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Effects of neuronal and inducible NOS inhibitor 1-[2-(trifluoromethyl) phenyl] imidazole (TRIM) in unpredictable chronic mild stress procedure in mice

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ABSTRACT

Nitric oxide is an intracellular messenger which is involved in several functions and pathologies such as depression, anxiety, learning and memory. In many studies nitric oxide synthase inhibitors (NOSI) were shown to possess antidepressant-like effects in animal models of depression. The aim of this study is to investigate the effects of a selective neuronal and inducible nitric oxide synthase inhibitor TRIM (30 mg/kg/day, 35 days) in mice subjected to unpredictable chronic mild stress and then compare it's effect with a conventional selective serotonin reuptake inhibitor fluoxetine (15 mg/kg/day, 35 days). Stressed vehicle animals showed a significant disturbed coat state when compared with nonstressed animals and this effect was reversed by TRIM or fluoxetine. Both TRIM and fluoxetine prevented the stress-induced deficit in the grooming behaviour in the splash test. TRIM and fluoxetine also significantly decreased the attack frequency when compared to the stressed control group in the resident–intruder test. These results support the assumption that NOS inhibitors can be a new class of antidepressant drugs possibly acting on neuronal NOS.
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1. Introduction

Depression is a common, chronic, recurrent and life debilitating illness with severe morbidity and mortality (Kiecolt-Glaser and Glaser, 2002). The mechanisms and brain areas underlying the pathophysiology of this disorder are not yet well understood. Clinical studies showed elevated plasma nitrate levels and increased nitric oxide synthase (NOS) expression in the hippocampus of depressed patients (De Oliveira et al., 2000, 2008).

Nitric oxide (NO), which is an important neurotransmitter in the nervous system, (Baranano et al., 2001) is synthesized from L-arginine aminoacid by the NOS enzyme (Schuman and Madison, 1994). It plays an important role in regulating many behavioural, cognitive and emotional processes such as learning, aggression, locomotion, anxiety and depression (Dzoljic et al., 1997; Harkin et al., 1999; Holscher 1997; Nelson et al., 1995; Wiley et al., 1995). In recent studies, inhibition of NOS enzyme elicited antidepressant-like behavioural effects in several animal experiments (Harkin et al., 1999; Jefferys and Funder, 1996; Da Silva et al., 2000; Yildiz et al., 2000a,b) and this effect was reversed by NOS substrate L-arginine suggesting that NO plays an important role in these behavioural responses (Harkin et al., 1999; Jefferys and Funder, 1996; Yildiz et al., 2000a,b). Further, NOS activity is involved in the mechanism of action of several antidepressants. For example, the selective serotonin reuptake inhibitor paroxetine inhibits in vitro NOS activity and decreases plasma nitrite and nitrate levels significantly in depressed patients (Finkel et al., 1996), whereas chronic therapy with imipramine or citalopram did not change NOS activity in the examined brain regions (cortex, hippocampus or cerebellum) (Jopek et al., 1999). Furthermore, Wegener et al. (2003) showed that, serotonergic antidepressants paroxetine, citalopram and tianeptine and mixed serotonergic–noradrenergic antidepressant imipramine decreased hippocampal NOS activity in vitro in rats although they don't have direct effects on NOS under clinically relevant conditions. It seems that there are controversial results for the effects of different antidepressants on NOS activity but the actions on NOS are common to a variety of structurally dissimilar serotonergic antidepressants.

The unpredictable chronic mild stress (UCMS) model is a promising and valuable animal model of depression which shows similar features to the depressive symptoms seen in human (Willner, 1997). It was also reported that, UCMS-mice exhibited a degradation in the physical coat state (Ducottet et al., 2003) and this effect was reversed by antidepressants such as fluoxetine (Ducottet et al., 2003; Santarelli et al., 2003). TRIM is a selective inhibitor of neuronal and inducible isoforms of NOS and it shows only a weak activity against endothelial

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NOS (Handy et al., 1995; 1996). In recent studies, it was shown that TRIM exerted antidepressant, anxiolytic and antinociceptive effects in rodents (Volke et al., 2003; Handy et al., 1995) and that it can be considered as an appropriate tool to clarify the role of nNOS in the central nervous system. However, these effects were experienced in a bioassay of depression, and not in a model that was mimicking the ethiopathogenic features of depression.

Literature search showed us that there had been no study investigating the effect of TRIM on depression after chronic injection in the UCMS model. This study was aimed to investigate the effects of 1-(2-trifluoromethylphenyl) imidazole (TRIM) in mice subjected to UCMS at a dose of 30 mg/kg which exhibited selectivity for inhibition of neuronal NO synthase in vitro and in vivo (Handy et al., 1995, 1996) and to compare it's effects with a conventional SSRI fluoxetine. For determining the effects of the UCMS regimen and drug therapy, coat state of the animals was recorded during UCMS. At the end of the UCMS regimen, splash and resident–intruder tests which are known to be the frequently used and effective methods to evaluate depression after UCMS model (Mineur et al., 2003; Yalcin et al., 2005, 2008) were applied to all of the animals in order to assess the ability of drugs to reverse stress-induced effects.

2. Methods

2.1. Animals

Seventy-two male, inbred BALB/c ByJ mice (Centre d'Elevage Janvier, France) aged of 7 weeks at their arrival to the laboratory were used in this study. The animals were kept in the laboratory for 2 weeks before the onset of the experiments. Animals were split into two groups: the non stressed mice and mice subjected to unpredictable chronic mild stress (UCMS) procedure. Non-stressed mice were group-housed (6 mice per cage) during the experiment while mice of the stressed group were singly-housed in cages (the dimensions: length: 268 mm, width: 135 mm, height: 81 mm) at the start of the chronic stress until the end of the study. Non-stressed mice were maintained under standard laboratory conditions (12-h-light:12-h-dark cycle, lights on at 08:00 h pm, $T=21\pm1$ C) in a separate room. All animals received food and water ad libitum. Such a group-housed control group was preferred to a singly-housing condition as social isolation is highly stressful for mice and thus should per se contribute to the chronic stress effects (Arbe et al., 2002; Muscat and Willner, 1992; Spani et al., 2003). All procedures described in this paper were conducted in accordance with European Community Council directive for the Ethical Treatment of Animals (86/609/EEC) and with the French legislation from Ministere de l'Agriculture concerning research involving animal subjects.

2.2. Experimental groups and drug administration

At the end of 2 weeks drug-free UCMS, mice were assigned to different experimental groups in a semi-randomized manner, so that the initial coat state and body weights were equivalent in all of the groups. We examined the effects of the chronic administration of TRIM and fluoxetine in stressed and non-stressed mice. Mice were randomly assigned to one of the 6 following experimental groups: non stressed NaCl ($n=11$), non stressed fluoxetine 15 mg/kg ($n=11$), non stressed TRIM 30 mg/kg ($n=12$), UCMS-ed NaCl ($n=10$), UCMS-ed fluoxetine 15 mg/kg ($n=12$), and UCMS-ed TRIM 30 mg/kg ($n=12$). All drugs were administered intraperitoneally (i.p.) every day at 11 a.m. in a volume of 0.1 ml/10 g body weight for 35 days. At the end of the UCMS, actograph, resident–intruder and splash tests were performed respectively. All of the nonstressed animals were isolated 1 day before the actograph test just like the UCMS-ed mice. Two days after the actograph test, the resident–intruder test was performed and 1 day later, the splash test was performed for all animals.

2.3. Unpredictable chronic mild stress model

The unpredictable chronic mild stress regimen used in this study was based on the procedure originally designed by Willner et al. (1992) and adapted to mice (Ducottet and Belzung, 2004). This stress model consists from repeated mild physical and psychological stressors. Mice were subjected several times a day for 7 weeks to different kinds of stressors in a chronic, inevitable and unpredictable way. Stressors were damp sawdust, changing the sawdust, placement in an empty cage or empty cage with water on the bottom (bath), periods of soiled cage with aversive odour (old rat or mice sawdust), social stress (switching the cages), cage tilting (45 °C), predator sounds for 15 min (sounds of different animals), inversion of light/dark cycle, lights on for a short time during the dark phase or lights off during the light phase, and confinement in tube (see Table 1). Stressors were administered in a pseudo-random manner, and could occur at any time of night and day. In this respect, the stressor sequence was changed every week in order to make the stress procedure unpredictable. During behavioural tests, the stress procedure was slightly modified: the number of stressors applied during the light period was reduced so as not to interfere with the tests. Non stressed mice were left undisturbed in their home cages. Ethically, stress procedure did not involve food and water deprivation or immobilization. The mice that were tested were not subjected to any stressors during 12 h before the behavioural tests, including splash test and actograph. In all experiments, the first 2 drug-free weeks of UCMS was followed by 5 UCMS applied weeks during which mice were treated with drug or vehicle. To determine the effects of the UCMS regimen and drug treatment, we examined the state of the coat in mice and performed the splash and the resident–intruder test. The actograph test was also performed to measure the locomotor activity. For further details on the procedure, see Yalcin et al. (2005).

2.4. Coat state and body weight

Before and during the UCMS, state of the coat and body weights of the animals were recorded once a week. The evaluation of the coat state was carried out by the assessment of eight different body parts: head (including eyes and nose), neck, dorsal coat, ventral coat, tail, forepaws, hindpaws and genital region (Ducottet et al., 2003; Ducottet and Belzung, 2004). A score of O for a coat in a good state or a score of 1 for a dirty coat (fur) or piloerection was given for each of these areas. Sum of these scores gave us an index of the general physical state of a mouse. Dirty state was characterized by a fluffy, greasy, less dense coat or piloerection. The state of the coat was evaluated by observers unaware of the treatment condition of the mice.

2.5. Splash test

This test was used to evaluate the grooming behaviour of mice. A 10% sucrose solution was squirted on the dorsal coat of mice in their homecage. The total time of grooming was recorded during 5 min after the vaporisation of sucrose solution (Ducottet and Belzung, 2004). All mice were then again placed in their home cage. The observer was unaware of the treatment conditions.

2.6. Actograph

Activity of the animals was recorded between 12 and 14 h using a photo-electric actimeter (Boissier and Simon, 1965). This test allowed us to evaluate the activity of mice in their homecage excluding the possible effects of the new environment on locomotor activity. The homecage was placed in the centre of the device, which consisted from a 20 cm×20 cm square plane with two electrical eyes. The infrared beams were placed outside of the cage at a height of 2.8 cm, sufficient to detect mice movements and elevated above the level of

Table 1
Unpredictable chronic mild stress procedure

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1	Coat state, weighing (9 h) Without sawdust (12h) Social stress (14h) New sawdust (16 h30)	3 sawdust changing (10–11–12 h) Damp sawdust (14 h) New sawdust (17 h)	Bain (10–11 h) Sounds of predators (15 h30) Cat hair (15–16 h) New sawdust (18 h)	Small tubes (9.30–10.30 h) 3 old sawdust changing (15–16–17 h) Without sawdust (22 h)	Cages tilt at 45° (9 h30–10 h30) New sawdust (15 h) Social stress (20 h)	Reversal of the light/ dark cycle (after 6 h) Social stress (15 h)	Reversal of the light/ dark cycle Old mice sawdust (12 h) New sawdust (20 h)
Week 2	Coat state, weighing (9 h) Cages tilt at 45° (12–14 h) Without sawdust (14 h) Small tubes (14–16 h) New sawdust (20 h)	Bain (12 h30–13 h30) Damp sawdust (15 h30–16 h30) Social stress (18–19–20 h)	Old sawdust (9 h30–11 h30) Sounds of predators (13 h) Horse hair (15 h–17 h) New sawdust (18 h)	Reversal of the light/dark cycle Social stress (10 h) Sounds of predators (12 h) Social stress (14 h, 15 h)	Small tubes (10.30–11.30 h) Old mice sawdust (16 h) New sawdust (22 h)	4 light/dark succession every 1 h (10–14.00 h) Without sawdust (14 h)	4 light/dark succession every 1 h (10–14.00 h) New sawdust (20 h)
Week 3	Coat state, weighing Treatment (11 h) Light (12–15 h) Damp sawdust (16–18 h)	Treatment (11 h) Damp sawdust (10.30–12.30 h) Without sawdust (14.30– 16.30 h)	Treatment (11 h) Cages tilt at 45° (12.30–14.30) Social stress (15, 16, 17 h)	Treatment (11 h) Reversal of the light/dark cycle (8–15 h) Damp sawdust (15–17 h)	Treatment (11 h) Without sawdust (10–12.30 h) Social stress (14–15–16 h)	Treatment (11 h) Reversal of the light/dark cycle Social stress (17 h)	Treatment (11 h) Reversal of the light/ dark cycle Damp sawdust (14– 16 h)
Week 4	Coat state, weighing Treatment (11 h) Without sawdust (13– 18 h) Dark (20–24 h)	Treatment (11 h) Damp sawdust (10.30–12.30 h) Small tubes (15–16 h)	Treatment (11 h) Cages tilt at 45° (11–14 h) Social stress (17 h)	Treatment (11 h) Without sawdust (13 h) Social stress (15 h)	Treatment (11 h) New sawdust (10 h) Social stress (15 h)	Treatment (11 h) Dark (01–04) Light (9–12)	Treatment (11 h) Dark (4–6) Light (15–17)
Week 5	Coat state, weighing Treatment (11 h) Small tubes (14–17 h)	Treatment (11 h) Old rat sawdust (10–12 h) Horse hair (14 h–16 h)	Treatment (11 h) New sawdust (10 h) Cages tilt at 45° (11 h–13 h) Old rat sawdust (14 h–17 h)	Treatment (11 h) New sawdust (10 h) Bain (10–11 h) Cat hair (13–16 h)	Treatment (11 h) Cages tilt at 45° (12–14 h) Sounds of predators (14 h30) Damp sawdust (15.30 h) New sawdust (17.30 h)	Treatment (11 h) 4 light/dark succession every 1 h (10–14 h) Without sawdust (13 h)	Treatment (11 h) 4 light/dark succession every 1 h (10–14 h) New sawdust (22 h)
Week 6	Coat state, weighing Treatment (11 h) Cages tilt at 45°(14–17 h) Sounds of predators (22 h)	Treatment (11 h) New sawdust (14 h) Old rat sawdust (16 h30) Social stress (17 h30)	Treatment (11 h) Social stress Cages tilt at 45°(14 h) Small tubes (15–17 h)	Treatment (11 h) Small tubes (12.30–13.30) Cat hair (13.30–14 h) Damp sawdust (14.30–16 h)	Treatment (11 h) New sawdust (11 h) Sawdust changing (11 h30)	Treatment (11 h) Reversal of the light/dark cycle Damp sawdust (14 h–17 h)	Treatment (11 h) Reversal of the light/ dark cycle
Week 7	Coat state, weighing (9 h) Treatment (11 h) Social stress (13, 14, 15 h) Old rat sawdust (20 h)	Treatment (11 h) New sawdust (14 h) Old rat sawdust (16.30) Social stress (17.30)	Treatment (11 h) Social stress (12 h) Cages tilt at 45° (14–16 h) New sawdust (17 h)	Treatment (11 h) Cages tilt at 45° (10 h–12 h) Without sawdust (15 h)	Treatment (11 h) New sawdust (10 h) Light (9–12 h) Bain (12–14 h) Small tubes (15–16 h)	Treatment (11 h) Reversal of the light/dark cycle Without sawdust (15 h)	Treatment (11 h) Reversal of the light/ dark cycle New sawdust (12 h) Social stress (15 h)

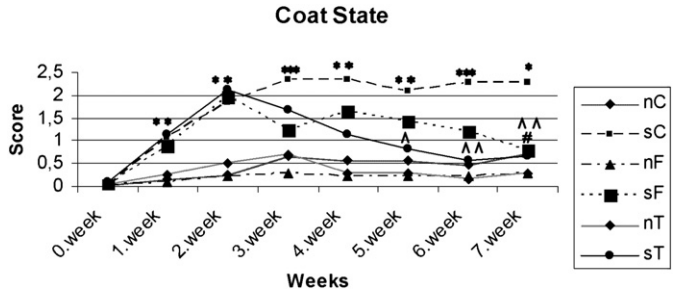

Fig. 1. Effects of fluoxetine (15 mg/kg, i.p.) and TRIM (30 mg/kg, i.p.) given for 35 days on coat state in non-stressed and stressed groups during UCMS. All of the treatments begun after 2 weeks of stress regimen and were administered during 5 weeks. Data are means ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$, difference between stressed and non stressed vehicle; ^$p<0.05$, ^^$p<0.01$ difference between stressed TRIM and stressed vehicle. # $p<0.05$, difference between stressed and non stressed fluoxetine group. nC = nonstressed control (vehicle) group, nF = nonstressed fluoxetine group, nT = nonstressed TRIM group, sC = stressed control (vehicle) group, sF = stressed fluoxetine group, and sT = stressed TRIM group.

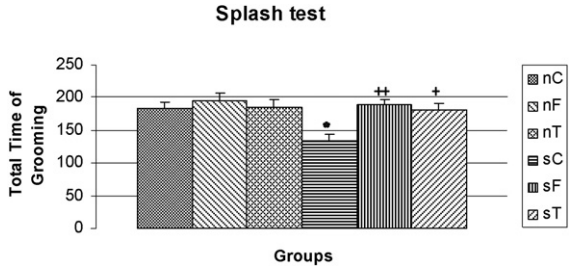

Fig. 2. Effects of fluoxetine (15 mg/kg, i.p.) and TRIM (30 mg/kg, i.p.) given for 35 days on total time of grooming in the splash test in the end of the unpredictable chronic mild stress regimen. Data are means ± SEM. *$p<0.01$, compared to non-stressed vehicle group, +$p<0.05$, ++$p<0.01$, compared to the stressed vehicle group. nC = nonstressed control (vehicle) group, nF = nonstressed fluoxetine group, nT = nonstressed TRIM group, sC = stressed control (vehicle) group, sF = stressed fluoxetine group, and sT = stressed TRIM group.

the sawdust in order to permit data recording. When mice crossed throughout, the movement of the animal was detected automatically.

2.7. Resident/intruder test

The resident–intruder test was carried out as previously described by Mineur et al. (2003). Non-stressed mice were isolated 48 h before the test during which the bedding was not changed in order to increase the amount of territorial cues within the cages. Mice were tested against an A/J intruder, known for it's high passivity and lack of aggression (Michard and Carlier, 1985). The opponent was placed into the cage of the test animal (resident) in such a way that mice were placed in opposite corners. The cage was then covered with a plastic lid and the test started immediately, lasting for a maximum of 5 min. The number of attacks between the resident and intruder mice was recorded during 5 min.

2.8. Drugs

TRIM was purchased from Tocris (Avonmouth, UK). Fluoxetine hydrochloride was supplied as a gift by Deva (İstanbul, Turkey). All of the drugs were dissolved in 0.9% NaCI and given intraperitoneally (i.p.) in a volume of 0.1 ml per 10 g body weight of mice. The control groups received the same volume of NaCI.

2.9. Statistics

The results of the coat state during 7 weeks and the results of locomotion and body weight in the end of UCMS regimen were compared by Kruskal–Wallis *H* followed by Dunn's test when significant differences were detected. The total time of grooming in the splash test and the attack frequency in resident–intruder test were analysed using a two-way ANOVA followed by the Dunnett-*t* post hoc test when differences were significant. Data are expressed as the mean values ± SEM. Differences were considered to be statistically significant when p was less than 0.05.

Table 2
The drugs were administered for 5 weeks

Environment	Treatment mg/kg (i.p.)	Body weight (g)	Locomotor activity
Non-stressed	Vehicle	30.33 ± 0.36	2254.33 ± 256.25
Non-stressed	Fluoxetine (15)	30.13 ± 0.57	2445.82 ± 321.57
Non-stressed	TRIM (30)	31.82 ± 0.29	2629.42 ± 295.70
Stressed	Vehicle	31.22 ± 0.54	2706.5 ± 420.9
Stressed	Fluoxetine (15)	31.30 ± 0.54	2462 ± 426.25
Stressed	TRIM (30)	31.45 ± 0.41	2324.83 ± 298.99

Results are shown as the means ± S.E.M.

3. Results

Fig. 1illustrates the total score of the coat state during 7 weeks of the UCMS regimen. Kruskal–Wallis *H* test revealed a significant difference between the groups from the beginning of the first week until the end of the UCMS ($H=41.415$, $p<0.0001$; $H=51.975$ $p<0.0001$; $H=39.965$, $p<0.0001$; $H=45.234$, $p<0.0001$; $H=39.537$, $p<0.0001$; $H=43.640$, $p<0.0001$; $H=33.951$, $p<0.0001$, respectively) (Fig. 1). We also observed a significant difference between non-stressed vehicle and stressed vehicle groups from the beginning of the first week until the end of the UCMS regimen ($p<0.01$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$, $p<0.001$, $p<0.05$, respectively). Effect of fluoxetine on coat state was statistically significant at the seventh week ($p<0.05$) whereas TRIM exerted its effect at the fifth week ($p<0.05$, $p<0.01$, $p<0.01$).

We did not observe a statistically significant difference between the body weight of all groups at the end of UCMS regimen ($H=10.275$, $p=0.068$). Furthermore, no significant impairment of locomotor activity due to the UCMS regimen or treatment was observed ($H=1.365$, $p=0.93$) (Table 2).

Effects of fluoxetine and TRIM on total time of grooming in the splash test are shown in Fig. 2. There was a significant difference between groups ($F_{(5,62)}=3.979$, $p=0.004$). Non-stressed vehicle mice groomed significantly more than stressed vehicle mice ($p<0.01$). Both fluoxetine and TRIM significantly augmented the latency of the grooming behaviour in stressed mice in the splash test ($p<0.01$ and $p<0.05$ respectively).

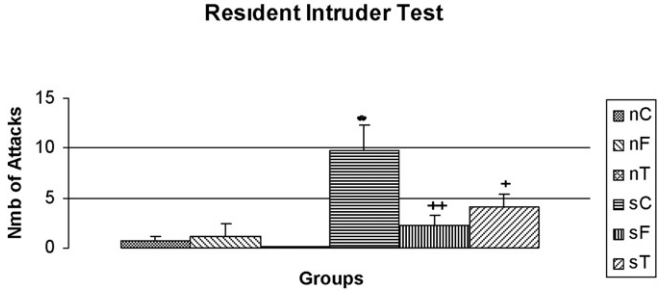

Fig. 3. Effects of fluoxetine (15 mg/kg, i.p/day, 35 days) and TRIM (30 mg/kg, i.p./day, 35 days) on attack frequency in the resident–intruder test after UCMS. Data are means ± SEM. *$p<0.0001$, compared to the non-stressed vehicle group, +$p<0.05$, ++$p<0.01$, compared to the stressed vehicle group. nC = nonstressed control (vehicle) group, nF = nonstressed fluoxetine group, nT = nonstressed TRIM group, sC = stressed control (vehicle) group, sF = stressed fluoxetine group, and sT = stressed TRIM group.

Effects of fluoxetine and TRIM on attack frequency in the resident–intruder test are shown in Fig. 3. There was a significant difference between groups ($F_{(5,62)}=7.664$, $p<0.0001$). In the resident–intruder test, the attack frequency was significantly increased in stressed vehicle animals compared to non-stressed animals ($p<0.0001$). This effect was reversed by both TRIM (30 mg/kg/day, 35 days) and fluoxetine (15 mg/kg/day, 35 days) ($p<0.05$, $p<0.01$ respectively compared to stressed vehicle).

4. Discussion

In this study, we showed that UCMS regimen induced a coat state degradation and this effect was reversed by a selective neuronal and inducible nitric oxide synthase inhibitor TRIM as well as by fluoxetine. Interestingly, the onset of this action was faster after TRIM then after fluoxetine. Indeed, coat state improvement occurred 3 weeks after TRIM treatment whereas 5 weeks therapy was necessary to observe fluoxetine's action. Similar effects were also observed in the splash test. Moreover, in the resident–intruder test, stressed mice demonstrated a larger degree of aggresivity, an effect abolished by both drugs. These results cannot be attributed to the effects of the drugs on activity since both TRIM and fluoxetine had no any effects on locomotor activity.

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. Furthermore, monoaminergic-acting antidepressants may exert their therapeutic effects by long-term persistent adaptations that serve as a form of drug induced neural plasticity (Santarelli et al., 2003; Surget et al., 2008) which needs several weeks to occur and may explain the delayed onset of action.

It is proposed that, NOS inhibitor 7-NI may increase serotonin (5-HT) levels in rat hippocampus after systemic therapy (Wegener et al., 2000), suggesting that antidepressant-like effects of NOS inhibitors can be related with the changes that occur in 5-HT levels in the brain. This is confirmed by the fact that NOS inhibition modulates central serotonin release (Kiss, 2000; Smith and Whitton, 2000; Wegener et al., 2000). In rats exposed to chronic mild stress, 5-HT and 5-HIAA levels decreased significantly in many brain regions, compared to nonstressed animals (Li et al., 2003; Vancassel et al., 2008). Therefore, it can be the fact that effects of TRIM might be underlied by its ability to reverse UCMS-induced alteration of 5-HT. Alternatively, a possible mechanism may also be related with NMDA receptors. Interestingly, competitive and non-competitive NMDA receptor antagonists induce antidepressant-like effects in animal models (Eckeli et al., 2000) and combined therapy of NMDA receptor antagonists with subactive doses of antidepressants such as fluoxetine, venlafaxine and imipramine, resulted in antidepressant response in the FST test (Rogóz et al., 2002). Indeed inhibition of NO synthesis may exert similar effects to the NMDA receptor antagonists (Wiley et al., 1995) and it can be suggested that NOS inhibition can have antidepressant effects similar to NMDA receptor antagonists.

In recent studies many investigators showed 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 (Ghasemi et al., 2008; Harkin et al., 2004; Volke et al., 2003; Yildiz et al., 2000a,b). It is suggested that nNOS plays a key role in the antidepressant-like effects of NOS inhibitors (Volke et al., 2003). This is in line with the observation that nNOS mRNA expression was increased after stress in several brain regions (De Oliveira et al., 2000) and that CMS exposure upregulates nNOS expression selectively in hippocampus while it doesn't change iNOS and eNOS expressions (Zhou et al., 2007). Furthermore, over-expression of nNOS suppresses hippocampal neurogenesis in the hippocampus of animals exposed to chronic stress while nNOS inhibition preserves and reverses CMS induced effects by promoting hippocampal neurogenesis (Zhou et al., 2007). Zhou et al. (2007) showed that CMS-induced behavioural despair and hippocampal neurogenesis impairment were prevented and reversed in mice receiving nNOS inhibitor 7-Nitroindazole (7-NI). TRIM had different pharmacokinetic and pharmacodynamic features when compared with 7-NI and there were controversial results for the selectivity on nNOS inhibition of these two compounds (Volke et al., 2003; Handy et al., 1995; Fidecka, 2003). TRIM at a dose of 50 mg/kg was known to inhibit nNOS and had clear antidepressive and anxiolytic effects although it also inhibited locomotion and motor activity at this dose (Volke et al., 2003).

Glutamate is a necessary neurotransmitter for long-term potentiation although it was proposed that neurotoxic elevations of glutamatergic and nitrergic activity should be the reason of the neurodegenerative pathology in the hippocampus of depressive patients (Sapolsky, 2000) and that hippocampal NOS activity is decreased by the local/direct administration of antidepressants in the hippocampus (Wegener et al., 2003). It was previously demonstrated that CMS exposure induces nNOS over-expression in the hippocampus and as a result, NO and it's metabolite peroxynitrite increases evidently. So, the decreased cell proliferation and the disturbed survival of the newborn cells in the dentate gyrus is formed especially due to the excess production of peroxynitrite which is attributed to the over-expression of nNOS in the hippocampus as a response to chronic stress. It was shown that stress and elevated glucocorticoid results in glutamate excitotoxicity, disturbed calcium homeostatis, inhibition of glucose transport and increase in oxygen radical generation (Sapolsky, 2000). TRIM, as a neuronal and inducible NOS inhibitor probably preserves cells from glutamate mediated neurotoxicity, necrosis and cell death. It can be that these effects might occur rapidly, thus explaining the rapid onset of drug action.

The effects of stress on feeding behaviour and body weight are still controversial. Although various studies indicated a decrease in these parameters (Haleem and Parveen, 1994), some recent studies indicated increase or no change (Sanchez et al., 1998). In our model there were no any effects of stress regimen and drugs on body weight. It is also known that TRIM has a selectivity to nNOS and iNOS but it showed very low selectivity to eNOS (Handy et al., 1995, 1996). So we didn't expect to observe any hypertensive effects of TRIM in this study. In recent studies it was also mentioned that TRIM had antinociceptive (Handy et al., 1995; Fidecka 2003) and anticonvulsant effects (Dzoljic et al., 1997) without changing arterial blood pressure (Handy and Moore 1997; Escott et al., 1998) with similar doses used in our study. Moreover another NOS inhibitor 7-NI used at the minimum dose required to inhibit nNOS (30 mg/kg) for 7 days in CMS mice model and exerted no side effects such as hypertension (Zhou et al., 2007).

In our study and in previous studies the results of the resident–intruder test showed that there is an increase in the aggresivity levels for defending the home territory against the intruder in the chronic mild stressed animals (Mineur et al., 2003; Yalcin et al., 2008). Serotonergic system can be the primer candidate for the underlying mechanisms. In recent studies, depressed patients showed changes in the serotonin levels (Tannenbaum and Anisman, 2003) and the therapy with serotonin reuptake inhibitors decreased aggression level in humans and rodents (Nelson and Chiavegatto, 2001).

It can be postulated that TRIM, selectively inhibiting nNOS, on the other hand can also selectively inhibit iNOS and thus may possibly contribute to the neuroprotective role against stress conditions (Harvey et al., 2004). Harvey et al. showed that stress induced NOS activation was blocked by selective iNOS inhibitors supporting that stres–restress-mediated glucocorticoid release activated iNOS. In recent studies, it was also postulated that increase in iNOS expression results in the formation of high amounts of NO and elevated NO

concentrations can then interact with superoxide anion, generated by the mitochondria or by other mechanisms, leading to the formation of the powerful oxidant species peroxynitrite. All these resulted with cell damage and changes in neuronal physiological functions (Lipton, 1999; Harvey et al., 2004).

5. Conclusion

In conclusion, NOS inhibitors can be a novel approach for antidepressant therapy exerting their effect possibly on neuronal NOS and TRIM can be a well-used kind of this group of drugs by the selective inhibition of both nNOS and iNOS. Further studies with different doses of TRIM should be done for the evaluation of iNOS on the antidepressant effect of TRIM.

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