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European Journal of Pharmacology 514 (2005) 165 – 174

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Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol

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Received 11 November 2004; received in revised form 17 February 2005; accepted 21 March 2005
Available online 4 May 2005

Abstract

This study investigated the antidepressant-like effects of a chronic treatment with either tramadol (20 mg/kg, i.p.) or desipramine (10 mg/kg, i.p.) in the unpredictable chronic mild stress model of depression in BALB/c mice. Mice were first submitted to a 2 week drug-free unpredictable chronic mild stress before the onset of the treatments. The unpredictable chronic mild stress regimen induced a degradation of the state of the coat and decreased the grooming behaviour in the splash test. These physical and behavioural abnormalities were counteracted by tramadol and desipramine. Furthermore, we observed neither a significant acceleration nor diminution by pindolol (5-$HT_{1A/1B}$ receptor antagonist, 10 mg/kg, i.p.) on the antidepressant-like actions of desipramine and tramadol whereas yohimbine (α_2-adrenergic receptor antagonist, 2 mg/kg, i.p.) antagonized the antidepressant-like effects of both drugs during the unpredictable chronic mild stress regimen.

The results of the study support the suggestion that antidepressant-like effect of tramadol and desipramine in mice in the unpredictable chronic mild stress model is mediated by the noradrenergic system rather than the serotonergic system.

Keywords: Unpredictable chronic mild stress; Tramadol; Desipramine; Pindolol; Yohimbine

1. Introduction

Tramadol is a centrally acting (Friderichs et al., 1978) and clinically effective analgesic, which is used mainly for the treatment of moderate or severe pain (Sindrup et al., 1999). It has a relatively weak opioid receptor affinity (Hennies et al., 1988) and selectivity for μ-subtype with a Ki in the micromolar range (Raffa et al., 1992). It has been shown that tramadol enhances the extraneuronal concentrations of the monoamine neurotransmitters, noradrenaline and serotonin, by interfering with the reuptake and release mechanisms (Driessen et al., 1993; Raffa et al., 1992). Unlike other opioid receptor agonists, tramadol is a racemic mixture of two enantiomers, each one having distinct but complementary mechanisms of action: (+) tramadol enantiomer is a selective agonist for the μ-opioid receptor which preferentially inhibits serotonin reuptake and enhances serotonin efflux in the brain whereas the (−) enantiomer mainly inhibits noradrenaline reuptake (Frink et al., 1996; Codd et al., 1995). The mechanism of action and structure of tramadol is very similar to that of some antidepressants such as venlafaxine (Markowitz and Patrick, 1998). In addition, tramadol elicits antidepressant-like effects in the forced swimming test in mice (Rojas-Corrales et al., 1998) and in the learned helplessness model in rats (Rojas-Corrales et al., 2002). But to our knowledge, the mechanism of antidepressant-like effect of tramadol still remains unclear.

Among the numerous serotonin and noradrenaline receptors, the 5-HT_{1A} receptors and the α_2-adrenoceptors seem to be particularly remarkable targets on to the action of antidepressants. Santarelli et al. (2003) reported that 5-HT_{1A} receptors are required for fluoxetine, a serotonin selective reuptake inhibitor (SSRI)-induced neurogenesis, a process

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doi:10.1016/j.ejphar.2005.03.029

necessary to its antidepressant effects, but not imipramine-induced neurogenesis. Furthermore, several studies have demonstrated that combination of a 5-HT_{1A} receptor antagonist with SSRIs potentiates the effect of antidepressant drug on the serotonin release (Romero and Artigas, 1997; Blier et al., 1997). On the other hand, the inhibition of neuronal activity of locus coeruleus by the noradrenaline reuptake inhibitors has been interpreted as a consequence of the increased concentration of synaptic noradrenaline, leading to an increased activation of α_2-adrenoceptors (Mongeau et al., 1998; Szabo et al., 2000). Furthermore, it has been shown that the effect of desipramine, a tricyclic antidepressant which preferentially blocks the reuptake of noradrenaline on extracellular noradrenaline in the brain cortex is modulated by α_2-adrenoceptors in the locus coeruleus (Mateo et al., 1998).

The unpredictable chronic mild stress is generally thought to be the most promising and valuable model to study depression in animals, mimicking several human depressive symptoms (Willner, 1997). It has been reported that the unpredictable chronic mild stress regimen decreases the consumption of or preference for a sucrose solution (anhedonia) (Willner et al., 1987) and induces a degradation of the physical state of the coat (Ducottet et al., 2003). However, these degradations can be reversed by the antidepressants such as fluoxetine (Ducottet et al., 2003; Santarelli et al., 2003).

The present study was designed to examine the possible antidepressant-like effects of tramadol and desipramine using the unpredictable chronic mild stress, a model that is more valid in terms of face and construct validity than the other tests such as the forced swimming test, the tail suspension and the learned helplessness model. Moreover, we aimed to clarify the mechanisms underlying the antidepressant-like actions of tramadol and desipramine. For this purpose, we tested the effects of the 5-$HT_{1A/1B}$ antagonist pindolol and the α_2-adrenergic receptor antagonist yohimbine, alone or in combination with either tramadol or desipramine, to reveal the contribution of the serotonergic and the noradrenergic systems on the antidepressant-like actions of these drugs. In order to determine the effects of the unpredictable chronic mild stress regimen and the drug treatment, we used two parameters in our experiments: the coat state and splash test. The state of the coat was used to evaluate grooming behaviour indirectly whereas the splash test determined this behaviour directly (Santarelli et al., 2003).

2. Materials and methods

2.1. Animals

Male, inbred BALB/c ByJ mice obtained from the Centre d'Elevage Janvier were used in this study. Indeed, this strain is very sensitive to the unpredictable chronic mild stress protocol (Ducottet and Belzung, 2005; Pothion et al., 2004; Mineur et al., 2003). They were kept in the laboratory for 2 weeks before the onset of the experiments. All stressed mice were maintained individually under the same standard conditions while non-stressed mice were housed five per cage and kept in regulated environment 24±1 °C, 12/12 light/dark cycle (lights on at 20:00). The dimensions of the home cages are 14 cm height, 29 cm longer and 17 cm larger. All animals received food and water ad libitum. This research was conducted in accordance with the European Community guidelines for the use of experimental animals.

2.2. Experimental groups and drug administration

At the end of 2 weeks of a drug-free stress exposure mice were assigned to the different experimental groups in a semi-randomized manner, so that the initial coat state and body weights were equivalent in all the groups. In experiment 1, we studied the effects of tramadol and desipramine in stressed and non-stressed mice. As no effects of the pharmacological treatments were observed in non-stressed mice, we did not include these non-stressed groups in experiments 2 and 3 which aimed at investigating the mechanisms underlying the actions of desipramine and tramadol in stressed mice. In order to investigate the contribution of α_2-adrenoceptors and 5-$HT_{1A/1B}$ receptors, tramadol and desipramine were combined either with pindolol or with yohimbine. This study included 3 main experimental studies. All main studies consisted of 6 subgroups. Subgroups were as follows in experiment 1: vehicle+vehicle, tramadol+vehicle, desipramine+vehicle for the stressed and non-stressed mice; in experiment 2: vehicle+vehicle, tramadol+vehicle, desipramine+vehicle, pindolol+vehicle, tramadol+pindolol, desipramine+pindolol for stressed mice; and in experiment 3: vehicle+vehicle, tramadol+vehicle, desipramine+vehicle, yohimbine+vehicle, tramadol+yohimbine, desipramine+yohimbine for stressed mice. In order to avoid multiple injections, we mixed the drugs before the administration in a same solution. Each group consisted of 10 stressed mice.

(±)-Tramadol 20 mg/kg, desipramine 10 mg/kg, pindolol 10 mg/kg and yohimbine 2 mg/kg were administered intraperitoneally (i.p.) 2 weeks after the beginning of the unpredictable chronic mild stress regimen. The doses used in the study were selected according to the literature. For yohimbine, we decided to use the dose which had been reported to have no effect on the noradrenaline release.Intraperitoneal injections were given daily at 1:30 p.m. in a volume of 0.1 ml/10 g body weight. Vehicle groups received the same volume of 0.9% NaCl with one drop of Tween 80.

2.3. Unpredictable chronic mild stress model

The unpredictable chronic mild stress regimen used in this study was based on the procedure originally designed

Table 1
Procedure of the unpredictable chronic mild stress model

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1	coat state, weighing (10 h) social stress (12 h)	social stress (9 h)	3 sawdust changing (10 h 30–11 h 30)	social stress (12 h)	damp sawdust (9 h 30–11 h)	reversal of the light/ dark cycle (after 8 h)	reversal of the light/dark cycle
	without sawdust (15–17 h)	sounds of predators (15 h)	social stress (15 h)	cages tilt at 45° (14–15 h 30)	social stress (14 h)	reversal of the light/ dark cycle	reversal of the light/ dark cycle
Week 2	end of reversal cycle (8 h) coat state, weighing (10 h)	without sawdust (9 h 30–12 h 30)+social stress (11 h)	damp sawdust (10–13 h)	social stress (12 h)	dark (5–6 h) 2 sawdust changing (10–11 h)	4 light/dark succession every 30 min (9 h 30–11 h 30)	4 light/dark succession every 30 min (9 h 30–11 h 30)
	sounds of predators (13 h) cages tilt at 45° (14–15 h 30)	cages tilt at 45° (15–16 h 30)	social stress (18 h)	sounds of predators (16 h) light (19–20 h)	social stress (15 h)	light (15–17 h) dark (4–6 h)	light (15–17 h) dark (4–6 h)
Week 3	coat state, weighing (10 h)	cages tilt at 45° (11–14 h)	damp sawdust (8 h 30–11 h 30)+ social stress (10 h)	sounds of predators (10 h)	light (9–9 h 30) sawdust changing (10 h)	reversal of the light/ dark cycle	reversal of the light/dark cycle
	treatment (13 h 30) without sawdust (15–16 h 30)	treatment (13 h 30) light (17–18 h)	treatment (13 h 30) cage changing (14 h 30–17 h 30)	treatment (13 h 30) bath (15min) (15 h)	treatment (13 h 30) without sawdust (14 h 30–17 h 30) Reversal of the light/dark cycle (after 20 h)	treatment (13 h 30) reversal of the light/ dark cycle	treatment (13 h 30) Reversal of the light/dark cycle. End of reversal cycle (after 20 h)
Week 4	coat state, weighing (10 h)	sounds of predators (11 h)	cage changing (9–12 h)	cage changing (9–9 h 30)	sawdust changing (10 h)	light (9–11 h)	end of reversal cycle (after 8 h)
	treatment (13 h 30) cages tilt at 45° (14 h 30–15 h 30) cages tilt at 45° (17–18 h)	treatment (13 h 30) damp sawdust (15–18 h)+ social stress (16 h 30)	treatment (13 h 30) lights (30 min) (15–16 h 30–18 h)	treatment (13 h 30) damp sawdust (14 h 30–17 h 30)	treatment (13 h 30) bath (15 min) (14 h 30)	treatment (13 h 30) reversal of the light/ dark cycle (after 20 h)	treatment (13h 30) Light 30 min (15 h 15–16 h 15–17 h 15–18 h 15–19 h 15)
Week 5	coat state, weighing (10 h)	cages tilt at 45° (10–13 h)	stress social (11 h)	sounds of predators (10 h 30)	dark (5–6 h) bath (15 min) (10 h)	4 light/dark succession every 30 min (9 h 30–11 h 30)	dark (4–6 h) 4 light/dark succession every 30 min (9 h 30–11 h 30)
	treatment (13 h 30) without sawdust (16–17 h 30)	treatment (13 h 30) social stress (15 h) light 30 min (17–18–19 h)	treatment (13 h 30) damp sawdust (14 h 30–17 h 30)	treatment (13 h 30) without sawdust (14 h 30–17 h 30)+ social stress (16–17 h 30)	treatment (13 h 30) 2 sawdust changing (15–16 h)	treatment (13 h 30) light (15–17 h)	treatment (13 h 30) light (15–17 h)
Week 6	dark (4–6 h), coat state, weighing (10 h)	behavioural tests	behavioural tests	behavioural tests	behavioural tests	behavioural tests	behavioural tests
	treatment (13 h 30)	treatment (13 h 30)	treatment (13 h 30)	treatment (13 h 30)	treatment (13 h 30)	treatment (13 h 30)	treatment (13 h 30)

by Willner et al. (1992) and adapted to mice (Ducottet and Belzung, 2004). Mice were subjected several times a day for 6 weeks to one of the following stressors such as damp sawdust, sawdust changing, placement in an empty cage, placement in an empty cage with water on the bottom, switching cages, cage tilting (45 °C), predator sounds for 15 min, inversion of light/dark cycle, lights on for a short time during the dark phase. To prevent habituation and to provide an unpredictable feature to the stressors, all the stressors and/ or sequences were administered at different time points every week (see Table 1). For ethical reason, the stress procedure did not involve food and water deprivation or immobilization.

2.4. Coat state and body weight

Before and during the unpredictable chronic mild stress, the state of coat and the body weights of the animals were recorded every Monday. The evaluation of the coat state was carried out by the assessment of eight different body parts: head, neck, dorsal coat, ventral coat, tail, forepaws, hindpaws and genital region (Ducottet et al., 2003; Ducottet and Belzung, 2004). A score of 0 for a coat in a good state or a score of 1 for a dirty coat were given for each of these areas. Total score was obtained from the sum of the score of each body parts. In all the experiments we present the total score that we obtained the last week of the stress regimen. However, in the second experiment aimed at investigating possible effects of pindolol on the onset of the antidepressant-like action of desipramine and tramadol, we also show the total score of each week.

The observers who scored the state of the coat were unaware of the treatment condition.

2.5. Splash test

At the beginning of the sixth week, the splash test was performed. This test was used to evaluate the grooming behaviour of both stressed and non-stressed mice. Grooming is cleaning the fur of animal by licking or scratching. Grooming bouts were recorded including nose/ face grooming (strokes along the snout), head washing (semicircular movements over the top of the head and behind the ears), body grooming (body fur licking) (Kalueff and Tuohimaa, 2004). An organiser (Psion Organiser Model LZ64, UK) was used to record the frequency of grooming, which refers to the number of licking during 5 min. The observer was unaware of the treatment conditions. For this purpose, 10% sucrose solution was squirted on the dorsal coat of mice in their homecage. The frequency and the duration of grooming were recorded during 5 min after the vaporisation of sucrose solution (Ducottet and Belzung, 2004). We display the number of grooming behaviour as total frequency in the graphics.

2.6. Actograph

Activity was recorded during 4 h using a photo-electric actimeter (Boissier and Simon, 1965). Actograph was used to examine the spontaneous locomotor activity during 4 h. This test allowed evaluating the activity of mice in their homecage excluding the possible effects of new environment on the locomotor activity. The home-cage was placed in the centre of the device, which consisted of 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 the mice movements and elevated above the level of the sawdust in order to permit the date recording. When the mice crossed throughout, the movement of the animal was detected automatically.

2.7. Drugs

We purchased desipramine hydrochloride, pindolol, yohimbine hydrochloride from Sigma. Tramadol was supplied as a gift by Abdi Ibrahim (Istanbul, Turkey). All the drugs were dissolved in 0.9% NaCl with one drop of Tween 80.

2.8. Statistics

Results are expressed as the means ± S.E.M. The results that we determined by observation such as the state of the coat and the frequency of grooming were compared by Kruskal–Wallis H followed by Mann–Whitney U test when significant differences were detected. Statistical analysis were performed to evaluate the locomotor activity and the body weight with one-way ANOVA followed by inter-group pair-wise comparisons with Tukey post hoc test when significant differences were detected. SPSS 10.0 for Windows was used for the statistics analysis. Significance was set at $P<0.05$.

3. Results

3.1. Experiment 1

Fig. 1 illustrates the total score of the coat state 6 weeks after the beginning of the unpredictable chronic mild stress regimen. The test of Kruskal–Wallis H revealed a significant difference between the groups ($H=20.989$, $P=0.001$). We observed a significant difference between non-stressed vehicle and stressed vehicle groups at the end of the unpredictable chronic mild stress regimen ($P=0.001$). Tramadol (20 mg/kg, $P=0.005$) and desipramine (10 mg/kg, $P=0.015$) significantly reversed the degradation on the coat state induced by unpredictable chronic mild stress in stressed mice when compared to vehicle group.

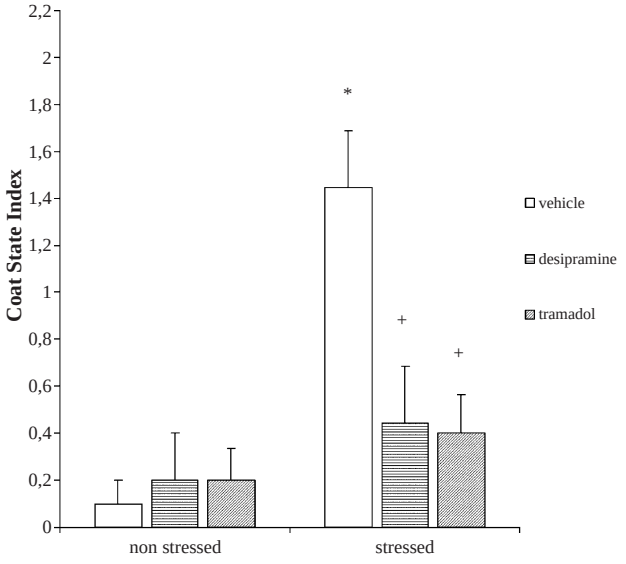

Fig. 1. Effects of desipramine (10 mg/kg, i.p.) and tramadol (20 mg/kg, i.p.) on the coat state in non-stressed and stressed groups after the end of the unpredictable chronic mild stress regimen. All of the treatments begun after 2 weeks of stress regimen and were administered during 4 weeks. *$P<0.05$, significantly different when compared to the non-stressed vehicle (0.9% NaCl+one drop Tween 80, i.p.), $^{+}P<0.05$, significantly different when compared to the stressed vehicle.

The effects of tramadol and desipramine on the grooming frequency are shown in Fig. 2. By the Kruskal–Wallis H test, we observed a significant difference between all the groups ($H=13.504$, $P=0.019$). Non-stressed mice groomed significantly more than stressed mice ($P=0.011$). Both tramadol ($P=0.007$) and desipramine ($P=0.002$) significantly augmented the frequency of the grooming behaviour in stressed mice in the splash test, but they did not elicit any effect in non-stressed mice ($P=0.389$, $P=0.411$, respectively).

We did not observe a significant difference between the body weight of non-stressed mice and stressed mice ($P=0.964$). Furthermore tramadol ($P=0.373$) and desipramine ($P=0.318$) treatment did not change the body weight when they compared with vehicle in stressed mice. Also, no significant impairment of locomotor activity due to unpredictable chronic mild stress regimen or treatment was observed ($F_{(5,52)}=2141$, $P=0.075$). These results are presented in the Table 2.

3.2. Experiment 2

The effect of pindolol (10 mg/kg, i.p.) on the antidepressant-like actions of tramadol and desipramine over the coat state are shown in Fig. 3. This figure illustrates the total score of each week to demonstrate whether pindolol acts on the onset of the antidepressant-like effects of tramadol and desipramine. The test of Kruskal–Wallis H revealed a significant differences between all the groups ($H=13.545$, $P=0.019$). In this experimental group, tramadol ($P=0.001$) and desipramine ($P=0.005$) also significantly reversed the degradation induced by unpredictable chronic mild stress regimen in stressed mice when compared to vehicle group. During the drug treatment regimen every week, we observed no effect on the onset by pindolol on the antidepressant-like actions of tramadol and desipramine. P values for tramadol and desipramine combined with

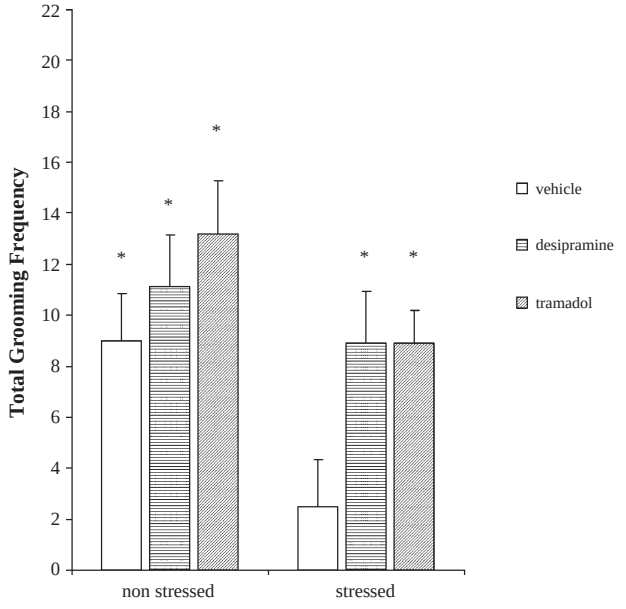

Fig. 2. Effects of a 4 week treatment with desipramine (10 mg/kg, i.p.) and tramadol (20 mg/kg, i.p.) on the total frequency of the grooming behaviour during the splash test after the end of the unpredictable chronic mild stress regimen. *$P<0.05$, significantly different when compared to the stressed vehicle.

Table 2
The effects of drugs on the body weight and locomotor activity

Experiment	Environment	Treatment	Body weight	Locomotor activity
1	Non-stressed	Vehicle	29.25±0.38	3275.10±252.11
1	Non-stressed	Desipramine	27.66±0.60	3350.40±241.43
1	Non-stressed	Tramadol	27.63±0.34	2634.30±328.12
1	Stressed	Vehicle	29.77±0.35	2255.44±283.07
1	Stressed	Desipramine	28.41±0.51	3195.88±379.77
1	Stressed	Tramadol	28.51±0.41	2942.40±228.29
2	Stressed	Vehicle	30.58±0.35	2427.70±309.74
2	Stressed	Pindolol	31.07±0.55	2477.90±317.53
2	Stressed	Desipramine	30.63±0.56	2860.90±257.19
2	Stressed	Desipramine/ Pindolol	29.26±0.53	3185.20±346.83
2	Stressed	Tramadol	29.64±0.57	2444.50±412.46
2	Stressed	Tramadol/ Pindolol	29.32±0.58	2077.70±328.32
3	Stressed	Vehicle	31.10±0.44	2308.70±346.92
3	Stressed	Yohimbine	30.90±0.43	1847.70±244.62
3	Stressed	Desipramine	30.50±0.45	2435.75±348.61
3	Stressed	Desipramine/ Yohimbine	31.05±0.47	2056.70±260.18
3	Stressed	Tramadol	30.95±0.31	2765.11±284.25
3	Stressed	Tramadol/ Yohimbine	31.05±0.47	2151.25±408.28

Results are shown as the means±S.E.M.

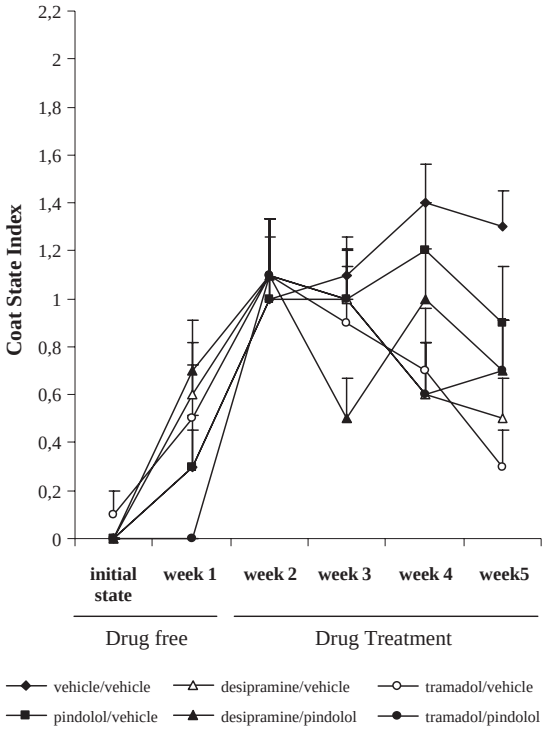

Fig. 3. Effect of pindolol (10 mg/kg, i.p.) on the antidepressant-like actions of desipramine (10 mg/kg, i.p.) and tramadol (20mg/kg, i.p.) over the coat state in stressed mice during the 4 weeks of treatment. Initial state shows the state of the coat before the unpredictable chronic mild stress regimen.

pindolol were: after 1 week drug administration $P=0.618$, $P=0.150$, after 2 weeks $P=0.836$, $P=0.188$ and after 3 weeks $P=0.156$, $P=0.522$, respectively. All the groups except pindolol ($P=0.181$) group significantly diminished

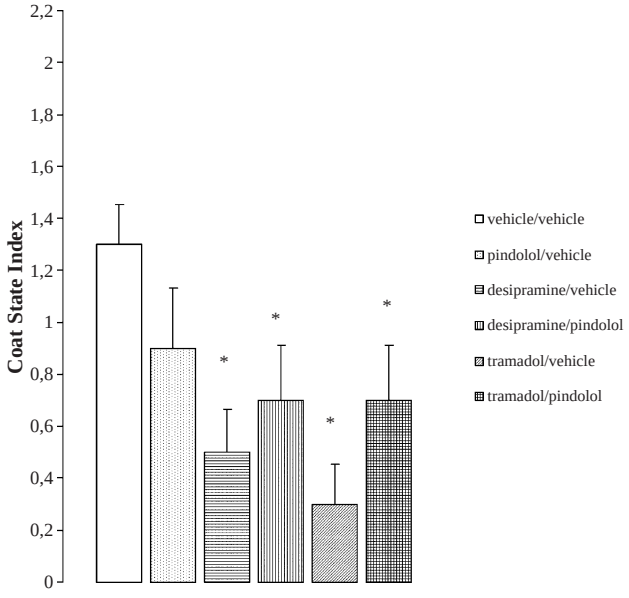

Fig. 4. Effect of pindolol (10 mg/kg, i.p.) on the antidepressant-like actions of desipramine (10 mg/kg, i.p.) and tramadol (20 mg/kg, i.p.) over the coat state in stressed mice after the end of the unpredictable chronic mild stress regimen. The drugs were administered during 4 weeks. $^{*}P<0.05$, significantly different when compared to the stressed vehicle.

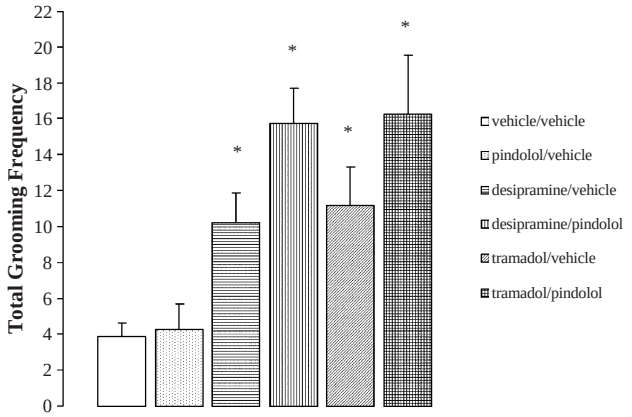

Fig. 5. Effect of pindolol (10 mg/kg, i.p.) on the antidepressant-like actions of desipramine (10 mg/kg, i.p.) and tramadol (20 mg/kg, i.p.) on the total frequency of the grooming behaviour during the splash test. The drugs were administered during 4 weeks. $^{*}P<0.05$, significantly different when compared to the stressed vehicle.

the total score of coat state at the end of the unpredictable chronic mild stress regimen when compared to the stressed vehicle group (Fig. 4; $P=0.019$).

The effect of pindolol on the actions of tramadol and desipramine on the total frequency of the grooming behaviour in the splash test are shown in Fig. 5. There was an overall treatment effect ($H=29.382$, $P=0.000$). Moreover, all of the groups except pindolol group significantly augmented the total frequency of the grooming behaviour when compared to the stressed vehicle ($P<0.05$). Pindolol did not modify the effects of tramadol ($P=0.322$) and desipramine ($P=0.06$) on this parameter.

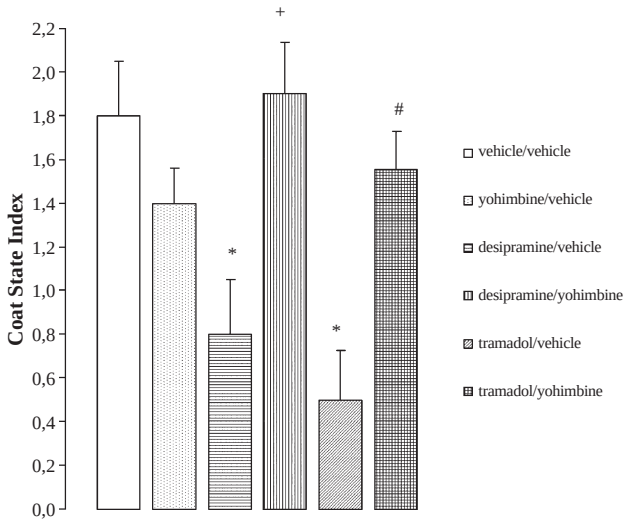

Fig. 6. Effect of yohimbine (2 mg/kg, i.p.) on the antidepressant-like actions of desipramine (10 mg/kg, i.p.) and tramadol (20 mg/kg, i.p.) over the coat state in stressed mice. The drugs were administered during 4 weeks. $^{*}P<0.05$, significantly different when compared to the stressed vehicle, $^{+}P<0.05$, significantly different when compared to the stressed desipramine, $^{\#}P<0.05$, significantly different when compared to the stressed tramadol group.

We did not observe any significant difference between the groups for body weight ($F_{(5,54)}=2.085$, $P=0.081$) and locomotor activity ($F_{(5,52)}=1.444$, $P=0.224$). These results are shown in Table 2.

3.3. Experiment 3

The effect of yohimbine (2 mg/kg, i.p.) on the antidepressant-like actions of tramadol and desipramine over the coat state are displayed in Fig. 6. We observed a significant difference between the groups by the test of Kruskal–Wallis H ($H=21.019$, $P=0.001$). Both tramadol ($P=0.003$) and desipramine ($P=0.017$) treatment induced a significant improvement of the coat state of the mice when compared to stressed vehicle group. The antidepressant-like effects of both drugs were significantly antagonized by yohimbine (P values for desipramine and tramadol combined with yohimbine; $P=0.009$ and $P=0.005$, respectively). However, yohimbine had no effect on the total score of the coat state compared to vehicle, when given alone ($P=0.240$).

The antagonism by yohimbine of the antidepressant-like effects of tramadol and desipramine in the splash test are shown in Fig. 7. There was an overall treatment effect ($H=16.669$, $P=0.05$). Desipramine and tramadol increased grooming behaviour ($P=0.017$ and $P=0.002$, respectively) and these effects were blocked by yohimbine. Yohimbine also did not elicit any effect compared to vehicle, when given alone ($P=0.448$).

Body weights of the mice didn't show significant difference between groups ($F_{(5,54)}=0.241$, $P=0.942$) and also no significant impairment of locomotor activity due to treatment was observed ($F_{(5,50)}=1.039$, $P=0.475$). These results are shown in Table 2.

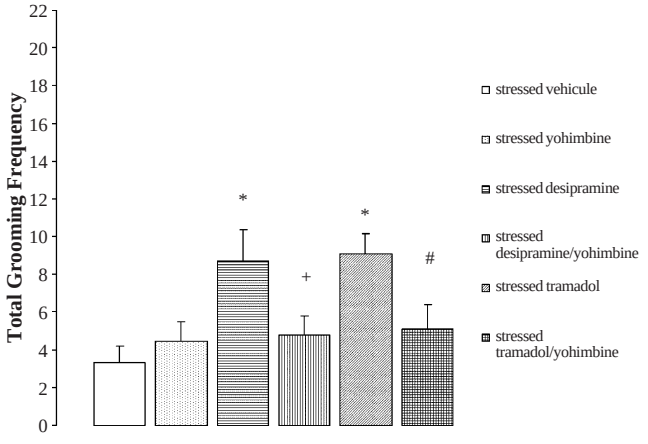

Fig. 7. Effect of yohimbine (2 mg/kg, i.p.) on the antidepressant-like actions of desipramine (10 mg/kg, i.p.) and tramadol (20 mg/kg, i.p.) on the total frequency of the grooming behaviour during the splash test. The drugs were administered during 4 weeks. $^{*}P<0.05$, significantly different when compared to the stressed vehicle, $^{+}P<0.05$, significantly different when compared to the stressed desipramine, $^{\#}P<0.05$, significantly different when compared to the stressed tramadol group.

4. Discussion

In this study, the antidepressant-like effects of tramadol and desipramine were investigated in the unpredictable chronic mild stress model in BALB/c mice. Tramadol and desipramine showed antidepressant-like effect. Pindolol did not change the antidepressant-like actions of desipramine and tramadol whereas yohimbine antagonized the antidepressant-like effects of both drugs during the unpredictable chronic mild stress regimen.

Analysis of data showed that the unpredictable chronic mild stress regimen induced a degradation in the state of the coat and decreased the grooming behaviour in the splash test in the stressed/vehicle mice when compared to the non-stressed/vehicle mice. This cannot be due to trivial effects of the treatments on activity, since locomotion remains unchanged. These physical and behavioural abnormalities were counteracted by 10 mg/kg desipramine and 20 mg/kg tramadol. These results indicate that desipramine and tramadol elicit antidepressant-like effects. Indeed, it has been already reported for fluoxetine, corticotropin releasing factor antagonists and vasopressin V1B receptor antagonists (Santarelli et al., 2003; Ducottet et al., 2003; Griebel et al., 2002) that they counteracted the effects of chronic stress on the state of the coat and in the splash test. In addition, this result is concordant with the study showing that desipramine increases the grooming behaviour in the chronic unpredictable mild stress model (D'Aquila et al., 2000). Our results also confirm previous studies showing that tramadol has potential antidepressant-like effects in rodents (Rojas-Corrales et al., 1998, 2002). Interestingly, as can be seen from experiment 2, these effects only appeared after a chronic treatment: indeed, no effect of these treatment is detected after one or two weeks of antidepressant administration. This further confirms the predictive validity of the unpredictable chronic mild stress model.

Moreover, we examined the effects of noradrenergic and serotonergic systems on the antidepressant-like effects of tramadol and desipramine by using pindolol and yohimbine. When given alone at the concentrations used in this work, pindolol and yohimbine didn't change the state of the coat and grooming behaviour in the splash test when compared to vehicle group. Furthermore, during the unpredictable chronic mild stress regimen, we observed neither a significant acceleration nor diminution of the onset of the antidepressant-like actions of desipramine and tramadol by pindolol. It is well known that 5-HT_{1A} receptors are intimately involved in the mechanism of action of antidepressant drugs. Most antidepressant drugs, such as SSRI, elicit their effects via an increase of the serotonin level by preventing its reuptake. However, this increase is offset by a negative feedback because of the activation of 5-HT_{1A} autoreceptors (Artigas et al., 2001). It has already been reported that 5-HT_{1A} receptor antagonists such as pindolol could accelerate the clinical effects of antidepressant like SSRI (paroxetine) by preventing this

negative feedback (Plenge and Mellerup, 2003). In contrast, two studies of pindolol combined with fluoxetine (Berman et al., 1999; Tatarczynska et al., 2002) found no acceleration of the antidepressant response. Furthermore, pindolol enhanced the analgesic effect of tramadol in two test of analgesia: the hot plate test in mice and the plantar test in rats (Rojas-Corrales et al., 2000). On the other hand, in our experiment setting pindolol did not change the antidepressant-like effects of desipramine, a tricyclic antidepressant that preferentially blocks the reuptake of noradrenaline. It has been shown that the combination of the tricyclic antidepressant drugs lacking effect on serotonin reuptake process (desipramine or trimipramine) with pindolol resulted in only one of ten patients achieving a 50% improvement after 28 days. In contrast, the combination of the SSRI fluvoxamine with pindolol produced a marked antidepressant effect (Blier et al., 1997). Moreover, in the forced swimming test, pre-treatment with (±) pindolol potentiated the effects of SSRI and was devoid of any activity on desipramine (Redrobe et al., 1996). These results provide further evidence that pindolol does not accelerate the antidepressant effect of drugs that alter the noradrenaline function.

Although pindolol can block the 5-HT_{1A} autoreceptor, it is inactive at post-synaptic 5-HT_{1A} receptors (Romero et al., 1996). It was also suggested that antidepressant-like effects of desipramine seem to be mediated by post-synaptic 5-HT_{1A} receptors (Redrobe et al., 1996). Furthermore, in our study pindolol did not modify the antidepressant-like effects of desipramine.

Yohimbine significantly diminished the antidepressant-like actions of both desipramine and tramadol over coat state and on total frequency of the grooming behaviour during the splash test. It has been recently shown that repeated treatment with tramadol (20 mg/kg, i.p., once daily for 21 days) induces statistically downregulation of [^{3}H]RX821002 binding sites, a selective α_2-adrenergic receptor ligand, in the rat brain (Faron-Gorecka et al., 2004). Subhash et al., 2003 reported that the density of rat cortical α_2-adrenergic receptors was significantly decreased upon repeated treatment with tricyclic antidepressants. Also, Giralt and Garcia-Sevilla, 1989 showed that chronic but not short term treatment with drugs which antagonize endogenous noradrenaline like yohimbine upregulated α adrenoceptors. Moreover, after the chronic administration of idazoxan (α_2-adrenoceptor antagonist), α_2-adrenoceptor number has been reported to be significantly increased and plasma 3-methoxy-4 hydroxy phenylglycol (MHPG) levels has been reported to be significantly reduced by chronic idazoxan (Glue et al., 1991). According to our results, it can be suggested that yohimbine may diminish the antidepressant-like effects of tramadol by reversing its down-regulative action on α_2-adrenergic receptor. However, further experiments evaluating the levels of noradrenaline and serotonin in different brain regions are necessary to confirm this hypothesis.

As we mentioned before, clinically active tramadol is a racemic mixture of two enantiomers: the (+) tramadol enantiomer preferentially inhibits 5-HT reuptake and enhances serotonin release, whereas the (−) enantiomer preferentially inhibits noradrenaline reuptake (Frink et al., 1996). Rojas-Corrales et al., 1998 reported that racemic tramadol and (−) tramadol, but not (+) tramadol, demonstrated antidepressant-like activity which was similar to that of imipramine by reducing dose-dependently the immobility time in the forced swimming test. It is possible to suggest that the serotonergic system doesn't have a major role in the antidepressant-like effects of tramadol. In accordance with our results, Rojas-Corrales et al. (1998) showed that pre-treatment with D,L-α-methyl-*p*-tyrosine (an inhibitor of noradrenaline synthesis) antagonized the immobility reducing action of tramadol while *para*-chlorophenyl alanine methylester hydrochloride (an inhibitor of serotonin synthesis) did not antagonize the reduction in immobility time produced by tramadol. The reduction in immobility induced by tramadol was reversed by both non-specific α antagonist phentolamine and by the α_2-adrenergic receptor antagonist yohimbine, although methysergide (non-specific serotonin receptor antagonist) was not capable of reducing the effect of tramadol.

Besides, it has been shown that the effect of desipramine on extracellular noradrenaline in the brain cortex is modulated by α_2-adrenoceptors in the locus coeruleus (Mateo et al., 1998). In addition, Reneric et al., 2001 reported that idazoxan produced no anti-immobility effects per se in the forced swimming test and antagonized the effects of desipramine. It is well known that yohimbine is highly selective for α_2 over α_1 adrenergic receptors and is frequently used to assess the involvement of α_2-adrenergic receptors in the mechanism of action of drugs (Goldberg and Robertson, 1983). Our study also showed that yohimbine significantly decreased the antidepressant-like effects of desipramine.

In conclusion, by interpreting the data of our study, we suggest that unpredictable chronic mild stress significantly induced physical and behavioural abnormalities such as a degradation of the coat state and decreased the grooming behaviour, which were reversed by a chronic administration of desipramine and tramadol. In addition, yohimbine was capable of reducing the effects of tramadol and desipramine, while pindolol was not. These findings can indicate that the noradrenergic mechanisms particularly α_2-adrenoceptors were responsible for the antidepressant-like effects observed with tramadol and desipramine in the unpredictable chronic mild stress model.

Acknowledgements

We would like to thank to Alexandre Surget for his kind help. The authors also extend their thanks to Prof. Dr. Nazan Alparslan for her comments on the statistical methods.

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