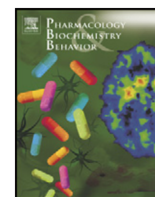
Pharmacology, Biochemistry and Behavior 95 (2010) 344–350

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh



Evidence for the involvement of the noradrenergic system, dopaminergic and imidazoline receptors in the antidepressant-like effect of tramadol in mice

Cristiano R. Jesse, Ethel A. Wilhelm, Cristiani F. Bortolatto, Cristina W. Nogueira *

Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

ARTICLE INFO

Article history:
Received 17 November 2009
Received in revised form 4 February 2010
Accepted 15 February 2010
Available online 23 February 2010

Keywords:
Tramadol
Antidepressant
Noradrenergic system
Imidazoline
Dopaminergic receptors

ABSTRACT

The involvement of the noradrenergic system, imidazoline, dopaminergic and adenosinergic receptors in the antidepressant-like action of tramadol in the mouse forced swimming test (FST) was evaluated in this study. The antidepressant-like effect of tramadol (40 mg/kg, per oral, p.o.) in the FST was blocked with yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist), α-methyl-para-tyrosine methyl ester (AMPT, 100 mg/kg, i.p., an inhibitor of tyrosine hydroxylase), efaroxan (1 mg/kg, i.p., an imidazoline I_1/α_2-adrenoceptor antagonist), idazoxan (0.06 mg/kg, i.p., an imidazoline I_2/α_2-adrenoceptor antagonist), antazoline (5 mg/kg, i.p., a ligand with high affinity for the I_2 receptor), haloperidol (0.2 mg/kg, i.p., a non selective dopamine receptor antagonist), SCH23390 (0.05 mg/kg, subcutaneously, s.c., a dopamine D_1 receptor antagonist), sulpiride (50 mg/kg, i.p., a dopamine D_2 and D_3 receptor antagonist) but was not reversed by prazosin (1 mg/kg, intraperitoneally, i.p., an α_1-adrenoceptor antagonist) and caffeine (3 mg/kg, i.p., a nonselective adenosine receptor antagonist). Monoamine oxidase-A and -B (MAO-A and MAO-B) activities were neither inhibited in the whole brain nor in specific brain regions of mice treated with tramadol. These data demonstrated that the antidepressant-like effect caused by oral administration of tramadol in the mouse FST is mediated by the noradrenergic system, dopaminergic and imidazoline receptors.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Tramadol, (1RS,2RS)-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride, is a centrally acting analgesic which is widely used in clinical practice. Tramadol is a synthetic opioid that binds weakly to μ-opioid receptors (Hennies et al., 1988). Nevertheless, a non-opioid component contributes to the mechanism of action of tramadol such as noradrenaline (NA) and serotonin (5-HT) neurotransmitters (Raffa et al., 1992). Tramadol elicits antidepressant-like effects in mice and rats (Rojas-Corrales et al., 1998, 2002, 2004; Jesse et al., 2008, 2009) and induces changes in the central nervous system (CNS) similar to those induced by conventional antidepressants; such as a decrease in the binding of frontocortical β-adrenoceptors, 5-HT_{2A} receptors (Hopwood et al., 2001) and α_2-adrenoceptors (Faron-Gorecka et al., 2004).

In clinical practice, tramadol has been used successfully in psychiatric disorders such as refractory major depression (Shapira et al., 2001), severe suicidal ideation and antidepressant potentiation (Spencer, 2000). Tramadol has been employed with positive effects in anxiety and anxiety-like disorders such as obsessive–compulsive disorders and in the treatment of Tourette's Syndrome (Shapira et al., 1997).

Numerous neural pathways are involved in the pathophysiology of depression. Therefore, a great number of neurotransmitters participate in the underlying mechanisms of drugs (Palucha and Pilc 2002). The causes of depression have been, in part, attributed to the dysregulation of neurotransmitters at the synapse (Prange, 1974). Monoamine neurotransmitters including 5-HT, NA and dopamine (DA) are believed to be involved in pathogenesis of depression and play important roles in mediating behavioral effects of antidepressant drugs (Millan, 2004; Lanni et al., 2009). Monoamine oxidase (MAO) is the key enzyme that is associated with metabolism of monoamines thus, regulating their intracellular concentrations in the brain. Therefore, the abnormal function of this enzyme is thought to be involved in several psychiatric disorders, such as depression (Deniker, 1984). Moreover, tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of NA and DA and its activity can be modulated in the antidepressant-like effect of different drugs (Kaster et al., 2007; O´ Leary et al., 2007).

The involvement of adenosine in the pathophysiology of depression and in antidepressant action is suggested by previous studies (El Yacoubi et al., 2001; Kaster et al., 2004, 2005). The imidazoline receptors, a family of non-adrenergic receptors, are widely distributed both centrally and peripherally and are involved in various physiological functions and depression (Regunathan and Reis, 1996). These

* Corresponding author. Tel.: +55 55 32209611; fax: +55 55 32208978.
E-mail address: criswn@quimica.ufsm.br (C.W. Nogueira).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.02.011

receptors have been classified into two main types: imidazoline I_1 and I_2 receptors (Bousquet et al., 2000). Study reports that the I_1 and I_2 binding sites have been implicated in mood disorders (Smith et al., 2009).

Considering that tramadol elicits an antidepressant-like effect in the forced swimming test (FST), a model predictive of antidepressant activity, (Rojas-Corrales et al., 1998; Jesse et al., 2008) and modulates a non-opioid mechanism that contributes to its pharmacological actions (Rojas-Corrales et al., 2004), the present study sought to extend previous findings by investigating the involvement of noradrenergic, imidazoline, dopaminergic and adenosinergic systems in the antidepressant-like action of tramadol in the mouse FST. We also investigated the activity of MAO-A and MAO-B in whole brain and in specific brain regions, implicated in the pathogenesis of depression, of mice treated with tramadol.

2. Materials and methods

2.1. Animals

The behavioral experiments were conducted using male adult Swiss mice (25–35 g, 3 months old) maintained at 22–25 °C with free access to water and food, under a 12:12 hour light/dark cycle, with lights on at 6:00 a.m. All manipulations were carried out between 08.00 a.m. and 04.00 p.m. All experiments were performed on separate groups of animals and each animal was used only once in each test. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

2.2. Chemicals and administration

Tramadol was a gift from Cristália (São Paulo, Brazil). The following drugs were used: α-methyl-para-tyrosine methyl ester (AMPT), prazosin hydrochloride, yohimbine hydrochloride, efaroxan hydrochloride, idazoxan hydrochloride, antazoline hydrochloride, haloperidol hydrochloride, SCH23390 hydrochloride, sulpiride, caffeine, selegiline, clorgiline, kynuramine dihydrobromide and 4-hydroxyquinoline (Sigma Chemical Co, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Drugs were administered by intraperitoneal (i.p.) route, except SCH23390 that was administered by subcutaneous (s.c.) route, in a constant volume of 10 ml/kg body weight. AMPT were dissolved in saline with 10% Tween 80, whereas all other drugs were dissolved in isotonic saline solution (NaCl 0.9%) immediately before use. Appropriate vehicle treated groups were also assessed simultaneously. The behavioral tests were performed by an observer blind to the drug treatment. Doses and administration schedule were chosen on the basis of experiments previously performed in our research group. The literature data confirm the selectivity and efficacy of the above mentioned dose regimen used (Brocardo et al., 2008; Kaster et al., 2007; Lobato et al., 2008; Machado et al., 2007).

2.3. Experimental procedure

Tramadol was administered (dose range: 1–40 mg/kg, p.o.) or vehicle 1 h before the FST in mice. The dose of 40 mg/kg was chosen based on previous published study (Jesse et al., 2009). To test the hypothesis that the antidepressant-like effect of tramadol is mediated through an interaction with the noradrenergic system, animals were pretreated with AMPT (100 mg/kg, i.p., an inhibitor of the enzyme tyrosine hydroxylase) or vehicle 4 h before tramadol administration (40 mg/kg, p.o.) (Brocardo et al., 2008; Kaster et al., 2007; Machado et al., 2007).

In a separate series of experiments, animals were pretreated with prazosin (1 mg/kg, i.p., an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist) or vehicle and after 1 h, they received tramadol (40 mg/kg, p.o.) and were tested in the FST.

The involvement of the imidazoline receptors in the anti-immobility effects of tramadol (40 mg/kg, p.o.) in the FST was investigated with mice pretreated with efaroxan (1 mg/kg, i.p., an imidazoline I_1/α_2-adrenoceptor antagonist), idazoxan (0.06 mg/kg, i.p., an imidazoline I_2/α_2-adrenoceptor antagonist), antazoline (5 mg/kg, i.p., a ligand with high affinity for the imidazoline I_2 receptor) or vehicle. After 1 h, they received an oral administration of tramadol (40 mg/kg, p.o.) or vehicle before the FST (Zeidan et al., 2007).

To assess the possible involvement of the dopaminergic or adenosinergic system in the antidepressant-like effect of tramadol in the FST, independent groups of animals were pretreated with haloperidol (0.2 mg/kg, i.p., a non selective dopamine receptor antagonist), SCH23390 (0.05 mg/kg, subcutaneous, s.c., a dopamine D_1 receptor antagonist), sulpiride (50 mg/kg, i.p., a dopamine D_2 and D_3 receptor antagonist), caffeine (3 mg/kg, i.p., a nonselective adenosine receptor antagonist) or vehicle (Lobato et al., 2008). After 1 h, they received tramadol (40 mg/kg, p.o.) or vehicle and were tested in the FST.

The open-field test (OFT) was carried out to rule out any psychostimulant effect of tramadol interaction with AMPT, prazosin, yohimbine, efaroxan, idazoxan, antazoline, haloperidol, SCH23390, sulpiride or caffeine. Appropriate vehicle treated groups were tested. The number of crossings and rearings of vehicle and tramadol groups were presented as means of all experiments.

2.4. Open-field test (OFT)

To assess the possible effects of tramadol on the locomotor and exploratory activities, mice were evaluated in the OFT. The open field was made of polywood and surrounded by walls 30 cm in height. The floor of the open field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 09 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 6 min to record the locomotor (number of segments crossed with the four paws) and exploratory activities (expressed by the number of time rearing on the hind limbs) (Walsh and Cummins, 1976).

2.5. Forced swimming test (FST)

The test was conducted using the method described by Porsolt et al. (1977), with minor modifications. Briefly, mice were individually forced to swim in open cylinders (25 cm height × 10 cm diameter) containing 19 cm of water at 25 ± 1 °C. The duration of immobility was scored during the 6 min test period as described previously (Zomkowski et al., 2006). Each mouse was recorded as immobile when floating motionless or making only those movements necessary to keep its head above water.

2.6. Ex vivo monoamine oxidase (MAO) assay

Mice were pretreated with tramadol (dose range: 1–40 mg/kg, p.o.) or vehicle after 1 h, animals were killed and the whole brain or specific brain regions implicated in the pathogenesis of depression (hippocampus, cortex and striatum) were removed for the determination of MAO activity.

2.6.1. Mitochondria preparation

A preparation of brain mitochondria was used for MAO assay (Soto-Otero et al., 2001). Cerebral cortices were immediately removed and washed in ice-cold isolation medium (Na_2PO_4/KH_2PO_4 isotonized with sucrose, pH 7.4). Cerebral mitochondria were then obtained by differential centrifugation. Briefly, after removing blood

vessels and pial membranes, cerebral cortices were manually homogenized with four volumes (w/v) of the isolation medium. Then, the homogenate was centrifuged at 900×*g* for 5 min at 4 °C. The supernatant was centrifuged at 12,500×*g* for 15 min. The mitochondria pellet was then washed once with isolation medium and recentrifuged under the same conditions. Finally, the mitochondrial pellet was reconstituted in a buffer solution (Na_2PO_4/KH_2PO_4 isotonized with KCl, pH 7.4) and stored in aliquots.

2.6.2. Enzyme assay

MAO activity was determined by Krajl (1965) with some modifications (Matsumoto et al., 1984). An aliquot of 200 µl of samples was incubated at 37 °C for 5 min in a medium containing buffer solution and specific inhibitors, selegiline (a MAO-B inhibitor, 250 nM) or clorgiline (a MAO-A inhibitor, 250 nM) at a final volume of 700 µl. Then, 20 µl of kynuramine dihydrobromide was added to the reaction mixture (90 µM (MAO-A) and 60 µM (MAO-B)) as substrate. Samples were then incubated at 37 °C for 30 min. After incubation, the reaction was terminated by adding 300 µl of 10% tricholor acetic acid. After cooling and centrifugation at 3000×*g* for 15 min, an aliquot of 800 µl of the supernatant was added to 1 ml of 1 M NaOH. The fluorescence intensity was detected spectrofluorimetrically with excitation at 315 nm and emission at 380 nm. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline. MAO activity was expressed as nmol of 4-hydroxyquinoline formed/mg protein.

The protein concentration was measured using bovine serum albumin as the standard (Bradford, 1976).

2.7. Statistical analysis

All experimental results are given as the mean ± S.E.M. Comparisons between experimental and control groups were performed by one-way (tramadol effect and MAO activity) or two-way ANOVA (AMPT, prazosin, yohimbine, efaroxan, idazoxan, antazoline, haloperidol or caffeine X tramadol) followed by Newman–Keuls test for post hoc comparison when appropriate. A value of $P<0.05$ was considered to be significant. Main effects of first order interactions are presented only when interaction was not significant.

3. Results

3.1. Effect caused by tramadol on MAO-A and MAO-B activities in mice

One-way ANOVA of MAO-A ($F(4,15)=1.44$, $P<0.27$) (Fig. 1A) and MAO-B ($F(4,15)=0.89$, $P<0.49$) (Fig. 1B) activities indicated that tramadol (dose range: 1–40 mg/kg, p.o.) did not change the activities in whole brain of mice.

One-way ANOVA of MAO-A ($F(1,6)=0.01$, $P<0.9312$) and MAO-B ($F(1,6)=0.06$, $P<0.8119$) data showed that tramadol (40 mg/kg, p.o.) did not change the enzyme activities in cortex of mice (Table 1).

One-way ANOVA of MAO-A ($F(1,6)=0.05$, $P<0.8251$) and MAO-B ($F(1,6)=0.16$, $P<0.7018$) activities demonstrated that tramadol (40 mg/kg, p.o.) had no effect on the enzymatic activities in hippocampus of mice (Table 1).

One-way ANOVA of MAO-A ($F(1,6)=0.39$, $P<0.5605$) and MAO-B ($F(1,6)=1.81$, $P<0.2272$) data showed that tramadol (40 mg/kg, p.o.) did not alter activities in striatum of mice (Table 1).

3.2. Mechanisms involved in the antidepressant-like effect of tramadol on mice evaluated in the FST

Results depicted in Fig. 2A demonstrate that the pretreatment of mice with prazosin (1 mg/kg, i.p., an α_1-adrenoceptor antagonist) did not reverse the reduction in immobility time elicited by tramadol (40 mg/kg, p.o.) in the FST ($F(1,24)=0.01$, $P<0.937$). No significant effects were observed on the number of crossing ($F(1,24)=0.01$, $P=0.971$) and rearing ($F(1,24)=0.01$, $P=0.956$) in the OFT in mice treated with tramadol and prazosin (Table 2).

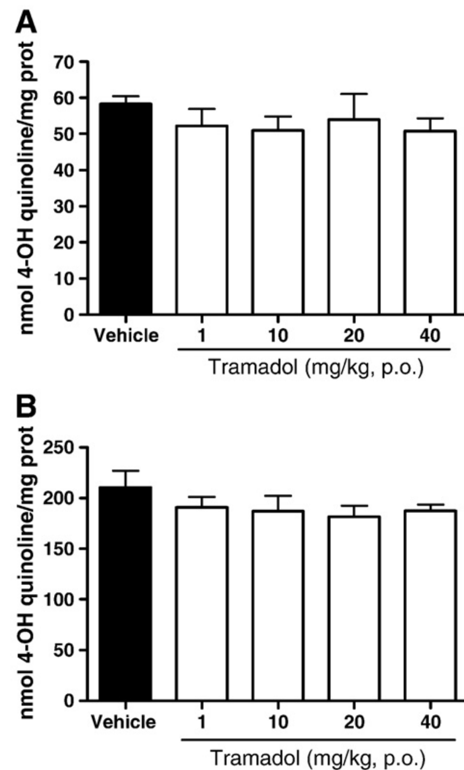


Fig. 1. Effect caused by oral administration of tramadol in MAO-A and MAO-B activities. Mice were pretreated with tramadol (1–40 mg/kg) or vehicle (saline, p.o.), after 1 h, animals were killed and the whole brain removed for the determination of MAO-A and MAO-B activities. Values are expressed as mean ± S.E.M. of 4 animals.

Fig. 2B shows that the pretreatment with yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist) was effective in reversing the antidepressant-like effect of tramadol (40 mg/kg, p.o.) in the mouse FST ($F(1,24)=21.10$, $P<0.001$). The number of crossing ($F(1,24)=0.59$, $P=0.448$) and rearing in the OFT ($F(1,24)=0.21$, $P=0.655$) were unmodified by tramadol and yohimbine administration (Table 2).

Fig. 2C shows that the pretreatment of mice with AMPT (100 mg/kg, i.p., an inhibitor of tyrosine hydroxylase) prevented the antidepressant-like effect of tramadol (40 mg/kg, p.o.) in the mouse FST ($F(1,24)=15.29$, $P<0.001$). Administration of tramadol and AMPT did not modify the number of crossing ($F(1,24)=0.13$, $P=0.723$) and rearing ($F(1,24)=0.01$, $P=0.926$) in the OFT (Table 2).

Table 1
Effect of oral administration of tramadol on MAO-A and MAO-B activities in cortex, hippocampus and striatum of mice.

Tissue	Treatment	MAO-A	MAO-B
Cortex			
	Vehicle	58.79 ± 6.50	143.80 ± 23.67
	Tramadol	58.93 ± 7.63	151.80 ± 21.49
Hippocampus			
	Vehicle	57.04 ± 8.77	124.30 ± 22.67
	Tramadol	54.52 ± 6.53	113.90 ± 12.60
Striatum			
	Vehicle	65.38 ± 10.52	137.70 ± 13.07
	Tramadol	70.80 ± 7.76	150.30 ± 13.04

The mice were pretreated with tramadol (40 mg/kg) or vehicle (saline, p.o.), after 1 h, animals were killed and the specific regions of brain were removed for the determination of MAO-A and MAO-B activities. Values are expressed as nmol 4-OH quinoline/mg protein, mean ± S.E.M. of 4 animals.

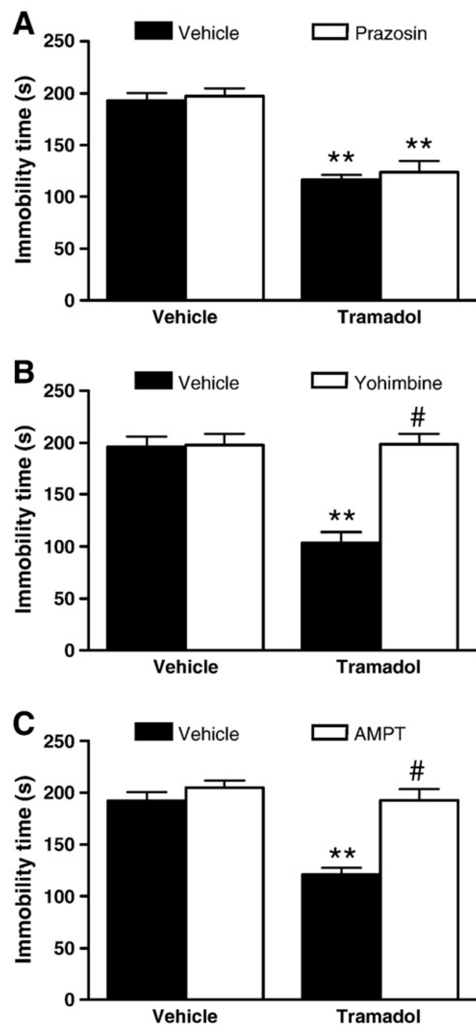


Fig. 2. Effect of pretreatment with prazosin (1 mg/kg, i.p., panel A), yohimbine (1 mg/kg, i.p., panel B) or AMPT (100 mg/kg, panel C) on the action of a dose of tramadol (40 mg/kg, p.o.) in the mouse FST. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by two-way analysis of variance (ANOVA) followed by Newman–Keuls test. **$P<0.01$ compared to the vehicle group and #$P<0.01$ compared to the tramadol group pretreated with vehicle.

Fig. 3A demonstrates that the pretreatment of mice with efaroxan (1 mg/kg, i.p., an imidazoline I_1/α_2-adrenoceptor antagonist) blocked the effect of tramadol (40 mg/kg, p.o.) in the mouse FST ($F(1,24) = 11.50$, $P<0.002$). No significant effects were observed on the number of crossing ($F(1,24) = 0.34$, $P = 0.563$) and rearing ($F(1,24) = 0.04$, $P = 0.850$) in the OFT in mice treated with tramadol and efaroxan (Table 2). Fig. 3B shows that the pretreatment with idazoxan (0.06 mg/kg, i.p., an imidazoline I_2/α_2-adrenoceptor antagonist) prevented the action of tramadol (40 mg/kg, p.o.) in the FST ($F(1,24) = 11.44$, $P<0.002$). The combined administration of tramadol with idazoxan did not produce any effect on the number of crossing ($F(1,24) = 0.29$, $P = 0.597$) and rearing ($F(1,24) = 0.58$, $P = 0.454$) in the OFT in mice (Table 2). Results depicted in Fig. 3C show that the pretreatment of mice with antazoline (5 mg/kg, i.p., a ligand with high affinity for the imidazoline I_2 receptor) was effective in reversing the antidepressant-like effect of tramadol (40 mg/kg, p.o.) in the FST ($F(1,24) = 11.44$, $P<0.002$). No significant differences were observed in the number of crossing ($F(1,24) = 0.58$, $P = 0.545$) and rearing ($F(1,24) = 0.29$, $P = 0.597$) when mice received tramadol and antazoline (Table 2).

As shown in Fig. 4A, pretreatment of mice with haloperidol (0.2 mg/kg, i.p., a non selective dopamine receptor antagonist) prevented the effect of tramadol (40 mg/kg, p.o.) in the FST ($F(1,24) = 7.83$, $P<0.001$). The number of crossing ($F(1,24) = 0.07$, $P = 0.787$) and rearing ($F(1,24) = 0.28$, $P = 0.602$) in the OFT was unmodified by tramadol and haloperidol administration (Table 2). Results depicted in Fig. 4B show that the pretreatment of mice with SCH23390 (0.05 mg/kg, s.c., a dopamine D_1 receptor antagonist) blocked the reduction in immobility time elicited by tramadol (40 mg/kg, p.o.) in the mouse FST ($F(1,24) = 20.45$, $P<0.001$). No significant effects were observed on the number of crossing ($F(1,24) = 0.70$, $P = 0.409$) and rearing ($F(1,24) = 0.03$, $P = 0.864$) in the OFT in mice that received tramadol and SCH23390 (Table 2). Fig. 4C shows that the pretreatment with sulpiride (50 mg/kg, i.p., a dopamine D_2 and D_3 receptor antagonist) reversed the antidepressant-like effect of tramadol (40 mg/kg, p.o.) in the FST ($F(1,24) = 9.89$, $P<0.001$). The number of crossing ($F(1,24) = 1.08$, $P = 0.309$) and rearing ($F(1,24) = 0.03$, $P = 0.872$) in the OFT was unmodified by tramadol and sulpiride administration (Table 2).

Table 2
Effect of tramadol, prazosin, yohimbine, AMPT, efaroxan, idazoxan, antazoline, haloperidol, SCH23390, sulpiride and caffeine on the number of crossings and rearings in the OFT.

Treatment	Number of crossings	Number of rearings
Control	79.84 ± 2.35	43.24 ± 1.63
Tramadol	80.20 ± 2.22	44.34 ± 1.62
Prazosin	86.00 ± 5.84	43.00 ± 4.05
Prazosin + tramadol	85.14 ± 7.13	45.29 ± 4.70
Yohimbine	83.86 ± 6.52	45.29 ± 5.51
Yohimbine + tramadol	83.86 ± 8.21	41.14 ± 4.02
AMPT	78.29 ± 7.02	41.00 ± 4.22
AMPT + tramadol	84.71 ± 8.05	42.43 ± 5.23
Efaroxan	76.43 ± 10.02	43.29 ± 4.59
Efaroxan + tramadol	74.00 ± 7.65	37.29 ± 4.95
Idazoxan	75.14 ± 6.76	40.00 ± 2.13
Idazoxan + tramadol	72.71 ± 8.30	44.23 ± 5.25
Antazoline	77.29 ± 8.52	41.71 ± 6.72
Antazoline + tramadol	82.57 ± 8.03	44.14 ± 4.09
Haloperidol	68.43 ± 10.37	45.86 ± 6.39
Haloperidol + tramadol	86.14 ± 10.01	40.57 ± 4.17
SCH23390	81.43 ± 9.50	44.43 ± 3.64
SCH23390 + tramadol	91.57 ± 7.81	41.29 ± 4.99
Sulpiride	83.43 ± 9.65	49.86 ± 6.98
Sulpiride + tramadol	73.29 ± 10.98	48.14 ± 4.39
Caffeine	73.71 ± 7.74	41.86 ± 5.04
Caffeine + tramadol	70.00 ± 5.42	39.29 ± 4.34

Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by two-way analysis of variance (ANOVA) followed by Newman–Keuls test.

The anti-immobility effect of tramadol (40 mg/kg, p.o.) was not prevented by pretreatment of mice with caffeine (3 mg/kg, i.p., a nonselective adenosine receptor antagonist; Fig. 5) ($F(1,24) = 0.001$, $P = 0.944$). No significant effects were observed on the number of crossing ($F(1,24) = 0.12$, $P = 0.729$) and rearing ($F(1,24) = 0.11$, $P = 0.747$) in the OFT when mice received tramadol and caffeine (Table 2).

4. Discussion

The antidepressant-like effect of tramadol in the FST in mice (Rojas-Corrales et al., 1998, Jesse et al., 2009) and rats (Rojas-Corrales et al., 2002, Jesse et al., 2008) has been reported. In addition to the effect on the animal's behavior, case reports and case series have illustrated a clinically antidepressant effect of tramadol in depressive states (Spencer, 2000), including resistant depression (Shapira et al., 2001). Although the antidepressant-like effect of tramadol is explained by its ability to modulate opioid receptors (Rojas-Corrales et al., 1998), the serotonergic system (Berrocoso et al., 2006, Yalcin et al., 2008), the L-arginine-nitric oxide-cyclic guanosine monophosphate pathway (Jesse et al., 2008) and potassium channels (Jesse et al., 2009) might also account for its action. The main finding from this study is the demonstration that noradrenergic (α_2-adrenoceptor), dopaminergic

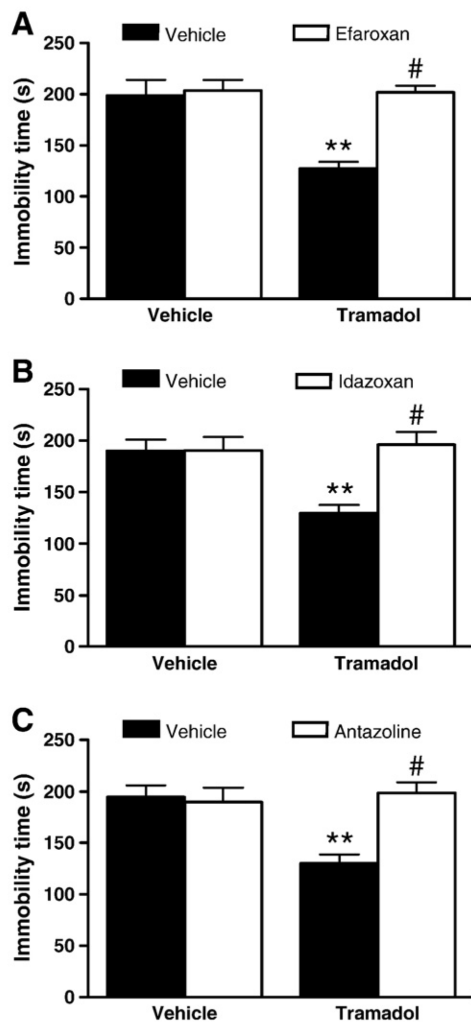


Fig. 3. Effect of pretreatment with efaroxan (1 mg/kg, i.p., panel A), idazoxan (0.06 mg/kg, i.p., panel B) or antazoline (5 mg/kg, i.p., panel C) on the action of a dose of tramadol (40 mg/kg, p.o.) in the mouse FST. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by two-way analysis of variance (ANOVA) followed by Newman–Keuls test. **$P < 0.01$ compared to the vehicle group and #$P < 0.01$ compared to the tramadol group pretreated with vehicle.

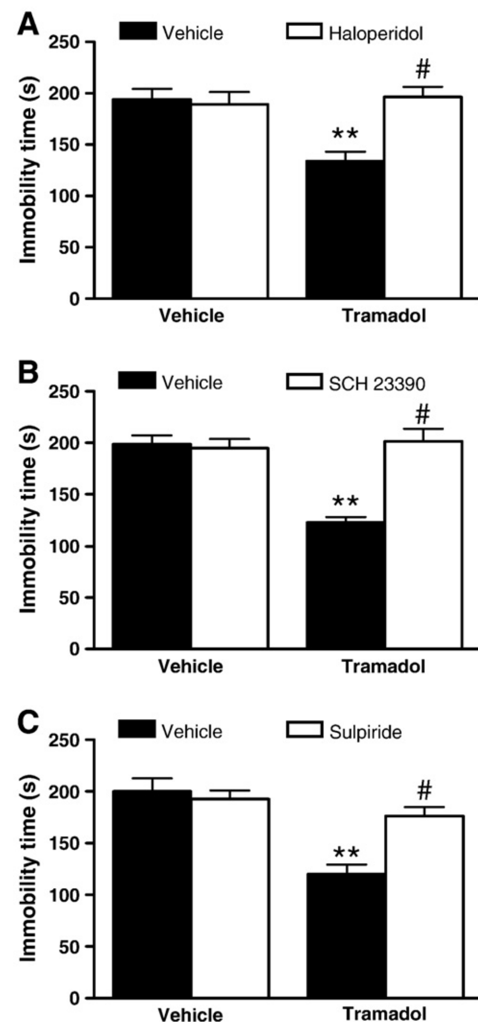


Fig. 4. Effect of pretreatment with haloperidol (0.2 mg/kg, i.p., panel A), SCH23390 (0.05 mg/kg, s.c., panel B) or sulpiride (50 mg/kg, i.p., a dopamine D_2 and D_3 receptor antagonist, panel C) on the action of a dose of tramadol (40 mg/kg, p.o.) in the mouse FST. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by two-way analysis of variance (ANOVA) followed by Newman–Keuls test. **$P < 0.01$ compared to the vehicle group and #$P < 0.01$ compared to the tramadol group pretreated with vehicle.

(D_1 and D_2 receptors) and imidazoline (I_1 and I_2 receptors) systems are related to the antidepressant-like effect of tramadol in mice.

An interesting finding of the current study was that oral treatment with tramadol neither inhibited MAO-A or MAO-B activities in the whole brain nor in specific brain regions, implicated in the pathogenesis of depression (hippocampus, cortex and striatum), of mice. Thus, we can suggest that MAO, an enzyme responsible for the metabolism of biologically important active amines (Krajl, 1965), is not implicated in the antidepressant-like effect caused by tramadol on the mouse FST. However, we cannot rule out the possibility that a chronic treatment with tramadol affects MAO-A and MAO-B activities.

Most of the antidepressants currently used exert their primary neurochemical effects by regulating synaptic concentrations of NA and DA (Brunello et al., 2003). In order to assess the importance of NA and/or DA pool in the antidepressant-like effect of tramadol, a selective inhibitor of tyrosine hydroxylase, AMPT, was used. Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of both NA and DA (Widerlov and Lewander, 1978). Mayorga et al. (2001) have demonstrated that AMPT reduces DA and NA levels (57 and 53%) in mice, without affecting the levels of 5-HT. In the present study, pretreatment of mice with AMPT (100 mg/kg, i.p.) prevented the anti-immobility effect of tramadol in the FST. It is well established that some antidepressant drugs increase the synaptic concentration of NA and some of these drugs were found to act directly at noradrenergic receptors (Elhwuegi, 2004).

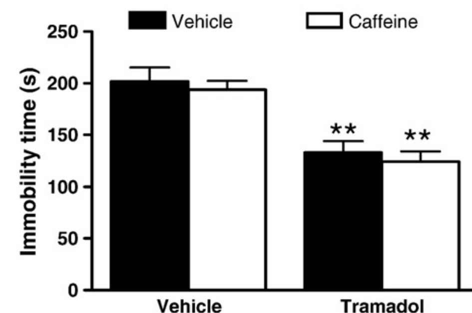


Fig. 5. Effect of pretreatment with caffeine (3 mg/kg, i.p.) on the action of a dose of tramadol (40 mg/kg, p.o.) in the mouse FST. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by two-way analysis of variance (ANOVA) followed by Newman–Keuls test. **$P < 0.01$ compared to the vehicle group and to the vehicle group pretreated with caffeine.

The decrease in the immobility time elicited by tramadol was reversed by pretreatment of mice with yohimbine. This effect should be linked to its α_2 antagonist properties, since the α_1 antagonist prazosin was uneffective; this is unexpected on account of the known antidepressant effect of either mianserin or mirtazapine. Mirtazapine, a new piperazinoazepine antidepressant, has chemical structure similar to mianserin but the mechanism of therapeutic action differs (De Boer and Ruigt, 1995; De Boer, 1996; Kelder et al., 1997). Both drugs have strong binding affinities for presynaptic α_2-adrenergic auto- and hetero-receptors but in contrast to mianserin, mirtazapine has little affinity for α_1 receptors. The reversed effect of yohimbine on antidepressant-like action of tramadol has been also demonstrated in an unpredictable chronic mild stress model in mice (Yalcin et al., 2005).

Results demonstrated that haloperidol, a non selective dopamine receptor antagonist, SCH23390, a selective dopamine D_1 receptor antagonist, and sulpiride, a dopamine D_2 and D_3 receptor antagonist, significantly antagonized the anti-immobility effect of tramadol in mice. A role for DA deficiency in the pathophysiology of depression is supported by studies demonstrating reduced levels of DA and its metabolite homovanillic acid in depressed and/or suicidal patients compared to normal individuals (Kapur and Mann, 1992; Reddy et al., 1992; Papakostas, 2006). Depressed patients have been reported regarding increased DA D_1/D_2 receptor binding (Shah et al., 1997) and reduced DA transporter activity (Neumeister et al., 2001). Pharmacological experiments suggest that the DA-like receptors play a key role in the response to biological antidepressant treatments (Maj et al., 1996; Rogoz and Dziedzicka-Wasylevska, 1999; Lammers et al., 2000).

In the present study, the anti-immobility effect elicited by tramadol in the FST was blocked by the pretreatment of mice with efaroxan (a preferential imidazoline I_1 receptor antagonist) and idazoxan, a preferential imidazoline I_2 receptor antagonist, suggesting the involvement of imidazoline I_1 and I_2 receptors in the antidepressant-like effect of tramadol. Pretreatment of mice with antazoline, a preferential imidazoline I_2 receptor ligand, was also effective in reversing the reduction of immobility time elicited by tramadol in the FST, further reinforcing the notion that its antidepressant-like effect is mediated, at least in part, by an interaction with the imidazoline I_2 receptors.

Clinical depression in human is associated with dysregulation or overexpression of imidazoline receptors (Garcia-Sevilla et al., 1996; Piletz et al., 2008) in platelets and brain tissues of depressed patients (Garcia-Sevilla et al., 1996; Holt, 2003; Piletz et al., 2008). Moreover, chronic treatment with several antidepressants like fluoxetine, citalopram, desipramine, imipramine or bupropion normalized the functioning of imidazoline receptors (Garcia-Sevilla et al., 1996; Zhu et al., 1999; Halaris et al., 2002).

The results of the present study show that the adenosinergic A_1 and A_{2A} receptors are not involved in the antidepressant-like effect of tramadol in the mouse FST, extending the knowledge about the mechanisms underlying the antidepressant-like effects of tramadol (Rojas-Corrales et al., 2002, Jesse et al., 2008, 2009). This assertion is supported by the demonstration that caffeine, an effective antagonist for A_1 and A_{2a} receptors, did not block the anti-immobility effects of tramadol.

Taken together, the results present pharmacological and biochemical evidences supporting the antidepressant-like action of tramadol in the FST. The tramadol antidepressant-like effect may be related to noradrenergic, dopaminergic (D_1 and D_2 receptors) and imidazoline (I_1 and I_2 receptors) mechanisms. Conversely, α_1-adrenoceptor, adenosinergic receptors (A_1 and A_{2A}) and MAO-A and MAO-B activities were not involved in the antidepressant-like effect of tramadol on the mouse FST. Further studies using other selective or highly specific analytical and pharmacological tools are necessary to define more reasonably the role of noradrenergic, dopaminergic and imidazoline systems in the antidepressant-like effect of tramadol.

Acknowledgements

The financial support by the UFSM, FAPERGS, CAPES and CNPq is gratefully acknowledged. This study was supported in part by the FINEP research grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” # 01.06.0842-00.

References

Berrocoso E, Rojas-Corrales MO, Mico JA. Differential role of 5-HT_{1A} and 5-HT_{1B} receptors on the antinociceptive and antidepressant effect of tramadol in mice. Psychopharmacology 2006;188:111–8.
Bousquet P, Bruban V, Schann S, Feldman J. Imidazoline receptors: a challenge. Pharm Acta Helv 2000;74:205–9.
Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
Brocardo PS, Budni J, Kaster MP, Santos AR, Rodrigues AL. Folic acid administration produces an antidepressant-like effect in mice: evidence for the involvement of the serotonergic and noradrenergic systems. Neuropharmacology 2008;54:464–73.
Brunello N, Blier P, Judd LL, Mendlewicz J, Nelson CJ, Souery D, et al. Noradrenaline in mood and anxiety disorders: basic and clinical studies. Int Clin Psychopharmacol 2003;18:191–202.
De Boer T. The pharmacologic profile of mirtazapine. J Clin Psychiatry 1996;57:19–25.
De Boer T, Ruigt GSF. The selective α_2 adrenoceptor antagonist mirtazapine (Org 3770) enhances noradrenergic and 5-HT_{1A} mediated serotonergic neurotranmission. CNS Drugs 1995;4:18–28.
Deniker P. The search for new antidepressant and related drugs. In: Tipton KF, Dostert D, Benedetti MS, editors. Monoamine oxidase and diseases, prospects for therapy with reversible inhibitors. New York, NY: Academic press; 1984. p. 3–8.
El Yacoubi M, Ledent C, Parmentier M, Bertorelli R, Ongini E, Costentin J. Adenosine A_{2A} receptor antagonists are potential antidepressants: evidence based on pharmacology and A_{2A} receptor knockout mice. Br J Pharmacol 2001;134:68–77.
Elhwuegi AS. Central monoamines and their role in major depression. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:435–51.
Faron-Gorecka A, Kusmider M, Inan SY, Siwanowicz J, Dziedzicka-Wasylewska M. Effects of tramadol on alpha2-adrenergic receptors in the rat brain. Brain Res 2004;1016:263–7.
Garcia-Sevilla JA, Escriba PV, Sastre M, Walzer C, Busquets X, Jaquet G. Immunodetection and quantitation of imidazoline receptor proteins in platelets of patients with major depression and in brains of suicide victims. Arch Gen Psychiatry 1996;53: 803–10.
Halaris A, Zhu H, Ali J, Nasrallah A, De Vane CL, Piletz JE. Downregulation of platelet imidazoline-1-binding sites after bupropion treatment. Int J Neuropsychopharmacol 2002;5:37–46.
Hennies HH, Friderichs E, Schneider J. Receptor binding, analgesic and antitussive potency of tramadol and other selected opioids. Arzneimittelforschung 1988;38: 877–80.
Holt A. Imidazoline binding sites on receptors and enzymes: emerging targets for novel antidepressant drugs? J Psychiatry and Neurosci 2003;28:409–14.
Hopwood SE, Owesson CA, Callado LF, McLaughlin DP, Stamford JA. Effects of chronic tramadol on pre- and post-synaptic measures of monoamine function. J Psychopharmacol 2001;15:147–53.
Jesse CR, Bortolatto CF, Savegnago L, Rocha JB, Nogueira CW. Involvement of L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect of tramadol in the rat forced swimming test. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:1838–43.
Jesse CR, Wilhelm EA, Barbosa NB, Nogueira CW. Involvement of different types of potassium channels in the antidepressant-like effect of tramadol in the mouse forced swimming test. Eur J Pharmacol 2009;613:74–8.
Kapur S, Mann JJ. Role of the dopaminergic system in depression. Biol Psychiatry 1992;32:1-17.
Kaster MP, Santos AR, Rodrigues AL. Involvement of 5-HT_{1A} receptors in the antidepressant-like effect of adenosine in the mouse forced swimming test. Brain Res Bull 2004;67:53–61.
Kaster MP, Rosa AO, Santos AR, Rodrigues AL. Involvement of nitric-oxide–cGMP pathway in the antidepressant-like effect of adenosine in the forced swimming test. Int J Neuropsychopharmacol 2005;8:601–6.
Kaster MP, Raupp I, Binfare RW, Andreatini R, Rodrigues AL. Antidepressant-like effect of lamotrigine in the mouse forced swimming test: evidence for the involvement of the noradrenergic system. Eur J Pharmacol 2007;565:119–24.
Kelder J, Funke C, De Boer T, Delbressine L, Leysen D, Nicholson V. A comparison of the physicochemical and biological properties of mirtazapine and mianserin. J Pharm Pharmacol 1997;49:403–11.
Krajl M. A rapid microfluorimetric determination of monoamine oxidase. Biochem Pharmacol 1965;14:1684–6.
Lammers CH, Diaz J, Schwartz JC, Sokoloff P. Selective increase of dopamine D_3 receptor gene expression as a common effect of chronic antidepressant treatments. Mol Psychiatry 2000;5:378–88.
Lanni C, Govoni S, Lucchelli A, Cinzia B. Depression and antidepressants: molecular and cellular aspect. Cell Mol Life Sci 2009;66:2985–3008.
Lobato KR, Binfare RW, Budni J, Rosa AO, Santos AR, Rodrigues AL. Involvement of the adenosine A_1 and A_{2A} receptors in the antidepressant-like effect of zinc in the

forced swimming test. Prog Neuropsychopharmacol Biol Psychiatry 2008;32: 994–9.
Machado DG, Kaster MP, Binfare RW, Dias M, Santos AR, Pizzolatti MG, et al. Antidepressant-like effect of the extract from leaves of *Schinus molle* L. in mice: evidence for the involvement of the monoaminergic system. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:421–8.
Maj J, Dziedzicka-Wasylewska M, Rogoz R, Rogoz Z, Skuza G. Antidepressant drugs given repeatedly change the binding of the dopamine D_2 receptor agonist, $^{[3H]}$N-0437, to dopamine D_2 receptors in the rat brain. Eur J Pharmacol 1996;304:49–54.
Matsumoto T, Furuta T, Nimura Y, Suzuki O. 3-(p-hydroxyphenyl)propionic acid as a new fluorogenic reagent for amine oxidase assays. Anal Biochem 1984;138:133–6.
Mayorga AJ, Dalvi A, Page ME, Zimov-Levinson S, Hen R, Lucki I. Antidepressant-like behavioral effects in 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) receptor mutant mice. J Pharmacol Exp Ther 2001;298:1101–7.
Millan MJ. The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. Eur J Pharmacol 2004;500:371–84.
Neumeister A, Willeit M, Praschak-Rieder N, Asenbaum S, Stastny J, Hilger E, et al. Dopamine transporter availability in symptomatic depressed patients with seasonal affective disorder and healthy controls. Psychol Med 2001;31:1467–73.
O´Leary OF, Bechtholt AJ, Bechtholt AJ, Crowley JJ, Hill TE, Page ME, et al. Depletion of serotonin and catecholamines block the acute behavioral response to different classes of antidepressant drugs in the mouse tail suspension test. Psychopharmacology 2007;192:357–71.
Palucha A, Pilc A. On the role of metabotropic glutamate receptors in the mechanisms of action of antidepressants. Pol J Pharmacol 2002;54:581–6.
Papakostas GI. Dopaminergic-based pharmacotherapies for depression. Eur Neuropsychopharmacol 2006;16:391–402.
Piletz JE, Baker R, Halaris A. Platelet imidazoline receptors as state marker of depressive symptomatology. J Psychiatr Res 2008;42:41–9.
Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730–2.
Prange Jr A. Proceedings: behavioral effects of hypothalamic polypeptides in animals and man. Psychopharmacol Bull 1974;10:11–5.
Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J Pharmacol Exp Ther 1992;260:275–85.
Reddy PL, Khanna S, Subhash MN, Channabasavanna SM, Rao BS. CSF amine metabolites in depression. Biol Psychiatry 1992;31:112–8.
Regunathan S, Reis DJ. Imidazoline receptors and their endogenous ligands. Annu Rev Pharmacol Toxicol 1996;36:511–44.
Rogoz R, Dziedzicka-Wasylevska M. Effects of antidepressant drugs on dopamine D_2/D_3 receptors in the rat brain differentiated by agonist and antagonist binding—an autoradiographic analysis. Naunyn Schmiedebergs Arch Pharmacol 1999;359: 178–86.
Rojas-Corrales MO, Gibert-Rahola J, Mico JA. Tramadol induces antidepressant-type effects in mice. Life Sci 1998;63:PL175–80.
Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Mico JA. Antidepressant-like effects of tramadol and other central analgesics with activity on monoamines reuptake, in helpless rats. Life Sci 2002;72:143–52.
Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Mico JA. Antidepressant-like effect of tramadol and its enantiomers in reserpinized mice: comparative study with desipramine, fluvoxamine, venlafaxine and opiates. J Psychopharmacol 2004;18: 404–11.
Shah BH, Saeed SA, Rashid F, Gilani AH. Dopamine potentiation of calcium ionophore, A-23187-induced platelet aggregation. Adv Exp Med Biol 1997;407:537–40.
Shapira NA, McConville BJ, Pagnucco ML, Norman AB, Keck Jr PE. Novel use of tramadol hydrochloride in the treatment of Tourette's syndrome. J Clin Psychiatry 1997;58: 174–5.
Shapira NA, Verduin ML, DeGraw JD. Treatment of refractory major depression with tramadol monotherapy. J Clin Psychiatry 2001;62:205–6.
Smith KL, Jessop DS, Finn DP. Modulation of stress by imidazoline binding sites: implications for psychiatric disorders. Stress 2009;12:97-114.
Soto-Otero R, Mendez-Alvarez E, Hermida-Ameijeiras A, Sanchez-Sellero I, Cruz-Landeira A, Lamas ML. Inhibition of brain monoamine oxidase activity by the generation of hydroxyl radicals: potential implications in relation to oxidative stress. Life Sci 2001;69:879–89.
Spencer C. The efficacy of intramuscular tramadol as a rapid-onset antidepressant. Aust N Z J Psychiatry 2000;34:1032–3.
Walsh RN, Cummins RA. The open-field test: a critical review. Psychol Bull 1976;83: 482–504.
Widerlov E, Lewander T. Tolerance to alpha-methyl-p-tyrosine in rats: studies on the antagonism of amphetamine-induced motor activity and excitatory behaviour. Psychopharmacology (Berl) 1978;60:41–5.
Yalcin I, Aksur F, Belzung C. Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. Eur J Pharmacol 2005;514:165–74.
Yalcin I, Coubard S, Bodard S, Chalon S, Belzung C. Effects of 5, 7-dihydroxytryptamine lesion of the dorsal raphe nucleus on the antidepressant-like action of tramadol in the unpredictable chronic mild stress in mice. Psychopharmacology 2008;200: 497–507.
Zeidan MP, Zomkowski AD, Rosa AO, Rodrigues AL, Gabilan NH. Evidence for imidazoline receptors involvement in the agmatine antidepressant-like effect in the forced swimming test. Eur J Pharmacol 2007;565:125–31.
Zhu H, Halaris A, Madakasira S, Pazzaglia P, Goldman N, De Vane CL. Effect of bupropion on immunodensity of putative imidazoline receptors on platelets of depressed patients. J Psychiatr Res 1999;33:323–33.
Zomkowski AD, Santos AR, Rodrigues AL. Putrescine produces antidepressant-like effects in the forced swimming test and in the tail suspension test in mice. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:1419–25.