Psychopharmacology (2007) 193:97–105
DOI 10.1007/s00213-007-0761-8

ORIGINAL INVESTIGATION

Role of serotonin 5-HT$_{1A}$ and opioid receptors in the antiallodynic effect of tramadol in the chronic constriction injury model of neuropathic pain in rats

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Received: 17 October 2006 / Accepted: 28 February 2007 / Published online: 29 March 2007

Abstract
Rationale Tramadol (1*RS*, 2*RS*)-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl)-cyclohexanol) is an atypical centrally acting analgesic agent with weak opioid receptor affinity that, like some antidepressants, enhances the extraneuronal concentrations of the monoamine neurotransmitters, noradrenaline and serotonin, by interfering with their re-uptake and release mechanisms.
Objectives The present study was undertaken to evaluate the potential role of 5-HT$_{1A}$ receptors and opioids receptors in the analgesic effect of tramadol in neuropathic pain. 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) or an opioid receptor antagonist (naloxone; naloxone hydrochloride dihydrate) was investigated in combination with tramadol by means of the cold-plate test in the chronic constriction injury model in rats.
Results The results showed that WAY-100635 (0.8 mg/kg) significantly enhanced the antiallodynic effect of non-effective doses of tramadol (5–10 mg/kg). In contrast, 8-OH-DPAT (0.5 mg/kg) counteracted the antiallodynic effect of an effective dose of tramadol (22 mg/kg). Naloxone (0.5 mg/kg) partially counteracted the antiallodynic effect of tramadol (22 mg/kg).
Conclusions These findings suggest the involvement of opioid and 5-HT$_{1A}$ receptors in the antinociceptive effect of tramadol and support the idea that the combination of tramadol with compounds having 5-HT$_{1A}$ antagonist properties could be a new strategy to improve tramadol-induced analgesia in neuropathic pain.

Keywords Serotonin · Serotonin 5-HT$_{1A}$ receptors · Neuropathic pain · Tramadol · Opioid receptors · Rat

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Introduction

Tramadol (1*RS*, 2*RS*)-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl)-cyclohexanol) is a centrally acting analgesic placed on step 2 pain ladder. Tramadol is a synthetic opioid that binds weakly to μ-opioid receptors (Hennies et al. 1988). In addition, tramadol is mainly metabolised by *O*-demethylation, and (+)-*O*-desmethyltramadol (M1) also binds to μ-opioid receptors with relatively high affinity (Raffa et al. 1993) participating to the analgesic activity. However, it has been shown that tramadol possesses a non-opioid mechanism that contributes to its pharmacological actions. It enhances the extraneuronal concentrations of the monoamine neurotransmitters noradrenaline and serotonin (5-HT) by interfering with their re-uptake and release mechanisms (Bamigbade et al. 1997; Driessen et al. 1993). In this sense, there are parallels between the mechanism of action of tramadol and that of antidepressants, which are believed to potentiate the effect of biogenic amines in endogenous pain-modulating systems and are used in the clinical treatment of neuropathic pain (Mico et al. 2006a). Furthermore, in addition to its analgesic effect, tramadol has been shown to be active in behavioural models predictive of antidepressant activity (Berrocoso et al. 2006b; Rojas-Corrales et al. 1998, 2002, 2004).

Peripheral nerve injury often leads to neuropathic pain, which is characterised by hyperalgesia (increased response to noxious stimuli), allodynia (painful sensation from a stimuli, which is not normally painful) and spontaneous pain. In these situations, classical opioid compounds have limited efficacy in alleviating neuropathic pain, both clinically and in animal models (Zimmermann 2001). In contrast to classical opioids, tramadol has shown a dose-related reversal of thermal hyperalgesia in mononeuropathic rats (Tsai et al. 2000), and it also relieves painful polyneuropathy and reduces allodynia in humans (Harati et al. 1998; Hollingshead et al. 2006; Sindrup et al. 1999). These results suggest the crucial role of the monoaminergic component of tramadol in alleviating neuropathic pain.

The monoaminergic system, specifically the one involving 5-HT, plays a multifaceted role in pain modulation, as it has not only produced antinociceptive but also pronociceptive actions (for review see Millan 2002). Among the many subtypes of 5-HT receptors potentially contributing to medullo-spinal modulation of pain, the 5-HT_{1A} receptor has a potentially significant role, as it is expressed both in supraspinal and spinal areas related with the modulation of nociception (Azmitia et al. 1996; Marlier et al. 1991). Administration of 5-HT_{1A} receptor agonists in the spinal cord has produced both pro- and antiallodynic effects (Millan 2002). In the rostroventromedial medulla, the administration of 5-HT_{1A} receptor agonists suppressed the release of 5-HT, probably by acting on 5-HT_{1A} autoreceptors of serotoninergic neurons in the raphe nucleus. Thus, it has been shown that the acute administration of selective 5-HT re-uptake inhibitors (SSRIs) preferentially increases extracellular levels of 5-HT in the raphe nuclei, resulting in an activation of somatodendritic 5-HT_{1A} receptors, slowing the spontaneous firing rate of its neurones and decreasing 5-HT release in projecting areas (Sprouse and Aghajanian 1987). Thus, the preventive blocking of somatodendritic 5-HT_{1A} autoreceptors by specific receptor antagonists could prevent the reduction of 5-HT release in terminal areas caused by 5-HT uptake blockers and potentiates their effects in terminal areas (Artigas 1995; Romero and Artigas 1997). In agreement with this, it has been reported recently that after medial medullary administration of a 5-HT_{1A} receptor antagonist, descending pain regulatory pathways are uninhibited, leading to a selective attenuation of neuropathic hypersensitivity (Wei and Pertovaara 2006). Tramadol, as well as SSRIs, enhances extracellular 5-HT levels in the vicinity of cell bodies and dendrites in the raphe nuclei (Bamigbade et al. 1997), and subsequently, it may indirectly activate presynaptic 5-HT_{1A} receptors, bringing into action a negative feedback control.

We have previously reported that the antinociceptive effect of tramadol is enhanced in acute pain models by the systemic administration of both pindolol, a putative antagonist of β-adrenergic and 5-$HT_{1A/B}$ receptors (Rojas-Corrales et al. 2000), and WAY-100635, a selective antagonist of 5-HT_{1A} receptors, and reduced by the selective agonist of 5-HT_{1A} receptors, 8-OH-DPAT, systemically injected (Berrocoso et al. 2006b; Rojas-Corrales et al. 2000, 2005). Therefore, considering that cold allodynia is one of the main clinical symptoms of neuropathic pain (Zimmermann 2001), the present study was designed to examine the role of 5-HT_{1A} receptors and opioid receptors in the cold-antiallodynic effects of tramadol in sciatic nerve-ligated rats, trying to elucidate the cooperative or non-cooperative role of 5-HT_{1A} and opioid receptors in tramadol antinociception.

Materials and methods

Animals

Experiments were performed using albino male Sprague–Dawley rats (body weight of 150–180 g at the time of surgery). 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 at 8:00 A.M.) with ad libitum food and water and constant temperature (21±1°C). All procedures and animal handling met the guidelines of the National Institutes of Health detailed in the "Principles of animal laboratory care" (National Institutes of Health 1996), European Communities directive 86/609/EEC and Spanish Law (RD 1201/2005) regulating animal research. The experimental protocols were reviewed and approved by the Committee for Animal Experimentation at the University of Cádiz. Animals were housed in groups of four, and a 7-day acclimatisation period was allowed before the experiments. Nine to 14 subjects were used per group. On each testing day, the rats were brought into the behaviour room 2 h before the test session to habituate them to the environment. The experiments were performed during the light phase between 9:00 A.M. and 4:00 P.M. by a single experimenter.

Drugs, treatment, and experimental procedure

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), 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamine) tetralin hydrobromide) and naloxone (naloxone hydrochloride dihydrate; provided by Sigma-Aldrich-Quimica, Spain).

The selective 5-HT_{1A} receptor antagonist (WAY-100635) and agonist (8-OH-DPAT) were subcutaneously injected 10 min after tramadol, which was intraperitoneally administered 15 min before the test. The antagonist and agonist of 5-HT_{1A} 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). The nonselective receptor antagonist naloxone was intraperitoneally injected 10 min after tramadol, which was intraperitoneally administered 15 min before the test. All drugs were freshly prepared just before use. They were dissolved in physiological saline (NaCl 0.9%) and injected at a volume of 2 ml/kg of body weight. Control animals received saline only. The treatments were administered under blind conditions.

Neuropathic pain model: chronic constriction injury

The chronic constriction injury was produced as described previously (Bennett and Xie 1988). Rats were anaesthetised with chloral hydrate (400 mg/kg, i.p., approved by the Committee for Animal Experimentation at the University of Cádiz) and the left sciatic nerve exposed at the mid-thigh level proximal to the sciatic trifurcation. Four chromic gut ligatures (4/0) were tied loosely around the nerve, 1–2 mm apart so that the vascular supply was not compromised. In all the animals, the overlying muscle was closed in layers with 4/0 synthetic absorbable surgical suture. The skin was closed and sutured with 4/0 silk thread. In sham animals, an identical dissection was performed in the left paw, except that the sciatic nerve was not ligated. Animals that presented autotomy of the distal phalanges of at least one toe of the injured paw were excluded from these studies. Self-mutilation was presented in 10.5% of the animals tested. Autotomy was never seen in the hind paw on the sham-operated animals.

Nociceptive test procedure: cold-plate test

Cold-induced ongoing pain was determined as described by Jasmin et al. (1998) and Sun et al. (2004). The rat was placed on a metal plate kept at a cold temperature (4±1°C) and covered with a transparent plastic box (280×250×210 mm). When the animal very briskly lifted its left hind paw, it was counted as a nociceptive response. Thus, the number of times that the rat strongly briskly lifted its left hind paw from the floor during the 2 min was recorded at a time interval of 15 min throughout a 60-min observation period. The steps accompanied with walking and slowly lifting related to locomotion were not counted. This behaviour was assessed at room temperature (22±1°C). Paw lifting was scored by an observer who was unaware of the treatment condition.

Expression of results and statistical analysis

All data are expressed as the mean values±SEM. The level of analgesia was expressed as the number of times the rat lifted its left paw. To investigate global effects, areas under the time–course curves ($AUC_{0–60\ min}$) of the antiallodynic effects were calculated from mean scores (±SEM) at each time, using the trapezoidal method.

Experimental data were analysed by using the computer program Graphd Prism (v. 3.0; GraphPad Software). Statistical significance was assessed by means of unpaired Student's *t* test for comparisons between sham and injured animals. Three-way and/or two-way repeated measures analysis of variance (ANOVA) followed by the Bonferroni test was used when the time–course of the effects was studied. To study the effect of the different treatments by the AUCs, one-way ANOVA followed by a Dunnett test was used to analyse the dose response curve for tramadol and two-way ANOVA followed by the Bonferroni test was used in the interaction studies. The number and type of factors of variance have been stated in each statistical analysis. A value of $p<0.05$ was considered to be significant.

Results

Effect of chronic constriction injury on nociceptive response

At 4°C, very few lifts were observed in sham animals (3.36±0.43, $n=11$) compared with the number of paw lifts in injured animals (24.45±2.47, $n=11$; $t=8.43$, $df=20$, $p<0.0001$, Student's *t* test).

Antiallodynic effect of tramadol

Tramadol induced a decrease in the number of times the paw was lifted in a dose-related manner over the time tested. Two-way repeated measures ANOVA revealed a significant effect of tramadol treatment (between subjects: $F_{(4,60)}=9.65$, $p<0.0001$), time (within subjects: $F_{(4,240)}=137.9$, $p<0.0001$) and the interaction factor ($F_{(16,240)}=7.20$, $p<0.0001$). Bonferroni post hoc test showed that tramadol at 22 mg/kg displayed a clear and significant antiallodynic effect at 15, 30 and 45 min after the drug injection compared with saline treatment ($p<0.01$, 0.05 and 0.05, respectively, for each time point; see Fig. 1a). Accordingly, one-way ANOVA analysis of the AUC data showed a significant effect of tramadol treatment (between subjects: $F_{(4,60)}=13.20$, $p<0.001$). The subsequent Dunnett post hoc test showed that tramadol at 22 mg/kg significantly decreased this parameter compared to control ($p<0.01$; see Fig. 1b).

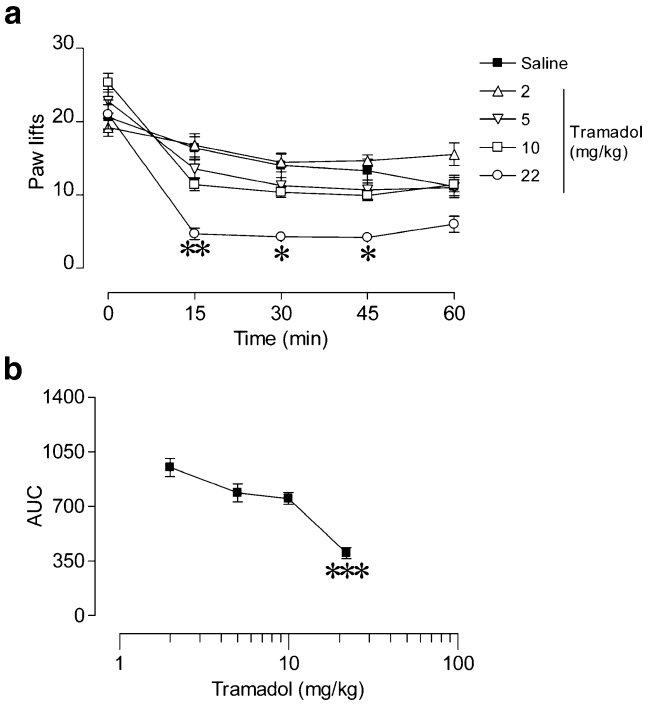

Fig. 1 Antiallodynic effect of tramadol in the cold-plate test in neuropathic rats. Tramadol (2–10, 22 mg/kg) was intraperitoneally administered 15 min before the beginning of the test. **a** Mean number of hind paw lifts per 2 min on the cold-plate test. *Asterisks* indicate a significant difference compared to saline group (*$p<0.05$, **$p<0.01$; Bonferroni post hoc test). **b** Mean areas under the curve (*AUC*) from 0–60 min after drug administration. AUC were calculated by summing the different scores obtained at each individual time using the trapezoidal method. AUC value for saline group is 893.84±88.03. *Asterisks* indicate significant difference respect saline group (***$p<0.001$ vs saline group; Dunnett post hoc test). Error bars denote the standard error of the mean; $n=9$–14 per value

Effect of selective 5-HT_{1A} blockade in the antiallodynic effect of tramadol

The combination of WAY-100635 (0.8 mg/kg) with tramadol (5–10 mg/kg) significantly increased the effect of tramadol. Three-way repeated measures ANOVA revealed a significant effect of tramadol treatment (between subjects: $F_{(2,54)}=10.90$, $p<0.001$), WAY-100635 treatment (between subjects: $F_{(1,54)}=22.50$, $p<0.001$) and time (within subjects: $F_{(4,260)}=107.93$, $p<0.001$). Significant interactions between tramadol and WAY-100635 treatment ($F_{(2,54)}=3.59$, $p<0.05$) and tramadol treatment and time ($F_{(8,216)}=4.89$, $p<0.001$) were also observed. No significant interactions between WAY-100635 and time ($F_{(4,216)}=1.03$, $p>0.05$) or among tramadol treatment, WAY-100635 treatment and time ($F_{(8,216)}=0.99$, $p>0.05$) were shown. Subsequently, two-way repeated measures ANOVA revealed a significant effect of tramadol and WAY treatment (between subjects: $F_{(5,54)}=10.31$, $p<0.0001$), time (within subjects: $F_{(4,216)}=107.09$, $p<0.0001$) and interaction ($F_{(20,216)}=2.56$, $p<0.001$). Bonferroni post hoc test showed that the combination of tramadol at 5 mg/kg plus WAY-100635 significantly decreased the number of paw lifting at min 30 respect saline ($p<0.05$). The combination with tramadol at 10 mg/kg significantly decreased the number of paw lifting at min 15 compared to tramadol (10 mg/kg), WAY-100635 and saline ($p<0.05$, 0.01 and 0.01, respectively), at min 30 compared to WAY-100635 and saline ($p<0.05$ and 0.001, respectively) and at min 60 compared to WAY-100635 ($p<0.05$; see Fig. 2a).

Two-way ANOVA analysis of the global effect through AUC data showed that there was a significant effect of tramadol treatment (between subjects: $F_{(2,54)}=14.30$, $p<0.0001$), WAY-100635 treatment (between subjects: $F_{(1,54)}=24.44$, $p<0.0001$) and interaction factor ($F_{(2,54)}=3.64$, $p<0.05$). Subsequently, Bonferroni post hoc test showed that WAY-100635 increased

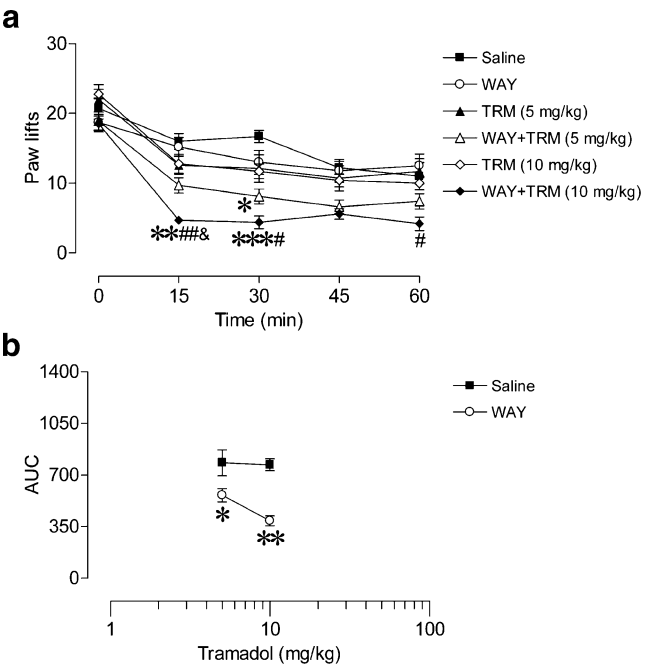

Fig. 2 5-HT1A receptor antagonist, WAY-100635, facilitated the antiallodynic effect of tramadol in the cold-plate test in neuropathic rats. Tramadol (5–10 mg/kg; *TRM*) was intraperitoneally administered 15 min before the beginning of the test. WAY-100635 (0.8 mg/kg; *WAY*) was subcutaneously injected 10 min after tramadol. **a** Mean number of hind paw lifts per 2 min on the cold-plate test. *Asterisks* indicate a significant difference compared to saline group (*$p<0.05$, **$p<0.01$, ***$p<0.01$), tramadol injected alone ($^{\&}p<0.05$) or WAY-100635 group ($^{\#}p<0.05$, $^{\#\#}p<0.01$; Bonferroni post hoc test). **b** Mean areas under the curve (*AUC*) from 0–60 min after drug administration. AUC were calculated by summing the different scores obtained at each individual time using the trapezoidal method. AUC values for saline+saline group = 911.25±40.65 and saline+WAY-100635 group = 834.25±69.79. *Asterisks* indicate a significant difference compared to saline (*$p<0.05$, **$p<0.01$; Bonferroni post hoc test). Errors bars denote the standard error of the mean; $n=10$ per value

the antiallodynic effect of tramadol at 5 mg/kg ($p<0.05$) and 10 mg/kg ($p<0.001$; see Fig. 2b).

Effect of selective 5-HT_{1A} agonism in the antiallodynic effect of tramadol

The combination of 8-OH-DPAT (0.5 mg/kg) with tramadol significantly decreased the antiallodynic effect of tramadol (22 mg/kg). Three-way repeated measures ANOVA revealed a significant effect of 8-OH-DPAT treatment (between subjects: $F_{(1,39)}=49.18$, $p<0.001$) and time (within subjects: $F_{(4,156)}=13.25$, $p<0.001$). No significant effect was reached for tramadol treatment (between subjects: $F_{(1,39)}=1.32$, $p>0.05$). There were significant interactions between tramadol and 8-OH-DPAT treatment ($F_{(1,39)}=23.43$, $p<0.001$), tramadol treatment and time ($F_{(4,156)}=5.57$, $p<0.001$), 8-OH-DPAT treatment and time ($F_{(4,156)}=27.35$, $p<0.001$) and among tramadol, 8-OH-DPAT treatment and time ($F_{(4,156)}=13.33$, $p<0.001$). Subsequently, two-way repeated measures ANOVA revealed a significant effect of treatment (tramadol and 8-OH-DPAT treatment; between subjects: $F_{(3,39)}=24.07$, $p<0.001$), time (within subjects: $F_{(4,156)}=13.25$, $p<0.001$) and interaction ($F_{(12,156)}=15.03$, $p<0.001$). Bonferroni post hoc test showed that the combination significantly increased the number of withdrawal reactions compared to tramadol injected alone at 15, 30, 45 and 60 min ($p<0.001$, 0.001, 0.001 and 0.001, respectively; see Fig. 3a).

Two-way ANOVA analysis of the global effect through AUC data showed that there was a significant effect of 8-OH-DPAT treatment (between-subjects factor: $F_{(1,39)}=53.85$, $p<0.0001$) and interaction factor ($F_{(1,39)}=27.80$, $p<0.0001$). No significant effect was reached for tramadol treatment (between subjects: $F_{(1,39)}=1.88$, $p>0.05$,). Subsequently, Bonferroni post hoc test showed that the combination of 8-OH-DPAT with tramadol at 22 mg/kg significantly increased the number of lifting reactions ($p<0.001$; see Fig. 3b).

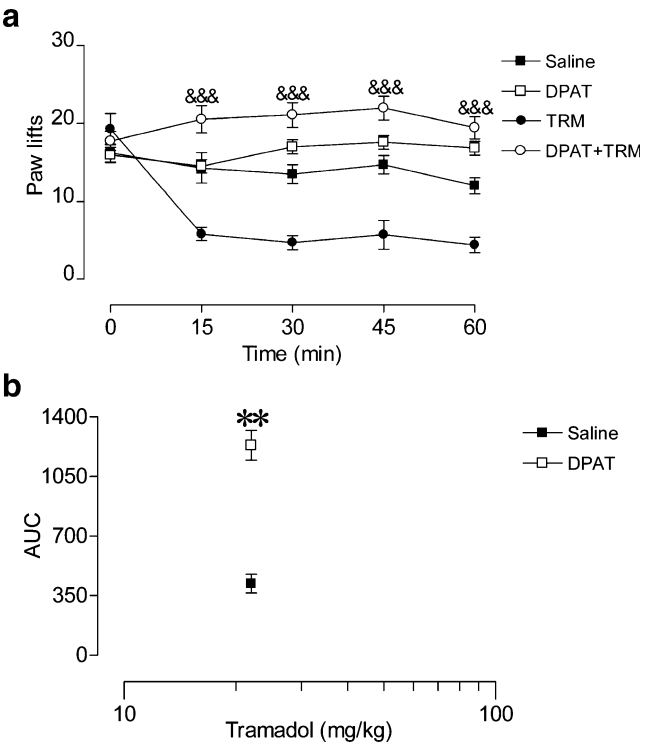

Fig. 3 5-HT1A receptor agonist, 8-OH-DPAT, counteracted the antiallodynic effect of tramadol in the cold-plate test in neuropathic rats. Tramadol (22 mg/kg; *TRM*) was intraperitoneally administered 15 min before test. 8-OH-DPAT (0.5 mg/kg; *DPAT*) was subcutaneously injected 10 min after tramadol. **a** Mean number of hind paw lifts per 2 min on the cold plate test. *Asterisks* indicate a significant difference compared to tramadol group ($^{\&\&\&}p<0.001$; Bonferroni post hoc test). **b** Mean areas under the curve (AUC) from 0–60 min after drug administration. AUC were calculated by summing the difference scores obtained at each individual time using the trapezoidal method. AUC values for saline+saline group = 849.00±71.11 and saline+8-OH-DPAT group = 982.19±35.92. *Asterisks* indicate a significant difference compared to saline (**$p<0.01$; Bonferroni post hoc test). Errors bars denote the standard error of the mean; $n=10$–12 per value

Effect of opioid receptors blockade in the antiallodynic effect of tramadol

The combination of naloxone (0.5 mg/kg) with tramadol decreased the antiallodynic effect of tramadol (22 mg/kg). Three-way repeated measures ANOVA revealed a significant effect of tramadol treatment (between subjects: $F_{(1,40)}=33.12$, $p<0.001$) and time (within subjects: $F_{(4,160)}=26.28$, $p<0.001$). No significant effect was reached for naloxone treatment (between subjects: $F_{(1,40)}=0.00$, $p>0.05$). There were significant interactions between tramadol and naloxone treatment ($F_{(1,40)}=4.31$, $p<0.05$) and tramadol treatment and time ($F_{(4,160)}=7.34$, $p<0.001$). No significant interactions between naloxone and time ($F_{(4,160)}=1.26$, $p>0.05$) or among tramadol treatment, naloxone treatment and time ($F_{(4,160)}=1.10$, $p>0.05$) were shown. Subsequently, two-way repeated measures ANOVA revealed a significant effect of treatment (tramadol and naloxone treatment; between subjects: $F_{(3,160)}=13.32$, $p<0.0001$), time (within subjects: $F_{(4,160)}=26.28$, $p<0.0001$) and interaction ($F_{(12,160)}=3.33$, $p<0.001$). Bonferroni post hoc test showed that the combination significantly increased the number of withdrawal reactions compared to saline at 45 min ($p<0.05$). No statistical differences were found between tramadol and the combination (tramadol+naloxone). As before, tramadol injected alone displayed a clear and significant antiallodynic effect at 15, 30 and 45 min after the drug injection compared with saline treatment ($p<0.05$, 0.01 and 0.05, respectively, for each time point; see Fig. 4a).

Two-way ANOVA analysis of the global effect through AUC data showed that there was a significant effect of

tramadol treatment (between-subjects factor: $F_{(1,40)}$=42.67, p<0.0001) and interaction factor ($F_{(1,39)}$=6.27, p<0.05). No significant effect was reached for naloxone treatment (between subjects: $F_{(1,40)}$=0.01, p<0.05,). However, subsequently Bonferroni post hoc test did not show any significant effect (see Fig. 4b).

Discussion

The current study shows that the antiallodynic effect of tramadol is enhanced by the systemic administration of WAY-100635, an antagonist of 5-HT_{1A} receptors, in neuropathic rats in the cold-plate test. Consistently, 8-OH-DPAT, an agonist of 5-HT_{1A} receptors, blocked the antiallodynic effect of an effective dose of tramadol in this test. Furthermore, the antiallodynic effect of tramadol was partially blocked by naloxone, an opioid receptor antagonist.

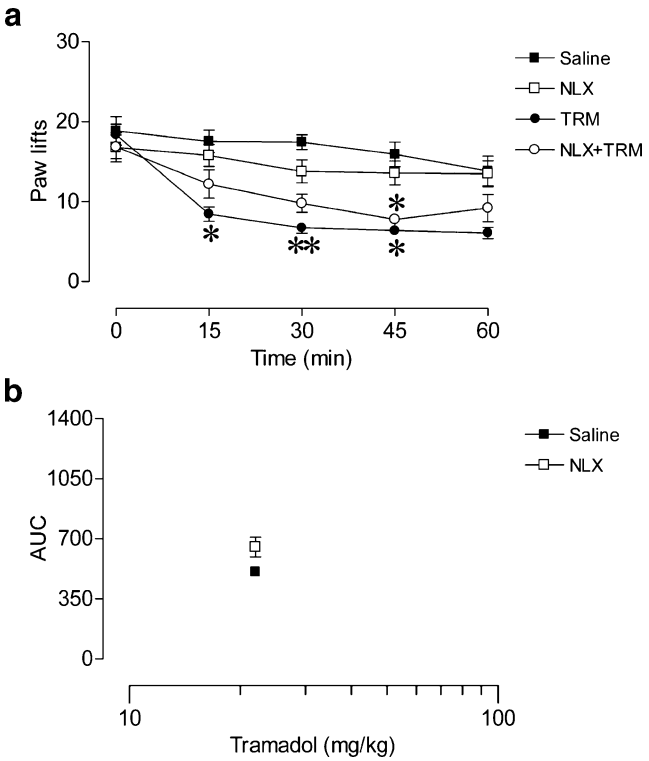

Fig. 4 Opioid receptor antagonist, naloxone, partially counteracted the antiallodynic effect of tramadol in the cold-plate test in neuropathic rats. Tramadol (22 mg/kg; *TRM*) was intraperitoneally administered 15 min before test. Naloxone (0.5 mg/kg; *NLX*) was intraperitoneally injected 10 min after tramadol. **a** Mean number of hind paw lifts per 2 min on the cold-plate test. *Asterisks* indicate a significant difference compared to saline group (*p<0.05, **p<0.01; Bonferroni post hoc test). **b** Mean areas under the curve (*AUC*) from 0–60 min after drug administration. AUC were calculated by summing the difference scores obtained at each individual time using the trapezoidal method. AUC values for saline+saline group = 1,009.04±58.97 and saline+naloxone group = 875.25±69.26. Errors bars denote the standard error of the mean; n=10–13 per value

This study confirms our results from previous studies with acute models of pain, the hot-plate test in mice and plantar test in rats (Berrocoso et al. 2006b; Rojas-Corrales et al. 2000) and contributes to support the evidence in favour of the role of the serotoninergic system in the analgesic effect of tramadol in healthy and mononeuropathic subjects. On serotonergic perikarya in the nucleus raphe magnus and other serotonergic nuclei, inhibitory 5-HT_{1A} autoreceptors exert a pronounced inhibitory influence upon the release of 5-HT throughout the CNS. 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 modulation of transmitter release in projecting areas, including the spinal cord (Barnes and Sharp 1999; Gobert et al. 1995; Pan et al. 1993). 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 (Mico et al. 2006b). This would lead to suppression of pain-related responses, consequently enhancing the analgesic effect displayed by tramadol.

Some changes in the serotoninergic system have been described in chronic pain states that may produce changes in the descending serotoninergic pathways. The release of 5-HT in the raphe nuclei is decreased in neuropathy (sciatic nerve ligation and diabetic neuropathy; Sounvoravong et al. 2004). In contrast, in animals suffering from inflammation, it is increased (Palazzo et al. 2004). This lower-than-normal release of 5-HT in the raphe nuclei in neuropathic animals could be implicated in the strong allodynic effect found in the combination of 8-OH-DPAT and tramadol. In the present study, s.c. administration of a 5-HT_{1A} receptor agonist alone had a slight tendency to increase allodynia in neuropathic animals, although not significantly. This is in line with our previous studies and other evidence indicating that systemic administration of a 5-HT_{1A} receptor agonist or antagonist had little effect on baseline nociception in healthy, diabetic and mononeuropathic animals (Ardid et al. 2001; Berrocoso et al. 2006b; Roca-Vinardell et al. 2003; Rojas-Corrales et al. 2000, 2005). However, some data indicate that WAY-100635, administered either systemically or into the rostroventromedial medulla, produces a selective attenuation of mechanical hypersensitivity in animals with an experimental neuropathy (Wei and Pertovaara 2006). In addition, electrophysiological studies have shown that 5-HT_{1A} agonists suppress the firing rate of serotoninergic raphe neurons in normal animals (Evrard et al. 1999). Therefore, these findings indicate that in animals treated

with 8-OH-DPAT plus tramadol, the activation of 5-HT_{1A} receptors in the raphe nuclei could lead to an inhibition of the descending serotoninergic pathways, leading to an amplification of pain behaviour in neuropathic animals. In addition, other studies have reported that in nerve-injured animals, there is an increase in the number of serotoninergic terminals in the spinal cord, indicating increased sprouting of descending serotoninergic pathways (Zhang et al. 1993). Concerning other changes in the 5-HT_{1A} receptors, inflammation (Zhang et al. 2002) but not nerve injury (Croul et al. 1995) has caused their up-regulation in the spinal cord. While inflammation may also produce up-regulation of 5-HT_{1A} receptors in the raphe magnus nucleus (Zhang et al. 2000), the effect that nerve injury has on them at this level still remains to be elucidated. All these changes might produce an influence in pain modulation that is different from that in normal animals.

The activation of 5-HT_{1A} receptors at spinal level has been related with both pronociceptive and antinociceptive effects depending on a number of experimental factors, such as sub-modality of test stimulation, dosage, duration of the treatment and pathophysiological condition. For example, F13640, a novel and highly selective agonist of 5-HT_{1A} receptors, has proved a potent antinociceptive in various experimental models of acute and chronic pain (Bardin et al. 2001; Colpaert et al. 2002, 2004). However, our present results fit with the hypothesis of a nociceptive effect due to the activation of spinal 5-HT_{1A} receptors. There is a considerable amount of evidence indicating that spinal administration of 5-HT_{1A} receptor agonists may promote nociception in different pain models (Alhaider and Wilcox 1993; Ali et al. 1994; Zemlan et al. 1983; Zhang et al. 2001), these 5-HT_{1A} receptors being located on inhibitory GABAergic/enkephalinergic interneurons in the spinal cord (Millan 2002). Therefore, the global effect found in this study could be explained in part by spinal action, in addition to the potentiation of the serotoninergic descending pathway.

Several studies have focused on the study of the analgesic mechanism of action of tramadol and the contribution of each component (i.e. opioid, noradrenergic and 5-HT). Regarding the opioid component, our study has shown that naloxone partially blocks its antinociceptive effect, in agreement with previous one (Raffa et al. 1992). Further, in contrast to other opioids, tramadol showed antinociceptive effects in homozygous μ-opioid receptor knockout mice, and the effects still remained under the influence of naloxone (Ide et al. 2006). Therefore, these data put forward that tramadol's opioid component is only one part of its antinociceptive effect and strengthen the contribution of the monoaminergic component. In this sense, it has been shown that the effects of tramadol in opioid receptor knockout mice were significantly reduced by pretreatment with yohimbine, an α_2-adrenoceptor antagonist (Ide et al. 2006), and yohimbine also reduced the antinociceptive effects of tramadol in wild-type mice, as previously reported (Desmeules et al. 1996; Driessen et al. 1993). These data suggest that tramadol induces analgesia via the norepinephrine nervous system too, especially via α_2-adrenoceptors. Electrophysiological studies support the idea that one of the possible mechanisms of tramadol antinociception via α_2-adrenoceptors may be an activation of the descending analgesic system that includes a noradrenergic pathway originating in the locus coeruleus and inhibits nociceptive responses at the level of the spinal cord (Berrocoso et al. 2006a). In addition, we have recently reported that the inhibitory effect of tramadol on locus coeruleus neurons in vivo is not modified by naloxone and it is potentiated by the i.v. pre-administration of 8-OH-DPAT in normal rats (Berrocoso et al. 2006a). Further electrophysiological studies in neuropathic animals will help to elucidate the role of locus coeruleus and its possible pharmacological modulation.

In conclusion, the present study makes clear that opioid and serotoninergic system, and specifically of 5-HT_{1A} receptors, contributes to the antiallodynic effect of tramadol in neuropathic pain. In addition, we propose that the combination of tramadol with compounds having 5-HT_{1A} antagonist properties could be a new strategy to improve the analgesia of tramadol in neuropathic pain, a chronic pain state not satisfactorily treated nowadays.

Acknowledgements This study has been supported by "Fondo de Investigación Sanitaria" (PI031430) from Instituto de Salud Carlos III and "Plan Andaluz de Investigación" (CTS-510) from Junta de Andalucía. The authors thank to Dr. Thomas Christoph for their help and cooperation in the revision of the manuscript.

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