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ORIGINAL INVESTIGATION

Differential role of 5-HT_{1A} and 5-HT_{1B} receptors on the antinociceptive and antidepressant effect of tramadol in mice

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Abstract
Rationale Tramadol, (1*RS*,2*RS*)-2-[(dimethylamine)-methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is an atypical analgesic which binds weakly to ì-opioid receptors and enhances the extra-neuronal concentration of noradrenaline and serotonin by interference with both the uptake and release mechanisms.
Objectives The present study was undertaken to evaluate the potential role of 5-HT_{1A} and 5-HT_{1B} receptors on the analgesic and antidepressant-like effect of tramadol.
Methods 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; 0.2–0.8, 8 mg/kg) or a selective 5-HT_{1B} receptor antagonist (SB 216641; *N*-[3-(3-dimethylamino) ethoxy-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-(1,1′-biphenyl)-4-carboxamide; 0.2–0.8, 8 mg/kg) was investigated in mice in combination with tramadol by means of the hot-plate test, a phasic nociceptive model, and the forced swimming test, a paradigm aimed at screening potential antidepressants.
Results The results showed that WAY 100635 enhanced the antinociceptive effect and produced a large decrease in the antidepressant-like effect of tramadol. In contrast, SB 216641 did not significantly modify either the analgesic or the antidepressant-like effects of tramadol.
Conclusions These findings suggest that 5-HT_{1A} receptors modulate the analgesic and the antidepressant-like effects of tramadol in differing ways. The results suggest the involvement of the 5-HT_{1A} autoreceptors from the raphe nuclei and spinal 5-HT_{1A} receptors in the antinociceptive effect. In contrast, the 5-HT_{1A} receptors located in the forebrain may be responsible for the blockade of the antidepressant-like effect of tramadol. 5-HT_{1B} receptors seem not to modify these effects in the models investigated.

Keywords Serotonin · Serotonin receptor · Antinociception · Antidepressant · Mice

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Introduction

Tramadol, (1*RS*,2*RS*)-2-[(dimethylamine)-methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is a centrally acting analgesic which is used mainly for the treatment of moderate or severe pain. It is a synthetic opioid in the aminocyclohexanol group that binds weakly to μ-opioid receptors (Hennies et al. 1988). However, it has been shown that tramadol possesses a non-opioid mechanism that contributes to its pharmacological actions. Indeed, it enhances the extraneuronal concentrations of the monoamine neurotransmitters, noradrenaline (NA), and serotonin (5-HT), by interfering with their reuptake and release mechanisms (Driessen et al. 1993; Bamigbade et al. 1997).

Considering the opioid and monoaminergic properties of tramadol, it has been claimed to have antidepressant-like effects, in addition to its analgesic action. It has previously been shown that tramadol is active in behavioural models predictive of antidepressant activity, such as, the forced swimming test and reserpine test in mice and the learned helplessness test in rats (Rojas-Corrales et al. 1998, 2002, 2004). In line with this evidence, other studies have shown that it induces changes in the central nervous system (CNS) similar to those induced with conventional antidepressants.

Whereas it decreases the binding of frontocortical β-adrenoceptors, 5-HT_{2A} receptors (Hopwood et al. 2001) and α_2-adrenoceptors (Faron-Gorecka et al. 2004a), it increases the binding of α_1-adrenoceptors and dopamine D_2/D_3 receptors (Faron-Gorecka et al. 2004b). Moreover, tramadol has been used successfully in several psychiatric disorders such as refractory major depression (Shapira et al. 2001), severe suicidal ideation (Spencer 2000), antidepressant potentiation (Fanelli and Montgomery 1998) and obsessive–compulsive disorder (Shapira et al. 1997; Goldsmith et al. 1999).

The 5-HT system plays a multifaceted role in the regulation of pain and depression. 5-HT pathways within the CNS arise from a series of nuclei situated in the midline of the brain stem, the raphe nuclei, which represent the richest source of neural 5-HT synthesized in the mammalian brain (Verge and Calas 2000). Moreover, they are in connection with many areas that are involved in pain and emotion. The raphe nuclei project, directly and indirectly, to the dorsal horn modulating pain processes, while they also have ascending projections to the forebrain area, where the regulation of affective states takes place, including the affective dimensions of pain (Wang and Nakai 1994; Adell et al. 2005). So, the raphe nuclei may represent crossroads in the integration of the pathways of nociception and emotion. In this complex pattern, the role of 5-HT_{1A} and 5-HT_{1B} receptors has attracted much attention (Millan 2002; Adell et al. 2005; Mico et al. 2006). The blockade of the 5-HT_{1A} autoreceptors, located in the raphe nuclei, and 5-HT_{1B} receptors, located in 5-HT nerve terminals, has been shown to modulate the antidepressant and the antinociceptive response of 5-HT reuptake inhibitors (Adell et al. 2005; Mico et al. 2006). In addition, the increase in the tonic activation of forebrain 5-HT_{1A} receptors has been correlated with the antidepressant effect of selective 5-HT reuptake inhibitors (SSRIs) and electroconvulsive shock therapy (Haddjeri et al. 1998). Finally, the 5-HT_{1A} and 5-HT_{1B} receptors located in the spinal cord have been shown to be involved with antinociception (Hains et al. 2003), pronociception (Alhaider and Wilcox 1993) or even to have no effect (Sasaki et al. 2001). Altogether, this suggests that different areas and different populations of 5-HT_{1A} and 5-HT_{1B} receptors could account in differing ways for the modulation of pain and depression. Considering that tramadol enhances extracellular 5-HT levels (Bamigbade et al. 1997), it may indirectly activate 5-HT_1 receptors modulating 5-HT transmission. Indeed, the antinociceptive effect of tramadol is enhanced in the hot-plate test in mice, by co-administration of both pindolol, a putative antagonist of β-adrenergic and 5-$HT_{1A/B}$ receptors, and WAY 100635, a selective agonist of 5-HT_{1A} receptors, and reduced by the selective agonist of 5-HT_{1A} receptors, 8-OH-DPAT (Rojas-Corrales et al. 2000, 2005). Moreover, neither the selective blockade nor selective agonist of 5-HT_{1B} receptors induced a net effect in the same test at the doses tested.

Therefore, considering the above mentioned data, the present study was designed to examine the role of both 5-HT_{1A} and 5-HT_{1B} receptors in the antinociceptive and antidepressant-like effects of tramadol by means of the hot-plate test, a phasic model of pain, and the forced swimming test, a model predictive of antidepressant activity.

Materials and methods

Animals

The experiments were performed using albino male CD1 mice (25–30 g). All the animals were provided by the "Servicio de Experimentación y Producción Animal" (SEPA) of the University of Cádiz. The animals were maintained under standard conditions: 12-h light–dark schedule (lights on at 8.00 a.m.) 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 of the School of Medicine at the University of Cádiz (License number 079604) and complied with the International Association for the Study of Pain ethical guidelines (Zimmermann 1983). Animal care and use procedures conformed to International European Ethical Standards (86/609-EEC), and Spanish Law (RD 223/1988) for the care and use of laboratory animals. Animals were housed in groups of 10 and a 7-day acclimatization period was allowed before the experiments. All the mice were experimentally naive and used only once and 10 subjects were used per group. The experiments were performed during the light phase between 9.00 a.m. and 4.00 p.m., by a single experimenter.

Drugs

The following drugs were used in the study: tramadol (1*RS*,2*RS*)-2-[(dimethylamine)-methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride (courtesy of Grünenthal-Andrómaco, Spain); WAY 100635 (*N*-2-[4-(2-methoxyphenyl-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexane carboxamide), (Sigma–Aldrich–Quimica, Spain) and SB 216641, (*N*-[3-(3-dimethylamino) ethoxy-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)- (1,1′-biphenyl)-4-carboxamide), (Tocris, UK).

Treatment and experimental design

The protocol of administration of drugs were chosen on the basis of our previous studies (Rojas-Corrales et al. 1998,

2005). The selective antagonists of 5-HT_{1A} receptors (WAY 100635) and 5-HT_{1B} receptors (SB 216641) were subcutaneously injected 15 min after tramadol, which was intraperitoneally administered 30 min before the hot-plate test and 60 min before the forced swimming test. All drugs were prepared immediately before use. They were dissolved in physiological saline (NaCl 0.9%) and injected at a volume of 10 ml/kg of body weight. Control animals received saline only. The treatments were administered under blind conditions.

To study the role of the blockade of 5-HT_{1A} and 5-HT_{1B} receptors on the antinociceptive effect of tramadol in the hot-plate test in mice, several doses of WAY 100635 (0.2–0.8, 8 mg/kg, s.c.) or SB 216641 (0.2–0.8, 8 mg/kg, s.c.) were co-administered with an effective dose of tramadol (32 mg/kg, i.p.). Secondly, to explore the role of the blockade of 5-HT_{1A} and 5-HT_{1B} receptors on the antidepressant-like effect of tramadol in the forced swimming test, several doses of WAY 100635 (0.2–0.8, 8 mg/kg, s.c.) or SB 216641 (0.2–0.8, 8 mg/kg, s.c.) were co-administered with tramadol (16–64 mg/kg, i.p.). The antagonists of 5-HT_{1A} and 5-HT_{1B} receptors have been shown to be selective ligands of these receptors in vivo and to exert their pharmacological actions in a very similar range of doses (Stamford et al. 2000).

Hot-plate test

The hot-plate test was used as a phasic pain model to evaluate the role of 5-HT_{1A} and 5-HT_{1B} receptors in the antinociceptive effect of tramadol. Antinociception was evaluated with a hot-plate apparatus (Digital DS-37 Socrel model; Milan, Italy) with a 25×25 cm metal surface maintained at 55.5±0.5°C surrounded by a 40-cm high Plexiglas wall (Woolfe and McDonald 1944). Latency was considered to be the time in seconds elapsed between when the animal was placed on the hot-plate surface and when it either licked or shook its hind paw or jumped. These actions are considered to be supraspinally integrated responses (Le Bars et al. 2001). Basal latency was determined as the mean of two or three trials with a delay of 30 min. After this, test latency was determined after drug injection. A cut-off time was established at 60 seconds to avoid tissue damage to the animal.

Forced swimming test

The forced swimming test was used to determine the role of 5-HT_{1A} and 5-HT_{1B} receptors in the antidepressant-like effect of tramadol following the classic method described by Porsolt (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 the 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-like activity.

Expression of results and statistical analysis

All data are expressed as the mean values±SEM. In the hot-plate test, the level of analgesia was expressed as a percentage of the maximal possible effect (%MPE) induced by the drugs related to basal latencies {%MPE=[(test latency−basal latency)/(cutoff−basal latency)]×100}±SEM. In the forced swimming test, the antidepressant-like effect was evaluated and expressed as immobility time (in seconds). The effects of tramadol when compared to the saline group were established by a Student *t* test for comparisons between two groups or by a one-way analysis of variance (ANOVA) followed by the Dunnett test. In the interaction studies, statistical analysis was performed using a two-way ANOVA. The factors of variance were tramadol treatment and 5-HT_{1A} and 5-HT_{1B} receptors antagonist treatment. To study the effect of the antagonist treatment on each dose of tramadol, a subsequent one-way ANOVA was used followed by the Dunnett test. A value of $p < 0.05$ was considered to be significant.

Results

Effect of 5-HT_{1A} receptor blockade on the antinociceptive effect of tramadol

The effect of administration of the selective 5-HT_{1A} receptor antagonist, WAY 100635 (0.2–1.6, 8 mg/kg), and tramadol (32 mg/kg) was studied in the hot-plate test (Fig. 1). Tramadol (32 mg/kg), as expected, significantly increased analgesia, compared with saline-treated animals (student *t* test; $p<0.001$). In the interaction study, a two-way ANOVA revealed a significant effect of WAY 100635 ($F_{(5,108)}=5.51$, $p<0.001$), tramadol ($F_{(1,108)}=209.02$, $p<0.001$), and interaction between WAY 100635 and tramadol ($F_{(5,108)}=3.27$, $p<0.01$). Subsequently, a one-way ANOVA showed that no dose of WAY 100635 significantly modified the effect with respect to saline ($F_{(5,54)}=1.05$, n.s.) and that WAY 100635 dose-dependently increased the antinociceptive response of tramadol. This combination reached statistical significance for WAY 100635 at 0.4, 0.8, 1.6 and 8 mg/kg compared with the administration of tramadol alone ($F_{(5,54)}=4.69$, $p<0.01$, Dunnett test).

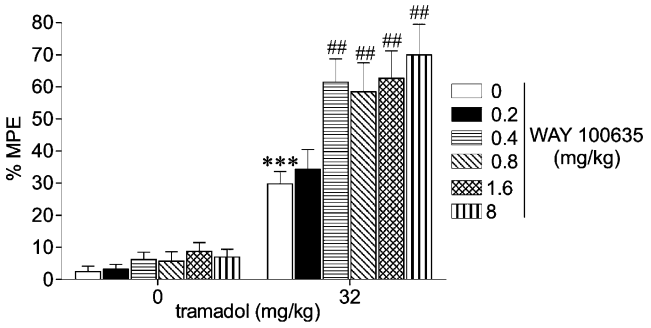

Fig. 1 Involvement of 5-HT$_{1A}$ receptor antagonist, WAY 100635, in the antinociceptive effect of tramadol in the hot-plate test in mice. Tramadol (32 mg/kg) was intraperitoneally administered 30 min before the test. WAY 100635 (0.2–1.6, 8 mg/kg) was subcutaneously injected 15 min after tramadol. Data are presented as mean±SEM of the percentage of maximum possible effect (%MPE); n=10 per value; ***p<0.001 vs saline group (Student t test), ##p<0.01 vs respective tramadol-treated group (Dunnett test)

Effect of selective 5-HT$_{1B}$ receptor blockade on the antinociceptive effect of tramadol

The effect of administration of the selective 5-HT$_{1B}$ receptor antagonist, SB 216641 (0.2–1.6, 8 mg/kg), and tramadol (32 mg/kg) was studied in the hot-plate test (Fig. 2).

First, tramadol (32 mg/kg) significantly increased analgesia, compared with saline-treated animals (student-t test; p<0.001). Next, a two-way ANOVA revealed a significant effect of tramadol ($F_{(1,108)}$=158.67, p<0.001), but neither SB 216641 ($F_{(5,108)}$=0.80, n.s.), nor the interactions between the two treatments reached statistical significance ($F_{(5,108)}$=1.69, n.s.). Subsequently, one-way ANOVA showed that no dose of SB 216641 significantly modified the percentage of maximal possible effect compared with control animals ($F_{(5,54)}$=0.92, n.s.) and that no dose of SB 216641 (0.2–1.6, 8 mg/kg) significantly modified the tramadol effect ($F_{(5,54)}$=1.30, n.s.).

Effect of selective 5-HT$_{1A}$ receptor blockade on the antidepressant-like effect of tramadol

The effect of administration of WAY 100635 (0.2–1.6, 8 mg/kg), and tramadol (16–64 mg/kg) was studied in the forced swimming test (Fig. 3). First, a one-way ANOVA showed a significant effect of the tramadol treatment ($F_{(3,36)}$=17.39, p<0.001; Dunnett test). Tramadol produced a dose-dependent reduction in the immobility time that differed significantly from control values at 32 and 64 mg/kg. Next, a two-way ANOVA demonstrated a significant effect of WAY 100635 ($F_{(5,216)}$=24.63, p<0.001), tramadol ($F_{(3,216)}$=16.67, p<0.001) and interaction between WAY 100635 and tramadol ($F_{(15,216)}$=4.23, p<0.001). Subsequently, a one-way ANOVA showed that no dose of WAY 100635 (0.2–1.6, 8 mg/kg) significantly modified the immobility time compared to saline ($F_{(5,54)}$=0.47, n.s.). However, the association of the lowest dose of tramadol, 16 mg/kg, with WAY 100635 at 8 mg/kg significantly increased the immobility time compared to tramadol injected alone ($F_{(5,54)}$=4.26, p<0.01, Dunnett test). The immobility effect of tramadol at 32 and 64 mg/kg was significantly increased by WAY 100635 at 0.4, 0.8, 1.6 and 8 mg/kg ($F_{(5,54)}$=10.15, p<0.001; $F_{(5,54)}$=15.50, p<0.001; Dunnett test, respectively).

Effect of selective 5-HT$_{1B}$ receptor blockade on the antidepressant-like effect of tramadol

The effect of administration of SB 216641 (0.2–1.6, 8 mg/kg), and tramadol (16–64 mg/kg) was studied in the forced swimming test (Fig. 4). First, a one-way ANOVA showed a

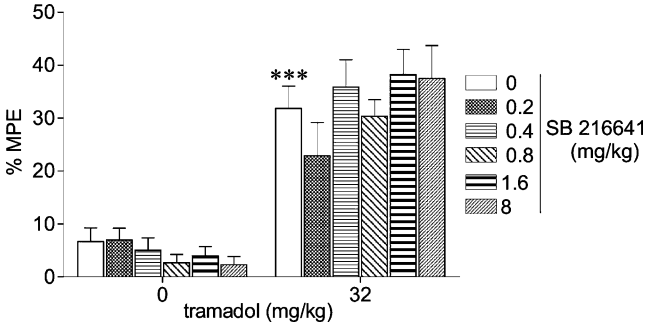

Fig. 2 Involvement of 5-HT$_{1B}$ receptor antagonist, SB 216641, in the antinociceptive effect of tramadol in the hot-plate test in mice. Tramadol (32 mg/kg) was intraperitoneally administered 30 min before the test. SB 216641 (0.2–1.6, 8 mg/kg) was subcutaneously injected 15 min after tramadol. Data are presented as mean±SEM of the percentage of maximum possible effect (%MPE); n=10 per value; ***p<0.001 vs saline group (Student t test)

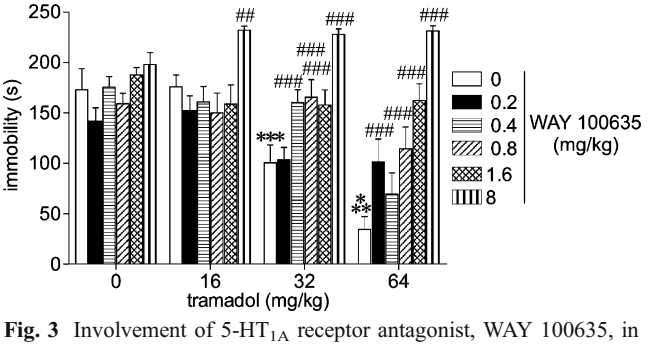

Fig. 3 Involvement of 5-HT$_{1A}$ receptor antagonist, WAY 100635, in the antidepressant-like effect of tramadol in the forced swimming test in mice. Tramadol (16–64 mg/kg) was intraperitoneally administered 60 min before test. WAY 100635 (0.2–1.6, 8 mg/kg) was subcutaneously injected 15 min after tramadol. Data are presented as mean± SEM of the immobility time; n=10 per value; ***p<0.001 vs saline group (Dunnett test), ##p<0.01 or ###p<0.001 vs respective tramadol-treated group (Dunnett test)

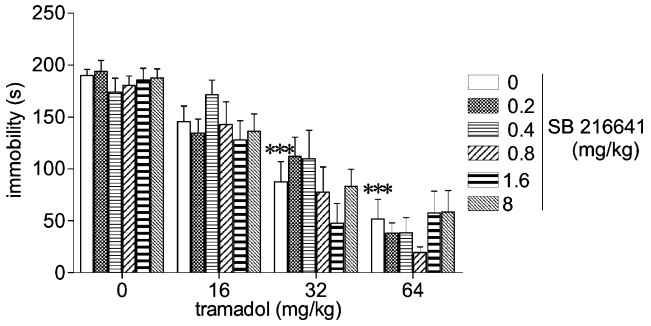

Fig. 4 Involvement of 5-HT_{1B} receptor antagonist, SB 216641, in the antidepressant-like effect of tramadol in the forced swimming test in mice. Tramadol (16–64 mg/kg) was intraperitoneally administered 60 min before test. SB 216641 (0.2–1.6, 8 mg/kg) was subcutaneously injected 15 min after tramadol. Data are presented as mean±SEM of the immobility time; n=10 per value; ***p<0.001 vs saline group (Dunnett test)

significant effect of the tramadol treatment ($F_{(3,36)}$=15.11, p<0.001; Dunnett test). Tramadol produced a dose-dependent reduction in immobility time that differed significantly from control values at 32 and 64 mg/kg. Next, a two-way ANOVA demonstrated a significant effect of tramadol ($F_{(3,211)}$=82.16, p<0.001), but neither SB 216641 ($F_{(5,211)}$=0.89, n.s.) nor the interaction between the two treatments ($F_{(15,211)}$=0.96, n.s.) significantly modified the immobility time. Subsequently, a one-way ANOVA showed that no dose of SB 216641 significantly modified the immobility time compared with control animals ($F_{(5,54)}$=0.51, n.s.) and that no dose of SB 216641 (0.2–1.6, 8 mg/kg) significantly modified the effect of tramadol compared to tramadol alone ($F_{(5,54)}$=0.80, n.s.).

Discussion

The present study shows that the acute administration of a selective 5-HT_{1A} receptor antagonist, WAY 100635, produces opposing effects on the analgesic and antidepressant-like effects of tramadol. Whereas the analgesic effect of tramadol is enhanced, the antidepressant-like effect is blocked by WAY 100635. Moreover, the selective 5-HT_{1B} receptor antagonist, SB 216641, did not modify significantly either the antinociceptive or antidepressant-like effect of tramadol.

The current study shows and confirms the study of Rojas-Corrales et al. 2005, that the antinociceptive effect of tramadol is enhanced by the administration of WAY 100635 in the hot-plate model. Tramadol both increases the release and inhibits the re-uptake of 5-HT in the raphe nuclei (Bamigbade et al. 1997) suggesting a possible implication of these nuclei in tramadol nociception. In the raphe nuclei, the 5-HT release is controlled by 5-HT_{1A} autoreceptors (Sprouse and Aghajanian 1987). Thus, it has been shown that the acute administration of SSRIs produces a local increase of 5-HT in the raphe nuclei, diminishes neuronal firing and produces a negative feedback regulation of transmitter release in projecting areas. Therefore, the blockade of these negative feedback mechanisms with 5-HT_{1A} receptor antagonists enhances the 5-HT increase produced by SSRIs (Adell et al. 2005). Considering that tramadol, like SSRIs, is able to enhance the 5-HT availability in the raphe nuclei (Bamigbade et al. 1997), it could be suggested that the combination with WAY 100635 blocked the negative feedback at raphe level and potentiated the descending monoaminergic pathway, consequently enhancing the analgesic effect displayed by tramadol (Mico et al. 2006). This hypothesis has been successfully demonstrated in compounds endowed with the ability to enhance serotonergic neurotransmission, such as the classic analgesic paracetamol (Roca-Vinardell et al. 2003), and the 5-HT/NA antidepressant venlafaxine (data not published) in the hot-plate test in mice, and also in the tricyclic antidepressant clomipramine in models of neuropathic pain (Ardid et al. 2001).

However, we cannot rule out the spinal level contribution in the antinociceptive effect of WAY 100635 plus tramadol. At this level, the activation of projection neurons or inhibitory GABAergic/enkephalinergic interneurons has been related with antinociceptive or pronociceptive actions, respectively (Millan 2002). In addition, the nature of the somatic noxious stimulus has been implicated with the activation of spinal 5-HT_{1A} receptors (Bonnefont et al. 2005). WAY 100635 intrathecally administered enhances the antinociceptive effect of sub-effective doses of 5-HT, paracetamol or venlafaxine in the paw pressure test, a phasic pain model. In contrast, WAY 100635 counteracts the antinociceptive effect in the formalin model, a tonic pain model (Bonnefont et al. 2005). In our study, the hot-plate test, a phasic pain model, was used to measure the antinociceptive action; thus, it could be suggested that spinal 5-HT_{1A} interneurons play a role in the effect of tramadol plus WAY 100635. Moreover, recent findings showed that intrathecal administration of 8-OH-DPAT produces pronociception in the paw pressure test and antinociception in the formalin test (Bardin and Colpaert 2004). We have previously demonstrated that the antinociceptive effect of tramadol is blocked by the systemic administration of 8-OH-DPAT in the hot plate test in mice and in the plantar test in rats, two models of phasic pain (Rojas-Corrales et al. 2000). Altogether, these data suggest that WAY 100635 enhanced the antinociceptive effect of tramadol due to the integration of both supraspinal and spinal sites.

Regarding the antidepressant effect, this study shows that the effect of tramadol is blocked by WAY 100635. The antidepressant effect of SSRIs has been attributed to the functioning of 5-HT_{1A} receptors located in the raphe nuclei

and forebrain. As previously mentioned, in the raphe nuclei, the 5-HT_{1A} receptors are located somatodendritically and act as autoreceptors controlling the release of 5-HT in terminal areas, such as the forebrain. In the forebrain, specifically in the dorsal hippocampus, it has been shown that the activation of postsynaptic 5-HT_{1A} receptors is necessary for the antidepressant effect of tricyclic antidepressants, SSRIs, IMAO and electroconvulsive therapy (Haddjeri et al. 1998). WAY 100635 possesses antagonistic properties at the level of both somatodendritic and postsynaptic 5-HT_{1A} receptors (Fletcher et al. 1996). Thus, its intravenous administration will act in the raphe nuclei and forebrain. That is, while WAY 100635 potentiates the rise in extracellular level of 5-HT in the raphe nuclei, it concurrently blocks forebrain postsynaptic 5-HT_{1A} receptors, thereby partly cancelling out the effect of the enhanced 5-HT concentration on overall 5-HT neurotransmission (Haddjeri et al. 1998; Beique et al. 2000). The blockade of forebrain 5-HT_{1A} postsynaptic receptors will mask the increase of 5-HT release due to the blockade of 5-HT_{1A} somatodendritic receptors from the raphe nuclei and consequently, a decrease in the antidepressant-like effect is evidenced for the combination of WAY 100635 and tramadol in the forced swimming test. However, a certain number of studies have shown that postsynaptic 5-HT_{1A} receptors in other forebrain areas (i.e. amygdala and medial prefrontal cortex) have some influence on the 5-HT raphe nuclei release via a feedback mechanism (Bosker et al. 1997; Casanovas et al. 1999). By means of this loop, blockade of these receptors can lead to increase in serotonergic cell firing and 5-HT release. If this were the case, then the antidepressant action of tramadol would be potentiated; but overall in our study, the global effect was an antagonism. The complexity of the mechanism of action of tramadol suggests that another class of receptor (other than the one examined in this study) could be implicated. In any case, our data could suggest that, in the absence of another possible mechanism, the 5-HT_{1A} receptors probably located in the forebrain (i.e. dorsal hippocampus) are responsible for the blockade of the antidepressant-like effect of tramadol. This contributes to reinforcing the pivotal role given to 5-HT_{1A} receptors in the forebrain in depression. It is interesting to note that venlafaxine, a dual antidepressant with analgesic effects, has shown the same results in these tests (data not published), which contributes to reinforcing the idea of the similarities, both molecular and pharmacological, between these compounds (Markowitz and Patrick 1998). In addition, we have recently reported that the pre-administration of 8-OH-DPAT enhanced the inhibitory effect of tramadol on locus coeruleus neurons in vivo (Berrocoso et al. 2006).

5-HT_{1B} receptors have been consistently implicated in mediating antinociception and depression (Millan 2002; Sari 2004). 5-HT_{1B} receptors are located on 5-HT nerve terminals where they act as autorreceptors responding to 5-HT released in the terminal fields by inhibiting further transmitter release (Adell et al. 2005). Thus, the blockade of 5-HT_{1B} receptors may facilitate the analgesic (Roca-Vinardell et al. 2003) and the antidepressant-like effect (Tatarczynska et al. 2004). In addition, the activation of 5-HT_{1B} receptors located in the spinal cord has been related to nociception. Indeed, the spinal administration of 5-HT_{1B} agonists produces antinociception in the tail flick and hot-plate test (Eide et al. 1990; Alhaider and Wilcox 1993; Ali et al. 1994; Sawynok and Reid 1994; Xu et al. 1994). Our study has not found a modulator role of 5-HT_{1B} receptors in the analgesic or the antidepressant-like effect of tramadol. Because we have previously shown that SB 21664 (0.1–0.8 mg/kg) did not modify the analgesic effect of tramadol, in this study the SB 21664 range was increased to (0.2–1.6, 8 mg/kg) confirming the initial study. As in the study of analgesia, with respect to the antidepressant profile, we have not found that 5-HT_{1B} receptors play any role in the effect of tramadol in the forced swimming test in mice, even using a supramaximal dose (8 mg/kg).

In summary, the current study provides evidence that WAY 100635, an antagonist of 5-HT_{1A} receptors, modulates the analgesic and the antidepressant-like effect of tramadol in opposing ways in the hot-plate and forced swimming tests. It could be contended that different 5-HT_{1A} receptor populations might be involved in the net effect displayed in these models. We hypothesize that the increase in the antinociceptive effect of tramadol is mainly due to the blockade of 5-HT_{1A} autoreceptors from the raphe nuclei increasing 5-HT release in the spinal cord, potentiating the descending serotonergic pathway. Additionally, spinal 5-HT_{1A} receptors may contribute to the analgesic effect. In contrast, 5-HT_{1A} receptors located in the forebrain are responsible for the blockade of the antidepressant-like effect of tramadol. Regarding 5-HT_{1B} receptors, they are not involved in either the analgesic or antidepressant-like effect of tramadol in these models. Moreover, considering the efficacy of tramadol in neuropathic pain, a pathology usually alleviated by antidepressants, the presently available data should be corroborated by further neuropathic pain models and other models of depression before a final conclusion can be reached.

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