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

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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 animals 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-HT_1 and 5-HT_2 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 para-chlorophenylalanine (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-HT_2 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-HT_2 receptors while non-significant effects were obtained with 5-HT_1 receptors.

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 prevalance 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 (monoaminooxidase 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; Yildiz 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-HT_2 receptors in the antidepressant-like behavioural effect of TRIM in the rat FST. Thus depletion of endogenous serotonin was asessed 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-HT_2 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-methoxphenyl)-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 seperate 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 previos 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-HT_1 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-HT_2 receptor subtype in the effect of TRIM, rats were pretreated with cyproheptadine (3 mg/kg, a 5-HT_2 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 seperate 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.