhibitors

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(Kelly and Leonard, 1994; Lacroix et al., 1996). The novel antidepressant reboxetine is a selective noradrenaline reuptake inhibitor that selectively inhibits the reuptake of synaptic norepinephrine without any marked affinity for other receptors or transporters (Wong et al., 2000).

The present study was undertaken to investigate the possibility of synergic antidepressant interaction between a potent nNOS inhibitor TRIM and currently used classical antidepressants of different classes in the FST in rats. In sight of this vision, behavioural effects of TRIM and antidepressants selectively acting on both serotonin and noradrenaline reuptake (imipramine), serotonin reuptake (fluoxetine, citalopram, tianeptine) and noradrenaline reuptake (reboxetine) alone, and in combination with TRIM were examined.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Animal Research Center, Kocaeli–Turkey) weighing 200–250 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-hour light/dark cycle (lights on at 07:00 h). Tap water and food pellets were provided ad libitum. All animals used for the experiments were naive to the swimming. 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 Kocaeli University of Ethics Committee (Number: 8-AEK 21/2, Kocaeli, Turkey).

2.2. Drugs

1-(2-trifluoromethylphenyl)-imidazole (TRIM), imipramine hydrochloride, fluoxetine hydrochloride, tianeptine and reboxetine were purchased from Sigma Chemicals (St Louis, Mo, USA). Citalopram HBr was obtained from Deva, Pharmaceutical Company Istanbul, Turkey. All drugs were dissolved in 0.9% physiological saline (PS) which was used as the vehicle control and given intraperitoneally (i.p.) in a volume of 0.2 ml per 100 g body weight of rats. The doses were chosen based on previous behavioural studies (Dhir and Kulkarni, 2007; File and Mabbutt, 1991; Harkin et al., 2004; Tatarczynska et al., 2004; Volke et al., 2003; Yildiz et al., 2000). Drugs were prepared freshly on the day of experiment.

2.3. Forced swimming test

The FST, the most widely 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 plexiglass 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 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 6–14 rats. All experiments were performed between 10.00 and 12:00 a.m.

In all experiments the drug effects were noted at the peak activity time. Measurement of FST was performed 50 min after IP injection of TRIM (10, 20, 50 mg/kg) or vehicle; imipramine (15, 30 mg/kg), citalopram (10, 20, 40 mg/kg), fluoxetine (20, 40 mg/kg), tianeptine (2.5, 5 mg/kg) and reboxetine (3, 10, 20 mg/kg) or their vehicles were given 30 min prior to testing.

2.4. Locomotor activity

Since compounds altering motor activity may give false positive/negative effects in FST, spontaneous locomotor activity of rats was evaluated by monitoring the activity of the animals in a locomotor activity cage (May 9803 Activity Monitoring System, Commat Iletisim Ltd. May Pentium Computer, Ankara, Turkey). Rats were individually placed in a plexiglass cage (42×42×30 cm) and the distance travelled by the animals was evaluated for a 5 min period. Immediately after the measurement of the locomotor activity, the same rats were assessed in the FST.

2.5. Data 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. Drug interaction data were statistically evaluated using two-way analysis of variance and post hoc comparisons between individual groups were carried out by means of Dunnet's test. Data are expressed as the mean±SEM with $p<0.05$ being considered statistically significant.

3. Results

3.1. Effects of imipramine and TRIM alone or in combination on immobility time in the FST

Imipramine (15, 30 mg/kg) dose dependently reduced the immobility time compared to vehicle-treated controls in the FST in

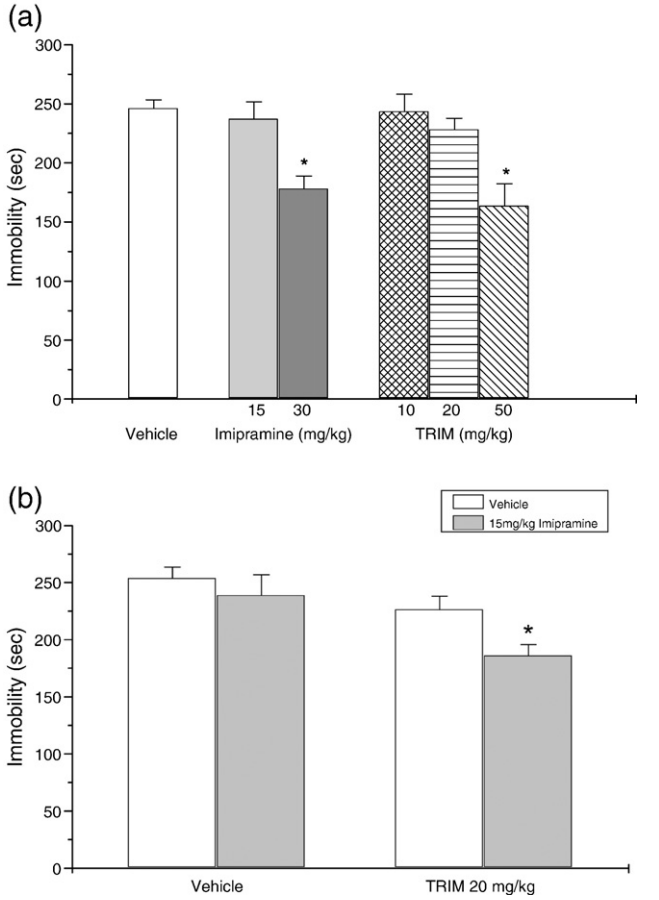

Fig. 1. (a). Effects of imipramine and TRIM on the immobility time in the rat FST. Each column represents the mean±SEM of 7–14 animals.*$p<0.001$ compared to vehicle control (Tukey test). (b). TRIM potentiates the activity of imipramine in the rat FST. Each column represents the mean±SEM of 7–10 animals.*$p=0.011$ compared to vehicle imipramine (15 mg/kg) (Dunnet test).

rats (Fig. 1a). One-way ANOVA showed a significant effect of drug treatment upon immobility time in FST [$F(5, 41)=8.64$, $p<0.001$]. Post-hoc comparisons revealed that 30 mg/kg imipramine significantly reduced immobility time compared to vehicle-treated group ($p<0.001$,Tukey test).

As shown in Fig. 1a, TRIM (10, 20, 50 mg/kg) given 50 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 [$F(5, 41)=8.64$, $p<0.001$]. Post-hoc comparisons revealed that 50 mg/kg TRIM significantly reduced immobility time compared to vehicle-treated group ($p<0.001$,Tukey test).

Two-way ANOVA showed a significant effect of drug treatment upon immobility time in FST in groups of animals given subeffective doses of imipramine and TRIM in combination [$F(3, 24)=6.934$, $p=0.002$]. Post hoc comparisons revealed that neither imipramine (15 mg/kg) nor TRIM (20 mg/kg) alone reduced immobility time compared to vehicle-treated group. Nevertheless in a group of animals where imipramine and TRIM was given in combination at subeffective doses, TRIM augmented the effects of imipramine in FST in rats (Fig. 1b).

3.2. TRIM augments the activity of citalopram or fluoxetine in the FST

Citalopram (10, 20, 40 mg/kg) failed to shorten the immobility time in the FST. No significant effect of drug treatment upon immobility time in FST in groups of animals given citalopram was indicated by one-way ANOVA [$F(3, 22)=0.34$, $p>0.05$]. Post hoc comparisons revealed that citalopram (10, 20, 40 mg/kg) had no effect on the immobility time in the FST (Fig. 2a). Two-way ANOVA showed a significant effect of drug treatment upon immobility time in FST in groups of animals given subeffective doses of citalopram and TRIM in combination [$F(3, 24)=6.02$, $p=0.003$]. Post hoc comparisons revealed that TRIM (20 mg/kg) given in combination with citalopram (20 mg/kg), revealed a significant reduction in the immobility time in rats (Fig. 2b).

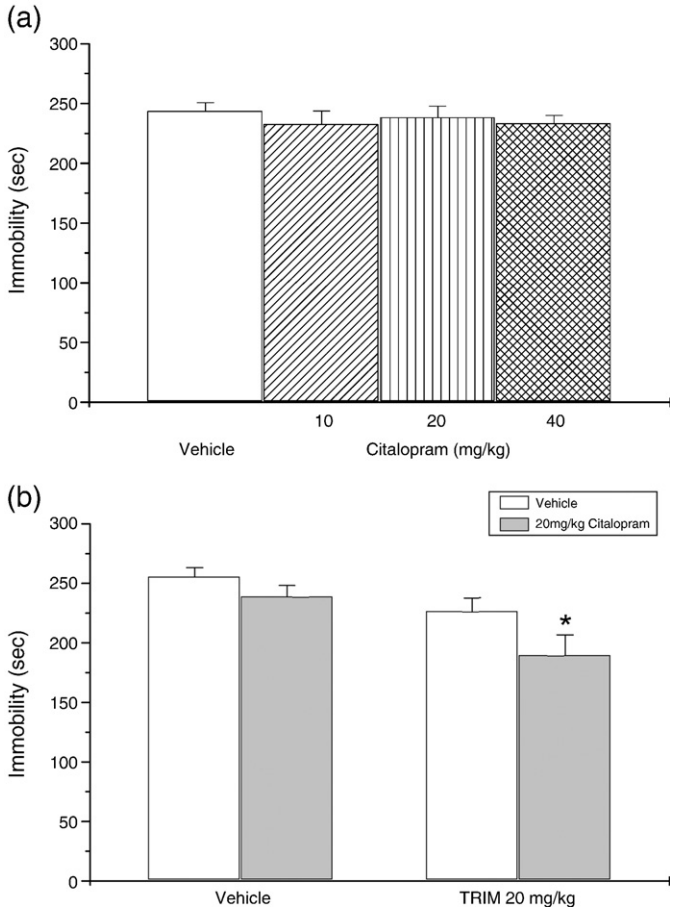

Fig. 2. (a). Effects of citalopram on the immobility time in the rat FST. Each column represents the mean±SEM of 7–9 animals. Citalopram failed to shorten the immobility time in the FST in rats. (b).TRIM (20 mg/kg) augments the activity of citalopram in the rat FST. Each column represents the mean±SEM of 7–9 animals.*$p=0.017$ compared to vehicle citalopram (Dunnet test).

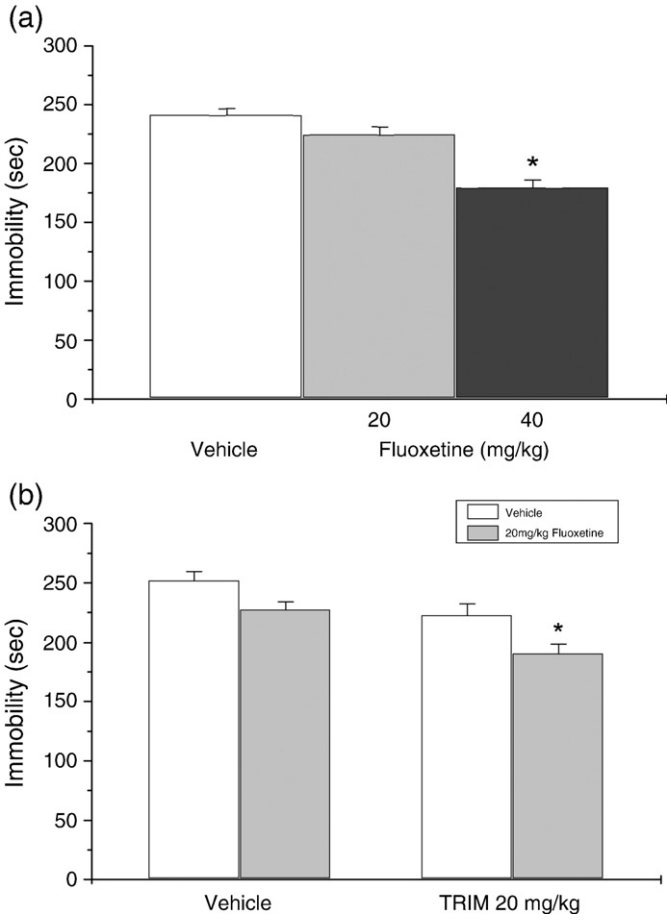

Fig. 3. (a). Effects of fluoxetine on the immobility time in the rat FST. Each column represents the mean±SEM of 7 animals. $p<0.001$ compared to vehicle control (Tukey test). (b).TRIM (20 mg/kg) augments the activity of fluoxetine in the rat FST. Each column represents the mean±SEM of 6–10 animals.*$p=0.01$ compared to vehicle fluoxetine (Dunnet test).

Fluoxetine (20, 40 mg/kg) reduced the immobility time in the FST. One-way ANOVA showed a significant effect of drug treatment [$F(2, 18)=26.28$, $p<0.001$]. Post hoc comparisons revealed that 40 mg/kg fluoxetine significantly reduced immobility time compared to vehicle-treated controls (Fig. 3a).

Two-way ANOVA showed a significant effect of drug treatment upon immobility time in FST in groups of animals given subeffective doses of fluoxetine and TRIM in combination [$F(3, 21)=9.3$, $p<0.001$]. Post hoc comparisons revealed that TRIM (20 mg/kg) given in combination with fluoxetine (20 mg/kg) augmented the effects of fluoxetine in the FST (3b).

3.3. TRIM augments the activity of tianeptine in the FST

Tianeptine (2.5, 5 mg/kg) reduced the immobility time compared to vehicle-treated controls in the FST (Fig. 4a). One-way ANOVA showed a significant effect of drug treatment upon immobility time in FST [$F(2,16)=7.91$, $p<0.01$]. Post hoc comparisons revealed that 5 mg/kg tianeptine significantly decreased the time spent in immobility compared to vehicle-treated controls. Two-way ANOVA showed a significant effect of subeffective doses of tianeptine and TRIM interaction [$F(3, 18)=6.9$,

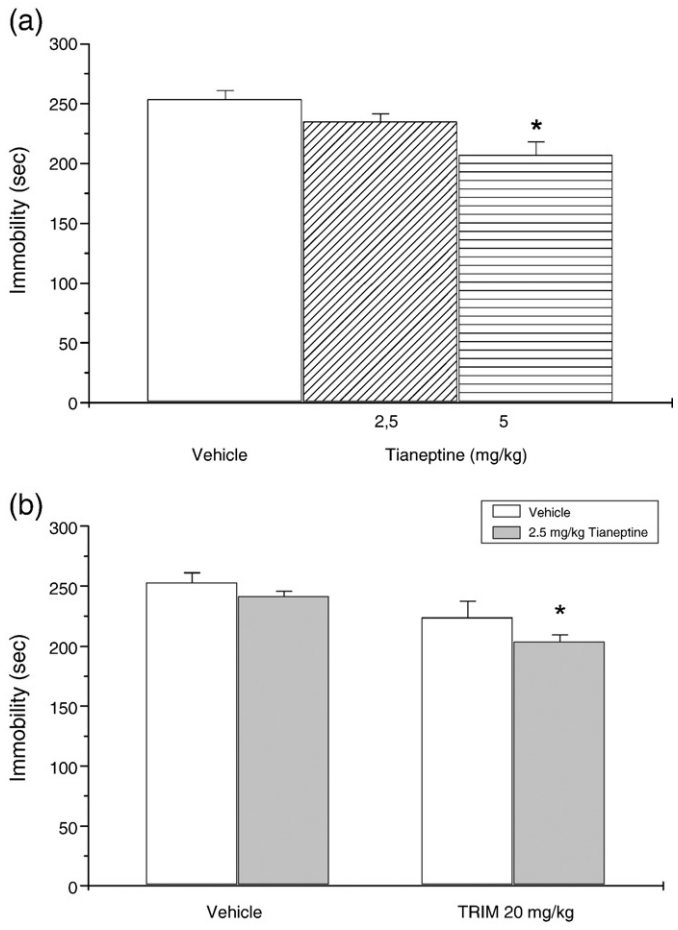

Fig. 4. (a). Effects of tianeptine on the immobility time in the rat FST. Each column represents the mean ± SEM of 6–7 animals.*$p<0.01$ compared to vehicle control (Tukey test). (b). TRIM (20 mg/kg) augments the activity of tianeptine in the rat FST. Each column represents the mean ± SEM of 6–9 animals.*$p=0.015$ compared to vehicle tianeptine (Dunnet test).

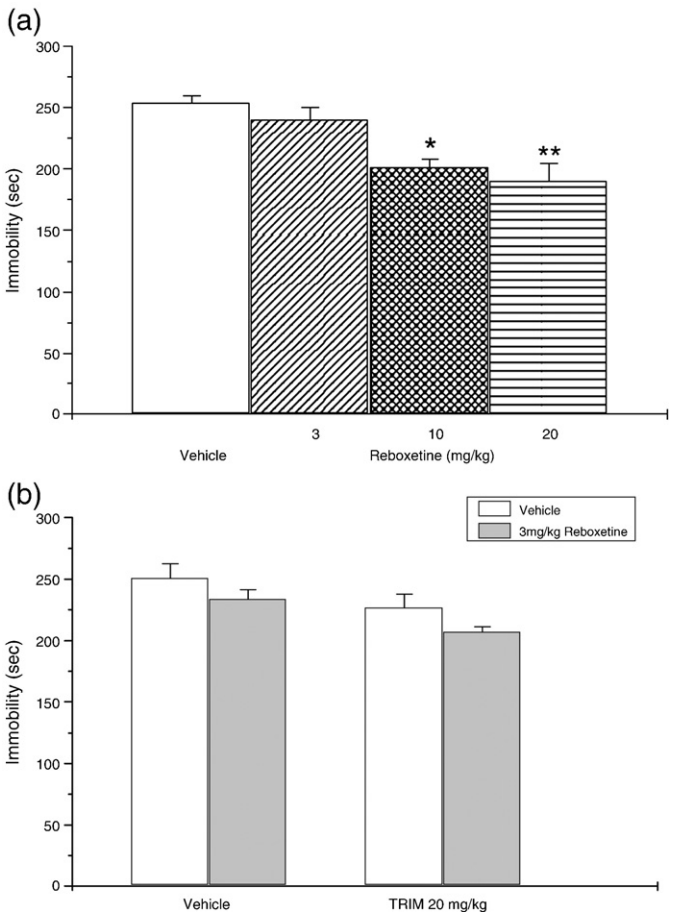

Fig. 5. (a). Effects of reboxetine on the immobility time in the rat FST. Each column represents the mean ± SEM of 7–9 animals.*$p<0.01$,**$p<0.001$ compared to vehicle control (Tukey test). (b).TRIM (20 mg/kg) failed to augment the activity of reboxetine in the rat FST. Each column represents the mean ± SEM of 8–12 animals. Reboxetine + TRIM combined group compared to vehicle reboxetine group ($p=0.066$, Dunnet test).

$p=0.003$]. Post hoc comparisons revealed that TRIM (20 mg/kg) augmented the effects of tianeptine 2.5 mg/kg in the FST (4b).

3.4. TRIM fails to augment the activity of reboxetine in the FST

Reboxetine (3, 10, 20 mg/kg) dose dependently reduced the immobility time compared to vehicle-treated controls in the FST in rats (Fig. 5a). One-way ANOVA showed a significant effect of drug treatment [$F(3, 22)=11.61$, *$p<0.01$, **$p<0.001$]. Post hoc comparisons showed that reboxetine significantly reduced the immobility time in the FST at 10 and 20 mg/kg doses compared to vehicle-treated controls. No significant interaction upon immobility time in FST in groups of animals given subeffective doses of reboxetine and TRIM was indicated by two-way ANOVA [$F(3, 24)=5.4$, $p=0.005$]. Post hoc comparisons showed that neither reboxetine (3 mg/kg) nor TRIM (20 mg/kg) reduced immobility time compared to vehicle-treated group. TRIM failed to augment the activity of reboxetine in the FST, in group of animals where TRIM was given in combination with reboxetine (Fig. 5b).

3.5. Effects on locomotor activity

It is well known that an antidepressant-like effect in the FST can be also evoked by drugs which induce hyperactivity (Maj et al., 1992), thus the influence of all the above treatments on the locomotor activity was concurrently evaluated. Neither TRIM (10, 20, 50 mg/kg) nor above treatments except fluoxetine (40 mg/kg) treated group

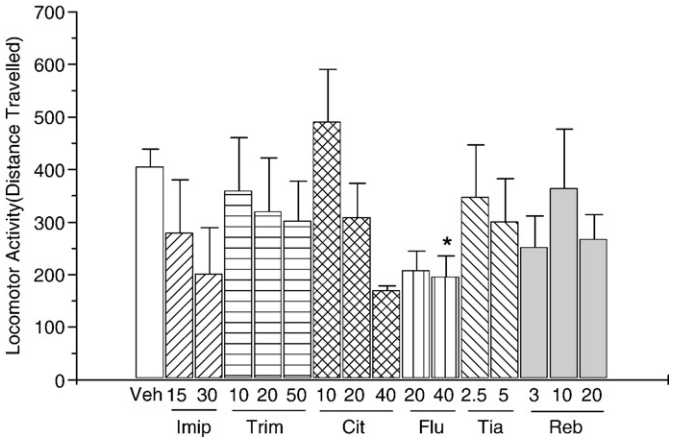

Fig. 6. Effect of TRIM (10, 20, 50 mg/kg), imipramine (Imip. 15, 30 mg/kg), citalopram (Cit. 10, 20, 40 mg/kg), fluoxetine (Flu. 20, 40 mg/kg), tianeptine (Tia. 2.5, 5 mg/kg) and reboxetine (Reb. 3, 10, 20 mg/kg) on the locomotor activity of rats. ANOVA showed no significant effect of drug treatment on locomotor activity test except fluoxetine ($F(3, 65)=0.6$, $p=0.61$ vehicle versus TRIM; $F(2,60)=2.8$, $p=0.06$ vehicle versus imipramine;; $F(3, 64)=2.15$, $p=0.07$ vehicle versus citalopram; $F(2, 59)=4.56$, $p=0.014$ vehicle versus fluoxetine; $F(2, 58)=0.65$, $p=0.5$ vehicle versus tianeptine; $F(3, 66)=1.5$, $p=0.22$ vehicle versus reboxetine). Results are expressed as the mean ± SEM of 6–10 animals. Post hoc comparisons revealed no significant effect of drugs on locomotor activity test except fluoxetine (Tukey test). *$p<0.05$ compared to vehicle control (Tukey test).

modified the total distance travelled by the rats (Fig. 6). ANOVA showed no significant effect of drug treatment on locomotor activity test [$F(3, 65)=0.6$, $p=0.61$ vehicle versus TRIM; $F(2,60)=2.8$, $p=0.06$ vehicle versus imipramine; $F(3, 64)=2.15$, $p=0.07$ vehicle versus citalopram; $F(2, 58)=0.65$,$p=0.5$ vehicle versus tianeptine; $F(3, 66)=1.5$, $p=0.22$ vehicle versus reboxetine]. Post hoc comparisons revealed no significant effect of drugs on locomotor activity test except fluoxetine 40 mg/kg significantly affected the locomotor activity compared to vehicle-treated group [$F(2, 59)=4.56$, $p=0.014$ vehicle versus fluoxetine].

4. Discussion

Noradrenaline and serotonin are two neurotransmitters widely reported to be involved in the mechanism of action of antidepressants and the development of drugs selectively affecting these transmitters has provided the opportunity to determine the role of these transmitter systems, alone and in combination, in an antidepressant response. In the present study, we examined effects of a potent nNOS inhibitor TRIM, 5-HT/NA reuptake inhibitor imipramine, SSRI citalopram and fluoxetine, serotonin reuptake enhancer tianeptine or selective NA reuptake inhibitor reboxetine alone or in combination in the FST in rats. As it was previously shown by Volke et al. (2003), TRIM decreased the immobility time at 50 mg/kg dose in the FST in rats. When given alone TRIM (10, 20 mg/kg), imipramine(15 mg/kg), citalopram (10, 20, 40 mg/kg), fluoxetine (20 mg/kg), tianeptine (2.5 mg/kg) and reboxetine (3 mg/kg) did not shorten the immobility time of rats. The higher doses of these drugs, except citalopram produced a significant reduction in the immobility of rats. Coadministration subeffective doses of TRIM (20 mg/kg) and reboxetine (3 mg/kg) did not affect the behavior of rats in the FST whereas subeffective dose of TRIM given in combination with imipramine (15 mg/kg), citalopram (20 mg/kg), fluoxetine (20 mg/kg) and tianeptine (2.5 mg/kg) significantly reduced the immobility time of rats in the FST. Our results indicate that the nNOS inhibitor TRIM, augments the effects of antidepressants acting via serotonergic system in the FST in rats.

In the current study, at the doses used neither TRIM nor the other antidepressants, except fluoxetine affected the locomotor activity of the animals while it has been reported that TRIM supresses locomotor activity only at 50 mg/kg dose (Volke et al., 2003). In previous studies (Dhir and Kulkarni 2007; Nowakowska et al., 2000) it is reported that fluoxetine had no effect on locomotor activity in rats. The diminished locomotor activity may be attributed to the environmental conditions, sex and strain differences. So the ability of TRIM to potentiate the behavioural activity of antidepressants acting via serotonergic system in the FST can not be attributed to a nonspecific locomotor stimulant effect of these drugs.

Other investigators and we (Ghasemi et al., 2008; Harkin et al., 2004; Volke et al., 2003; Yildiz et al., 2000) have previously shown that various inhibitors of NOS such as competitive nonspecific NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-L-arginine (L-NA), selective nNOS inhibitors 7-nitroindazole (7-NI) and N^W-propyl-L-arginine (L-NPA) possess antidepressant-like properties in animal models. Thus it is claimed that NOS is an important target in FST, a pre-clinical behavioural method widely used for studying the antidepressant activity of drugs. NO modulates the extracellular levels of various neurotransmitters such as serotonin, noradrenaline, dopamine and glutamate in the central nervous system while serotonin reuptake may be influenced by the NO pathway. It is suggested that, 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 relatively selective inhibitor of nNOS 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.

The classical antidepressant imipramine exerts its effect by inhibiting both 5-HT and noradrenaline reuptake (Carrodi and Fuxe, 1968). In order to clarify whether the interaction between TRIM and imipramine is due to serotonergic or noradrenergic system, selective serotonin reuptake inhibitor citalopram and fluoxetine; selective serotonin reuptake enhancer tianeptine; and selective noradrenaline reuptake inhibitor reboxetine was examined in the FST in rats.

Selective serotonin re-uptake inhibitors are believed to exert their clinical antidepressant effects by blocking the re-uptake 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. Citalopram is established to be one of the most selective of the SSRIs (Goodnick and Goldstein, 1998) and shown that citalopram administration did not alter hippocampal NOS activity under basal conditions (Wegener et al., 2004).

Tianeptine is an atypical antidepressant which is reported to increase serotonin re-uptake and decrease extracellular 5-HT in the brain (in contrast with most antidepressant agents).

(Datla and Curzon, 1993; Fattacini et al., 1990; Mennini et al., 1987). How tianeptine exerts its effect is a question that remains. Although the neurochemical properties of tianeptine and of selective serotonin reuptake inhibitors differ, they demonstrate similar antidepressant efficacy (Wilde and Benfield, 1995). It is interesting how these two antidepressants with opposite molecular mechanisms act. The fact that both inhibitors and enhancers of the serotonin reuptake are potent antidepressants, challenges the hypothesis on the central mechanisms of actions of these drugs. Nowakowska et al. (2000) claimed that in reference spatial memory test (food finding time in the maze), tianeptine had no effect whereas fluoxetine caused a very marked improvement of reference memory. So, besides the effect on serotonin re-uptake, other mechanisms must play an important role in the action of these drugs.

The novel antidepressant reboxetine is a selective noradrenaline reuptake inhibitor. It selectively inhibits the reuptake of synaptic norepinephrine without any marked affinity for other receptors or transporters (Wong et al., 2000). Our findings using the forced swimming test are in agreement with other reports describing the antidepressant-like activity of reboxetine in other animal models of depression (Harkin et al., 1999b; Wong et al., 2000). There is a complex interaction between ventral and dorsal noradrenergic bundle projecting neurons in modulating the antidepressant-like effects of reboxetine in the forced swimming test (Cryan et al., 2002b). It is established that forced swimming test exposure increases serotonergic activity in the amygdala, frontal cortex and hippocampus and dopamine turnover in the striatum; and reboxetine attenuates forced swimming test-induced increases in amygdaloid and cortical serotonin turnover and striatal dopamine turnover (Connor et al., 1999).The anti-immobility effect of reboxetine may be more closely related to its ability to antagonise the stressor-induced increase in dopaminergic turnover in the striatum since it is known that mesocorticolimbic dopaminergic activity may play a role in the behaviour of rats in the forced swimming test (Willner, 1995).

Following reasons can be suggested to explain the augmentation of the effects of antidepressant drugs acting via serotonergic system by nNOS inhibitor TRIM in the FST in rats:

One is that activation of NMDA receptors result in the formation of NO and increases cGMP levels (East and Garthwaite, 1991). Administration of NMDA antagonists has been shown to produce antidepressant-like effects in animal models (Trullas, 1997; Trullas and Skolnick, 1990). Therefore, NOS inhibition may exert similar effects to that NMDA receptor antagonists. Since it has been shown that NMDA receptor antagonists augment the activity of antidepressants such as fluoxetine, venlafaxine and imipramine when given in combination (Rogoz et al., 2002) it is the fact that interruption of the NMDA-NO synthase pathway may result in antidepressant-like and/or augmented antidepressant activity (Harkin et al., 2004). Consistent with our

findings, Harkin et al. (2004) showed that NO synthase inhibitor N^G-nitro-L-arginine (L-NA) augmented the effects of imipramine, fluoxetine, sertraline and citalopram but not reboxetine in the mouse FST; moreover this synergistic effect was also tested between 7-nitroindazole and imipramine or fluoxetine. Thus it was claimed that NOS inhibitors augment the effects of serotonin reuptake inhibitors in the FST. Moreover it has been shown that L-NA and 7-NI exert similar antidepressant-like behavioural profile as SSRI in FST in rats. Although it is not clear whether NMDA receptor antagonists exert antidepressant-like effects in the FST via serotonergic mechanism, it has been postulated that this antidepressant-like behaviour of L-NA and 7-NI is endogen serotonin dependent (Harkin et al., 2003). So it is postulated that the antidepressant augmenting effects of NOS inhibitors may be attributed to the modulation of serotonin release (Harkin et al., 2004). 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.

A second explanation for this result is that paroxetine, a selective serotonin reuptake inhibitor, is also a potent inhibitor of NOS enzyme activity (Finkel et al., 1996). Later, Wegener et al. (2003) showed that serotonergic antidepressants paroxetine, citalopram and tianeptine and the mixed serotonergic–noradrenergic antidepressant imipramine decrease NOS activity in vivo suggesting that actions on NOS are common to a variety of structurally dissimilar serotonergic antidepressants. From this point of view, it may be speculated that, NOS inhibition in the brain plays some role in the antidepressant effect of drugs acting via serotonergic system. Since NMDA receptor plays an important role in brain NOS activation, these effects may be due to secondary to inhibitory effects on NMDA receptors.

5. Conclusion

Thus the pharmacological mechanism seems to be more due to serotonergic than adrenergic neurotransmission and co-administration of antidepressants acting via serotonergic system by TRIM, a selective nNOS inhibitor may enhance beneficial effects in therapy-resistant depression and thus represent a potential source of novel drugs for antidepressant therapy.

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