

Role of serotonin 5-HT_{1A} receptors in the antidepressant-like effect and the antinociceptive effect of venlafaxine in mice

Esther Berrocoso and Juan A. Mico

Pharmacology and Neuroscience Research Group, Department of Neuroscience (Pharmacology and Psychiatry), School of Medicine, University of Cádiz, Cádiz, Spain

Abstract

The present study was undertaken to evaluate the potential role of 5-HT_{1A} receptors in the antidepressant-like effect and antinociceptive effect of venlafaxine. With this aim, the effect of either a selective 5-HT_{1A} receptor antagonist (WAY-100635; *N*-2-[4-(2-methoxyphenyl-1-piperazinyl)ethyl]-*N*-2-pyridinylcyclohexane carboxamide) or a selective 5-HT_{1A} receptor agonist (8-OH-DPAT; 8-hydroxy-2-(di-*n*-propylamine) tetralin hydrobromide) was investigated in mice in combination with venlafaxine by means of the forced swimming test, a paradigm aimed at screening potential antidepressants, and the hot-plate test, a phasic pain model. Surprisingly, the results showed that WAY-100635 produced a large decrease in the antidepressant-like effect of venlafaxine, while 8-OH-DPAT rendered effective a non-effective dose of this antidepressant. However, in the hot-plate test WAY-100635 significantly enhanced the antinociceptive effect of venlafaxine, whereas 8-OH-DPAT counteracted its antinociceptive effect. These findings show that 5-HT_{1A} receptors play differing roles in modulating the antidepressant-like and antinociceptive effects of venlafaxine in the models investigated. The results imply that blockade of the 5-HT_{1A} receptors in the forebrain will counteract the favourable (antidepressant-like) effect at raphe nuclei level, and consequently, the overall effect evidenced is an antagonism. This suggests a predominant role of 5-HT_{1A} receptors located in the forebrain area for the antidepressant-like effect. In contrast, the antinociceptive effect of venlafaxine is probably potentiated due to the blockade of somatodendritic 5-HT_{1A} receptors in the same raphe nuclei, facilitating the descending monoaminergic pain control system.

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Introduction

Pain is one of the most prevalent physical symptoms in depression, which makes the treatment and the prognosis of the illness difficult (Greden, 2003). Antidepressants are the first-line drugs for the treatment of depression, but they are also among the first choices for the treatment of some painful conditions. Specifically, evidence exists that dual antidepressants [serotonin (5-HT) and noradrenaline (NA) reuptake inhibitors] have greater efficacy than selective ones for

pain relief, with the older tricyclics still being the first choice (Eschalier et al., 1994; Mico et al., 2006a). Recently, a number of controlled trials with the new dual class of antidepressants (venlafaxine, duloxetine and milnacipran) suggest that these compounds could have a therapeutic analgesic potential (Briley, 2004; Saarto and Wiffen, 2007; Urquhart et al., 2008). In the case of venlafaxine, it has been shown to be effective in relieving pain of several aetiologies such as diabetic neuropathy, migraine and fibromyalgia (Ozyalcin et al., 2005; Rowbotham et al., 2004; Sayar et al., 2003), and it also alleviates pain as a main or secondary physical symptom in some cases of clinical depression (Barkin and Barkin, 2005). In spite of these favourable clinical perspectives its analgesic mechanism of action remains elusive.

Based on reports of the known comorbidity between chronic pain and depressive illness, it is

Address for correspondence: Dr J. A. Mico, Pharmacology and Neuroscience Research Group, Department of Neuroscience (Pharmacology and Psychiatry), School of Medicine, University of Cádiz, Plaza Fragela 9, 11003 Cádiz, Spain.
Tel.: +34 956015247 Fax: +34 956015225
E-mail: juanantonio.mico@uca.es

possible that these disease states are linked, i.e. via a monoaminergic system dysfunction (Delgado, 2004). In particular, it has been suggested that 5-HT_{1A} receptors play a role in the regulation of cognitive, emotional, and pain states due to their elevated presence in specific brain areas, such as the raphe nuclei, cerebral cortex, limbic system, hypothalamus and spinal cord. In the raphe nuclei they function as somatodendritic autoreceptors, controlling 5-HT release and can be activated with an acute injection of an inhibitor of 5-HT reuptake, such as venlafaxine, limiting the increase in extracellular 5-HT in projection areas such as the forebrain (Bortolozzi et al., 2004; Riad et al., 2004). Consequently, antidepressants' greater or lesser degree of clinical effectiveness has been related to the blockade of 5-HT_{1A} receptor activity in the raphe nuclei (Adell et al., 2005; Millan, 2006; Shelton, 2007). A similar mechanism, which involves the raphe spinal pathway (descending monoaminergic pain control system), has been suggested for 5-HT_{1A} autoreceptors in analgesia (Mico et al., 2006b). In forebrain areas, the activation of post-synaptic 5-HT_{1A} receptors has been related to the antidepressant effect of tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), venlafaxine and electroconvulsive shock treatment (Beique et al., 2000b; Blier and Ward, 2003; Millan, 2006). Moreover, in the spinal cord, the activation of 5-HT_{1A} receptors has been related to both pro- and antinociceptive effects (Millan, 2002). Considering these findings, different pharmacological consequences may be triggered by either the activation or antagonism of different 5-HT_{1A} receptor populations, this being of particular interest in the coexistence and treatment of pain and depression.

From a preclinical point of view, conflicting data exist with respect to the effect of the modulation of 5-HT_{1A} receptors on the action of antidepressants. For instance, in the forced swimming test (FST), one of the most used tests to detect antidepressant-like activity, the blockade of 5-HT_{1A} receptors enhanced the antidepressant-like activity of the selective and dual reuptake inhibitors (Millan et al., 1998; Tatarczynska et al., 2004), whereas other studies failed to demonstrate this (Redrobe et al., 1996; Tatarczynska et al., 2004). These differing effects have also been reported in patients with clinical depression. In this sense, it has been demonstrated recently that pindolol (a partial 5-HT_{1A} receptor antagonist) accelerates the antidepressant response but does not increase the effectiveness of SSRIs in unresponsive patients (for review see Artigas et al., 2006). In rats, different dosage ranges or experimental procedures might account

for these differences. Regarding pain models, we and others have demonstrated an augmentation of the antinociceptive effect of different compounds with antidepressant-like effects when they are combined with 5-HT_{1A} antagonists (Ardid et al., 2001; Rojas-Corrales et al., 2000). Unfortunately, at the present time no clinical data have been reported regarding this approach in humans with chronic pain.

Taking into account that depression and pain are two entities which are sometimes interrelated and that venlafaxine has antidepressant and analgesic effects, and in view of the importance of 5-HT_{1A} receptors in modulating both states, the aim of the present study has been to investigate the role these receptors play in the modulation of these functions in the effect of venlafaxine.

Method

Animals

Experiments were performed using male albino CD1 mice (25–30 g). All the animals were provided by the 'Servicio de Experimentación y Producción Animal' (SEPA) of the University of Cádiz. Animals were maintained under standard conditions: 12-h light–dark schedule (lights on 08:00 hours) with ad libitum food and water and a constant temperature (21 ± 1 °C). The experimental protocols were reviewed and approved by the Local Committee for Animal Experimentation at the University of Cádiz and complied with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983). Animal care and use procedures conformed to European Ethical Standards (86/609-EEC) and Spanish Law (RD 1201/2005) for the care and use of laboratory animals. Animals were housed in groups of 10, and a 7-d acclimatization period was allowed before the experiments began. All mice were experimentally naive and used only once and 9–10 subjects were used per group. The experiments were performed during the light phase between 09:00 and 16:00 hours, by a single experimenter.

Drugs and treatment

The following drugs were used in the study: venlafaxine hydrochloride (1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol; Wyeth, NJ, USA); WAY-100635 (N-2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexane carboxamide; Sigma-Aldrich-Quimica, Madrid, Spain) and 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamine) tetralin hydrobromide; Sigma-Aldrich-Quimica).

The selective 5-HT_{1A} receptor antagonist (WAY-100635) and agonist (8-OH-DPAT) were subcutaneously injected 15 min after venlafaxine, which was intraperitoneally administered 30 min before all tests. All drugs were freshly prepared immediately prior to use. They were dissolved in physiological saline (0.9% NaCl) and injected at a volume of 10 ml/kg of body weight. Control animals received saline only. The treatments were administered under blind conditions.

Antidepressant-like test

The FST was used to determine the role of 5-HT_{1A} receptors in the antidepressant-like effect of venlafaxine, following the classic method described by Porsolt et al. (1977). Mice were placed individually into glass cylinders (height 18 cm, diameter 10 cm) containing water 10 cm deep at 23 °C, and left there for 6 min. Tests were video-recorded and subsequently a highly trained observer, who was unaware of the treatment, evaluated the duration of immobility during the last 4 min of the 6-min testing period. A mouse was judged to be immobile when it remained floating in the water making only the movements necessary to keep its head above the water. Reduction of immobility in this test was considered to indicate antidepressant activity.

Hot-plate test (Woolfe and McDonald, 1944)

The hot-plate test was used as a phasic pain model in order to evaluate the role of 5-HT_{1A} receptors in the antinociceptive effect of venlafaxine. Animals were treated under the same conditions as for antidepressant testing. Antinociception was evaluated with a hot-plate apparatus (Digital DS-37 Socrel model; Milan, Italy), with a 25 cm × 25 cm metal surface maintained at 55.5 ± 0.5 °C surrounded by a 40-cm high Plexiglas wall. Latency was considered as the time in seconds between when the animal was placed on the hot-plate surface and when it either licked or shook its hind paw or jumped. These responses are considered to be supraspinally integrated (Le Bars et al., 2001). Basal latency was determined as the mean of two trials with a delay of 30 min between each one in order to randomize animal groups. After this, test latency was determined after drug injection. A cut-off time was established at 60 s in order to avoid tissue damage to the animal.

Locomotor activity

Motor activity of the mice was measured by spontaneous motor activity recording and tracking

apparatus (SMART; Letica Scientific Instruments, Barcelona, Spain). A mouse was placed in a Plexiglas chamber (22 × 22 × 20 cm) and allowed to explore freely. The activity was monitored for 10 min. Motor activity was assessed following the arbitrary units established by the SMART device.

Experimental design

First, dose–response relationships were established for venlafaxine in the FST (2.5–20 mg/kg) and the hot-plate test (20–80 mg/kg). Second, the role of blockade of 5-HT_{1A} receptors on both the antidepressant-like and antinociceptive effects was studied. WAY-100635 (0.2–0.8 mg/kg) was co-administered with effective and ineffective doses of venlafaxine (5–20 mg/kg in the FST; 20–80 mg/kg in the hot-plate test). Third, based on the results obtained in this phase, we tested the role of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT in venlafaxine-induced effects in the FST and the hot-plate test. 8-OH-DPAT (0.03125–0.125 mg/kg) was combined with an ineffective dose of venlafaxine (5 mg/kg) in the FST. In the hot-plate test 8-OH-DPAT (0.0625–0.25 mg/kg) was combined with an effective dose of venlafaxine (80 mg/kg). Fourth, locomotor activity was assessed in order to explore the possible role of motor activity in the results obtained in the FST and hot-plate test.

The antagonist/agonist of 5-HT_{1A} receptors chosen have been shown to be selective ligands of these receptors in vivo (Stamford et al., 2000). WAY-100635/8-OH-DPAT doses were chosen based on data available in the literature and previous successful experiments performed in our laboratory into pharmacological activity in 5-HT_{1A} receptors, the results of which showed a minimum effect on baseline values.

Expression of results and statistical analysis

All data are expressed as mean ± S.E.M. values. In the FST, the antidepressant-like effect was evaluated and expressed as immobility time (in seconds). Locomotor activity was expressed as the mean activity counted (arbitrary units). In the hot-plate test, the level of analgesia was expressed as latency time (in seconds). The effects of venlafaxine compared to the saline group were established by a one-way analysis of variance (ANOVA) followed by Dunnett's test. In the interaction studies, statistical analysis was performed using a two-way ANOVA. The factors of variation were venlafaxine treatment and 5-HT_{1A} receptor antagonist or agonist treatment. To study the effect of either antagonist or agonist treatments on each dose of venlafaxine, a subsequent unpaired Student's *t* test for

comparisons between two groups was used. Further, a one-way ANOVA followed by Dunnett's test was used for comparisons between more than two groups. A value of $p < 0.05$ was considered significant.

Results

Antidepressant-like effect of venlafaxine

The antidepressant-like effect of venlafaxine (2.5–20 mg/kg) was evaluated in the FST. A one-way ANOVA showed a significant effect of the treatment ($F_{4,45} = 6.43$, $p < 0.001$). Venlafaxine produced a dose-dependent reduction in immobility time that differed significantly from control values at 10 mg/kg and 20 mg/kg, indicating it has an antidepressant-like effect.

Role of 5-HT_{1A} receptors in the antidepressant-like effect of venlafaxine

WAY-100635 (0.2–0.8 mg/kg), was co-administered with venlafaxine (5–20 mg/kg) in the FST to evaluate the involvement of 5-HT_{1A} receptors in the antidepressant-like effect of venlafaxine (Figure 1a). A two-way ANOVA demonstrated a significant effect of WAY-100635 ($F_{3,144} = 6.93$, $p < 0.001$), venlafaxine ($F_{3,144} = 11.55$, $p < 0.001$) and an interaction between WAY-100635 and venlafaxine ($F_{9,144} = 3.67$, $p < 0.001$). Subsequently, a one-way ANOVA showed that no dose of WAY-100635 (0.2–0.8 mg/kg) significantly modified the immobility time compared with saline ($F_{3,36} = 0.47$, n.s.) and that WAY-100635 (0.2–0.8 mg/kg) did not alter the changes induced in the immobility time for venlafaxine at 5 mg/kg ($F_{3,36} = 0.41$, n.s.). However, WAY-100635 at 0.4 mg/kg and 0.8 mg/kg significantly increased the immobility time induced by venlafaxine at 10 mg/kg and 20 mg/kg compared with each dose of venlafaxine administered alone ($F_{3,36} = 3.52$, $p < 0.05$; $F_{3,36} = 9.51$, $p < 0.001$, respectively; Dunnett's test), indicating a reduction in the antidepressant-like effect of venlafaxine.

First, the effect on locomotor activity was measured for the combination of WAY-100635 (0.2–0.8 mg/kg) and venlafaxine (5–20 mg/kg) (Figure 1b). A two-way ANOVA demonstrated a significant effect of WAY-100635 ($F_{3,144} = 3.13$, $p < 0.05$), but neither the factor venlafaxine ($F_{3,144} = 1.79$, n.s.), nor the interaction between the two treatments ($F_{9,144} = 1.27$, n.s.) reached statistical significance. Next, the one-way ANOVA did not show any statistical difference between WAY-100635 (0.2–0.8 mg/kg) and saline ($F_{3,36} = 1.47$, n.s.). Similarly, the combination of WAY-100635 (0.2–0.8 mg/kg) and venlafaxine at 5 mg/kg and

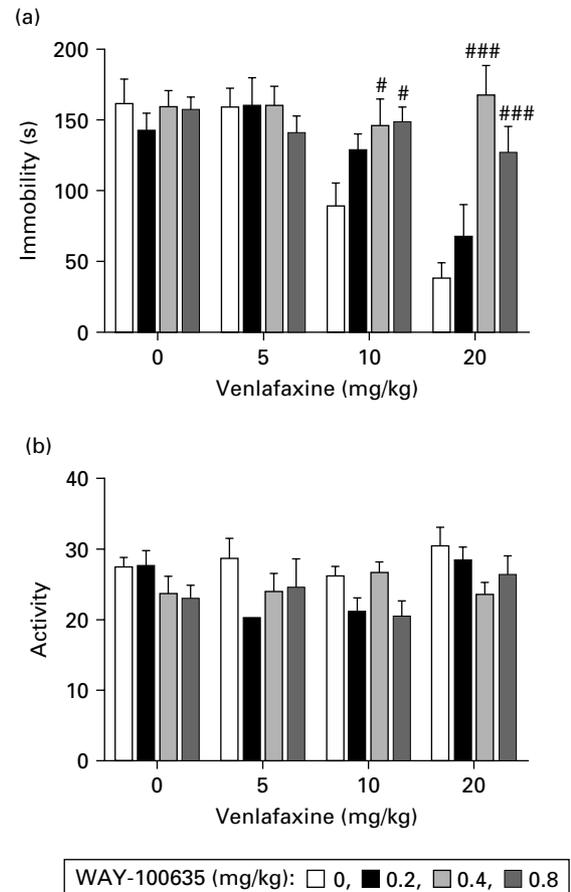


Figure 1. Involvement of the 5-HT_{1A} receptor antagonist, WAY-100635, in the antidepressant-like effect of venlafaxine in the forced swimming test in mice (a). Effect on locomotor activity of the combination of WAY-100635 and venlafaxine (b). Venlafaxine (5–20 mg/kg) was intraperitoneally administered 30 min before the test. WAY-100635 (0.2–0.8 mg/kg) was subcutaneously injected 15 min after venlafaxine. Data are presented as mean \pm S.E.M. of the immobility time or locomotor activity; $n = 10$ per value; # $p < 0.05$, ### $p < 0.001$ vs. respective venlafaxine-treated group.

20 mg/kg did not significantly modify the locomotor activity compared to venlafaxine alone ($F_{3,36} = 1.31$, n.s.; $F_{3,36} = 1.70$, n.s., respectively). However, combined with 10 mg/kg venlafaxine, there was a marked effect, but no group of treatment reached statistical significance ($F_{3,36} = 3.25$, $p < 0.05$, Dunnett's test).

Second, 8-OH-DPAT (0.03125–0.125 mg/kg), was co-administered with an ineffective dose of venlafaxine (5 mg/kg) in the FST to evaluate the involvement of 5-HT_{1A} receptors in the antidepressant-like effect of venlafaxine (Figure 2a). A two-way ANOVA revealed a significant effect of both 8-OH-DPAT ($F_{3,72} = 9.46$,

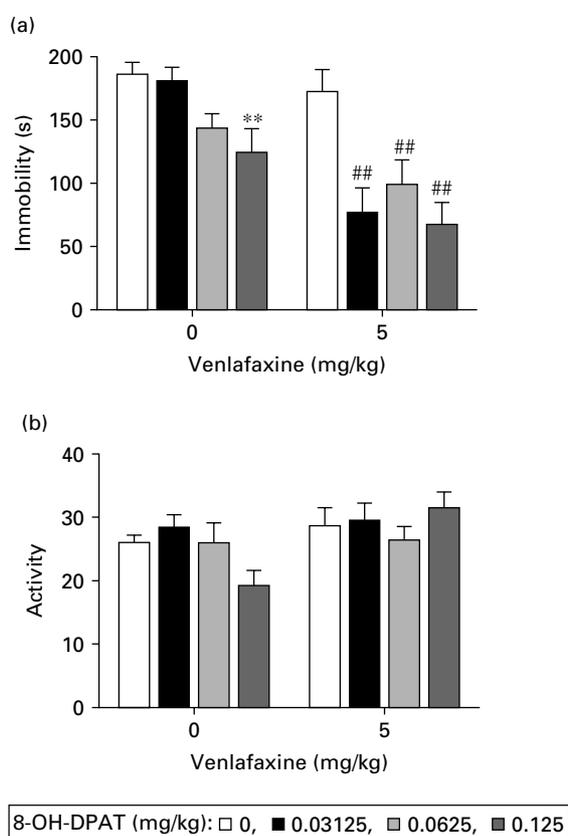


Figure 2. Involvement of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, in the antidepressant-like effect of venlafaxine in the forced swimming test in mice (a). Effect on locomotor activity of the combination of 8-OH-DPAT and venlafaxine (b). Venlafaxine (5 mg/kg) was intraperitoneally administered 30 min before the test. 8-OH-DPAT (0.03125–0.125 mg/kg) was subcutaneously injected 15 min after venlafaxine. Data are presented as mean \pm S.E.M. of the immobility time or locomotor activity; $n=9-10$ per value; ** $p < 0.01$ vs. saline group; ## $p < 0.01$ vs. venlafaxine-treated group.

$p < 0.001$) and venlafaxine ($F_{1,72}=23.25$, $p < 0.001$) and an interaction between 8-OH-DPAT and venlafaxine ($F_{3,72}=2.75$, $p < 0.05$). A one-way ANOVA showed that 0.125 mg/kg 8-OH-DPAT significantly decreased the immobility time compared with saline-treated animals ($F_{3,36}=5.13$, $p < 0.01$, Dunnett's test) and that the combination of 8-OH-DPAT (0.03125–0.125 mg/kg) and venlafaxine (5 mg/kg) significantly decreased the immobility time compared with venlafaxine alone ($F_{3,36}=6.60$, $p < 0.01$, Dunnett's test).

The effect on locomotor activity was measured for the combination of 8-OH-DPAT (0.03125–0.125 mg/kg) with venlafaxine (5 mg/kg) (Figure 2b). A two-way ANOVA demonstrated a significant effect of venlafaxine ($F_{1,70}=5.87$, $p < 0.05$), but neither the factor

8-OH-DPAT ($F_{3,70}=0.85$, n.s.), nor the interaction between the two treatments ($F_{3,70}=2.44$, n.s.) reached statistical significance. A one-way ANOVA showed that although 8-OH-DPAT (0.03125–0.125 mg/kg) slightly reduced spontaneous activity compared with control animals, it neither reached statistical significance ($F_{3,35}=2.81$, n.s.) nor modified the locomotor activity of venlafaxine (5 mg/kg) ($F_{3,35}=0.68$, n.s.).

Antinociceptive effect of venlafaxine

The antinociceptive effect of venlafaxine (20–80 mg/kg) was studied in the hot-plate test in mice. A one-way ANOVA showed a significant effect of the treatment ($F_{3,36}=5.64$, $p < 0.01$). Venlafaxine induced an increase in latency time in a dose-related manner. At doses of 20 mg/kg and 40 mg/kg, it induced a non-significant increase, but at 80 mg/kg, it reached statistical significance in comparison with saline controls.

Role of 5-HT_{1A} receptors on the antinociceptive effect of venlafaxine

First, the effect of administration of WAY-100635 (0.2–0.8 mg/kg) and venlafaxine (20–80 mg/kg) was studied in the hot-plate test in mice to determine the involvement of 5-HT_{1A} receptors in the antinociceptive effect of venlafaxine (Figure 3a). A Two-way ANOVA revealed a significant effect of WAY-100635 ($F_{3,144}=6.92$, $p < 0.001$), venlafaxine ($F_{3,144}=58.09$, $p < 0.001$) and interaction between WAY-100635 and venlafaxine ($F_{9,144}=2.04$, $p < 0.05$). Subsequently, a one-way ANOVA showed that no dose of WAY-100635 (0.2–0.8 mg/kg) significantly modified the latency time compared with saline ($F_{3,36}=0.85$, n.s.). The combination of WAY-100635 (0.2–0.8 mg/kg) with the lowest dose of venlafaxine (20 mg/kg) slightly increased the latency time although no drug combination reached statistical significance ($F_{3,36}=0.57$, n.s.). However, the combination of WAY-100635 at 0.2 mg/kg and 0.8 mg/kg with 40 mg/kg venlafaxine significantly increased the latency time compared with venlafaxine alone ($F_{3,36}=10.06$, $p < 0.001$, Dunnett's test). The combination of WAY-100635 with venlafaxine at the highest dose (80 mg/kg) did not significantly increase the antinociceptive effect compared with venlafaxine alone ($F_{3,36}=1.29$, n.s.).

The effect on locomotor activity was measured for the combination of WAY-100635 (0.8 mg/kg) and venlafaxine (40–80 mg/kg) (Figure 3b). A two-way ANOVA demonstrated a significant effect of venlafaxine ($F_{2,52}=15.78$, $p < 0.001$), but neither the factor WAY-100635 ($F_{1,52}=3.14$, n.s.), nor the interaction

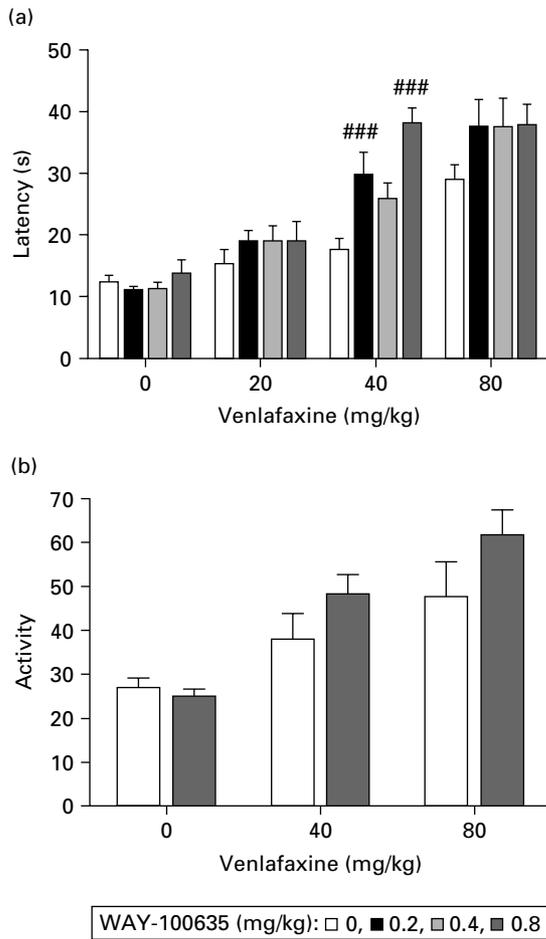


Figure 3. Involvement of the 5-HT_{1A} receptor antagonist, WAY-100635, in the antinociceptive effect of venlafaxine in the hot-plate test in mice (a). Effect on locomotor activity of the combination of WAY-100635 and venlafaxine (b). Venlafaxine (20–80 mg/kg) was intraperitoneally administered 30 min before the test. WAY-100635 (0.2–0.8 mg/kg) was subcutaneously injected 15 min after venlafaxine. Data are presented as mean \pm S.E.M. of the latency time or locomotor activity; $n=9$ –10 per value; ### $p < 0.001$ vs. respective venlafaxine-treated group.

between the two treatments ($F_{2,52}=1.33$, n.s.) reached statistical significance. WAY-100635 (0.8 mg/kg) did not modify spontaneous activity compared with saline (Student's t tests: $t=0.74$, d.f. = 17, n.s.). Furthermore, the combination of WAY-100635 (0.8 mg/kg) and venlafaxine at 40 mg/kg and 80 mg/kg did not significantly modify the activity compared to venlafaxine alone (Student's t tests: $t=-1.43$, d.f. = 17, n.s.; $t=-1.45$, d.f. = 18, n.s., respectively).

Second, the effect of administration of 8-OH-DPAT (0.0625–0.25 mg/kg), and an effective dose of venlafaxine (80 mg/kg) was evaluated in the hot-plate

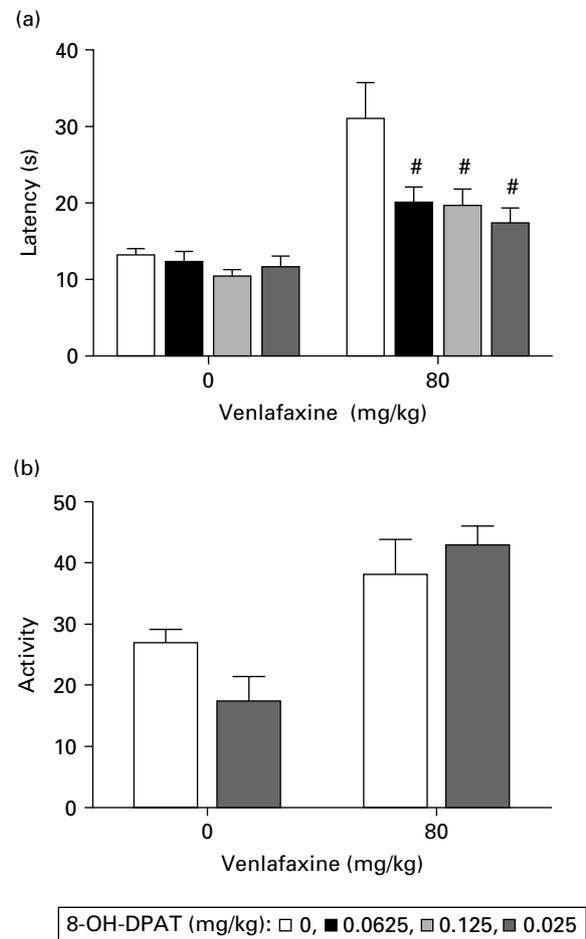


Figure 4. Involvement of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, in the antinociceptive effect of venlafaxine in the hot-plate test in mice (a). Effect on locomotor activity of the combination of 8-OH-DPAT and venlafaxine (b). Venlafaxine (80 mg/kg) was intraperitoneally administered 30 min before the test. 8-OH-DPAT (0.0625–0.25 mg/kg) was subcutaneously injected 15 min after venlafaxine. Data are presented as mean \pm S.E.M. of the latency time or locomotor activity; $n=9$ –10 per value; # $p < 0.05$ vs. respective venlafaxine-treated group.

test in mice to determine the involvement of 5-HT_{1A} receptors in the antinociceptive effect of venlafaxine (Figure 4a). A two-way ANOVA revealed a significant effect of 8-OH-DPAT ($F_{3,72}=5.00$, $p < 0.01$), venlafaxine ($F_{1,72}=41.63$, $p < 0.001$) and an interaction between the two treatments ($F_{3,72}=2.90$, $p < 0.05$). Next, the one-way ANOVA showed no significant effect of 8-OH-DPAT compared with control animals ($F_{3,36}=1.07$, n.s.). Moreover, all combinations of 8-OH-DPAT (0.0625–0.25 mg/kg) with venlafaxine counteracted the immobility time displayed by venlafaxine alone ($F_{3,36}=4.38$, $p < 0.05$, Dunnett's test).

The effect on locomotor activity was measured for the combination of 8-OH-DPAT (0.25 mg/kg) with venlafaxine (80 mg/kg) (Figure 4b). A two-way ANOVA demonstrated a significant effect of venlafaxine ($F_{1,35}=21.91$, $p<0.001$), but neither the factor 8-OH-DPAT ($F_{1,35}=2.06$, n.s.), nor the interaction between the two treatments ($F_{1,35}=0.26$, n.s.) reached statistical significance. 8-OH-DPAT (0.25 mg/kg) slightly reduced spontaneous activity compared with control animals, but it did not reach statistical significance (Student's t tests: $t=2.03$, d.f. = 17, n.s.). In addition, the combination of 0.25 mg/kg 8-OH-DPAT and 80 mg/kg venlafaxine did not significantly modify the locomotor activity vs. venlafaxine alone (Student's t tests: $t=0.54$, d.f. = 18, n.s.).

Discussion

The present study shows that the antidepressant-like effect of venlafaxine is blocked by WAY-100635, while the antinociceptive effect is enhanced. Consistent with these findings, 8-OH-DPAT rendered effective a non-effective dose of venlafaxine in the antidepressant test and blocked the antinociceptive effect of an effective dose of the drug in the hot-plate test. In addition, the effects induced by the 5-HT_{1A} receptor antagonist or agonist, which modulates the action of venlafaxine, do not seem to be due to a putative locomotor effect, given that these pharmacological combinations do not affect this behaviour. This is especially relevant as it rules out unspecific-like effects (sedative/muscle relaxant effect) responsible for the FST results, a test which evaluates the mobility of rodents.

It has recently been suggested that venlafaxine, in addition to its antidepressant profile, could have a therapeutic role in analgesia therapy (Briley, 2004). Venlafaxine inhibits the reuptake of both 5-HT and NA, although to varying degrees. It has been shown that venlafaxine inhibits preferentially the reuptake of 5-HT at low doses, whereas at higher doses it inhibits both monoamine carriers (Beique et al., 2000a; Redrobe et al., 1998; Stahl et al., 2005). In the models used in the present study, venlafaxine displays both antidepressant-like and antinociceptive effects. However, interestingly, the antidepressant-like effect is reached at lower doses than the antinociceptive effect. Thus, it could be contended that venlafaxine exhibits antidepressant-like effects starting with doses that have been related to 5-HT reuptake whereas the contribution of both monoamines are necessary for the antinociceptive effect (Marchand et al., 2003; Redrobe et al., 1998). These data contribute to supporting the accumulating evidence suggesting that

dual antidepressants are more effective analgesics than selective ones (Lynch, 2001). In addition, these preclinical results regarding dose range and the onset of the analgesic and antidepressant effect are in agreement with clinical data about venlafaxine (Briley, 2004), contrasting with the existing knowledge from clinical experience with tricyclics where the analgesic effect appears at lower doses than the antidepressant effect (Lynch, 2001).

As mentioned above, venlafaxine increases the concentration of 5-HT in the extracellular brain space as a consequence of blockade of the 5-HT carrier. As a result, acute administration of venlafaxine is able to activate feedback mechanisms mediated by somatodendritic 5-HT_{1A} receptors located in the raphe nuclei. Proof of this is that the systemic administration of WAY-100635 completely reverses the venlafaxine inhibition of the firing rate at raphe nuclei level (Bjorvatn et al., 2000), and that WAY-100635 enhances the venlafaxine-induced extracellular 5-HT concentrations in the frontal cortex (Dawson et al., 1999). The blockade of these feedback mechanisms should supposedly lead to an enhancement of both 5-HT availability and the antidepressant effect. However, in the present study the blockade of 5-HT_{1A} receptors surprisingly led to an antagonism of the antidepressant-like effect of venlafaxine, which might imply the participation of other mechanisms. It has been suggested that the antidepressant effect of venlafaxine is mediated by the activation of post-synaptic 5-HT_{1A} receptors in the dorsal hippocampus (Beique et al., 2000a). WAY-100635 possesses antagonistic properties at the level of both somatodendritic and post-synaptic 5-HT_{1A} receptors (Fletcher et al., 1996). Therefore our findings suggest that while the blockade of somatodendritic 5-HT_{1A} receptors in the raphe nuclei potentiates the rise in extracellular levels of 5-HT, the simultaneous blockade of forebrain post-synaptic 5-HT_{1A} receptors partly cancels out the consequences of the enhanced 5-HT concentration (Beique et al., 2000b; Haddjeri et al., 1998). Thus, blockade of the 5-HT_{1A} receptors in the forebrain will counteract the facilitating antidepressant-like effect at raphe nuclei level, and consequently, in the FST, the overall effect evidenced is an antagonism of the antidepressant-like effect of venlafaxine. In addition to depression, our results in the FST could be relevant for the affective dimension of pain (emotional feelings) (Price, 2002). It has been shown that the augmentation of serotonergic transmission reduces the activation of forebrain areas during nociceptive stimuli (Nemoto et al., 2003). Overall, data support the accumulating evidence suggesting a pivotal role of the post-synaptic

5-HT_{1A} receptors in the forebrain in depression and probably in the affective dimension of pain (Beique et al., 2000b; Blier and Ward, 2003).

In the present study, WAY-100635 significantly enhances the antinociceptive effect of venlafaxine in the hot-plate test, whereas 8-OH-DPAT counteracts it. This could be explained by considering the role of monoaminergic descending projections from raphe nuclei to the spinal cord in mediating analgesia. That is, the acute administration of venlafaxine leads to a local increase of 5-HT in the raphe nuclei that would activate 5-HT_{1A} autoreceptors (Bjorvatn et al., 2000). Therefore, activation of these receptors leads to a decrease in 5-HT release in projecting areas such as the spinal cord (Sprouse and Aghajanian, 1987). Thus, it may be suggested that the acute administration of WAY-100635 blocked this negative feedback mechanism at raphe level, yielding an increase in 5-HT release in projecting areas, consequently, enhancing the analgesic effect displayed by venlafaxine.

Venlafaxine, in addition to blocking the 5-HT carrier, inhibits the reuptake of NA, mainly at higher doses. Therefore, a contribution of the noradrenergic system in the interaction of 5-HT_{1A} receptors and venlafaxine could be hypothesized. Some microdialytical and electrophysiological data have suggested that noradrenergic cell bodies in the brainstem, notably in the locus coeruleus, are subject to the influence of 5-HT_{1A} receptors (for review see Millan, 2002). In this sense, we have previously shown that the activation of 5-HT_{1A} receptors by 8-OH-DPAT enhanced the inhibitory effect of venlafaxine on locus coeruleus neurons *in vivo* (Berrocoso and Mico, 2007). This fact is very relevant to our study of the antinociceptive effect because 8-OH-DPAT blocked the analgesic effect of venlafaxine. This suggests that the combination of venlafaxine and an agonist of 5-HT_{1A} receptors will lead to them cooperating to decrease NA neurotransmission and inhibit the descending noradrenergic pain pathway and consequently block the analgesic effect of venlafaxine. Regarding the antidepressant effect, our data demonstrated that the activation of 5-HT_{1A} receptors significantly enhanced, instead of counteracting, the antidepressant-like effect of venlafaxine. Similarly, Mayorga's study (Mayorga et al., 2001) showed that the antidepressant-like effect of the 5-HT reuptake inhibitors fluoxetine and citalopram is blocked in 5-HT_{1A} receptor knockout mice. In contrast, the antidepressant-like effect of the NA reuptake inhibitor desipramine remained effective. Thus, overall data would rule out a link between 5-HT_{1A} receptors and the inhibition of the reuptake of NA for the

antidepressant-like effect of 8-OH-DPAT plus venlafaxine and would imply a noradrenergic contribution in the antinociceptive effect. That is, the antagonism of 5-HT_{1A} receptors at both raphe and locus coeruleus level will lead to an increase in 5-HT and NA release in the spinal cord, which will contribute to a greater analgesic effect of venlafaxine. Finally, the affinity of 8-OH-DPAT for other 5-HT receptor subtypes should be considered in the present study. 8-OH-DPAT is a moderate agonist of 5-HT₇ receptors (Hedlund et al., 2004), but data available suggest that the blockade, rather than the agonism, of these receptors is involved in the antidepressant-like or nociceptive responses (Dogrul and Seyrek, 2006; Guscott et al., 2005). Therefore, their contribution seems unlikely because in the present study 8-OH-DPAT facilitated the antidepressant-like effect and blocked the antinociceptive effect of venlafaxine.

The main aim of the present study was to explore and compare the role of 5-HT_{1A} receptors in depression and pain, two very interrelated clinical entities and extend the study of this modulation to the antidepressant and analgesic effects of venlafaxine. For this purpose we chose two behavioral tests with similar procedures (e.g. acute models, animal manipulation, drug administration). Nevertheless, one limitation of the present study is that we have used an acute model instead of a chronic one that resembles the clinical situation. Furthermore, the role of various subtypes of 5-HT and adrenergic receptors in the action of venlafaxine, or other antidepressants, depends on the parameters and components of the model under study. Thus, methodology can determine the conclusions drawn from every study, making them test-dependent (Millan, 2002, 2006). However, this approach was chosen in order to minimize the physiological adaptations in the 5-HT_{1A} receptors induced by a stressful chronic manipulation (Grippo et al., 2005), which would probably interfere with the purpose of our study. Moreover, models of depression based on chronic stress induce changes in pain thresholds (Hammack et al., 1999; Quintero et al., 2000). Therefore, the use of more ethological models of depression and pain and long-term pharmacological treatments will give us the opportunity to further investigate the modulation of 5-HT_{1A} receptors in the antidepressant and analgesic effects of antidepressants. Additionally, considering the great interest in the development of add-on strategies for the antidepressant effect based on the combination of 5-HT_{1A} receptor antagonists with antidepressants (Adell et al., 2005), it would be very attractive to extend these studies to analgesia in order to get an

original, mechanistic-based analgesia-augmentation strategy.

In conclusion, the present study provides evidence that 5-HT_{1A} receptors differentially modulate the antidepressant-like and the antinociceptive effects of the antidepressant venlafaxine in rodents in the behavioural and pain models investigated. We contend that the blockade of 5-HT_{1A} receptors in the forebrain counteracts the positive antidepressant effect at raphe nuclei level, and consequently the global effect evidenced is an antagonism of the effect of venlafaxine in the antidepressant-like test. In contrast, in the hot-plate test the antinociceptive effect of venlafaxine is potentiated, probably due to blockade of 5-HT_{1A} receptors situated in the raphe nuclei. These results suggest that blockade of 5-HT_{1A} receptors is an innovative augmentation strategy for the antinociceptive effect in antidepressants which act on noradrenergic and serotonergic neurotransmission, since the participation of both monoamines is necessary for the clinical analgesic effect.

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Statement of Interest

None.

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