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In vivo effect of tramadol on locus coeruleus neurons is mediated by α_2-adrenoceptors and modulated by serotonin

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Abstract

Tramadol is a centrally-acting analgesic endowed with opioid, noradrenergic and serotonergic properties. Various data suggest that, in addition to its analgesic effect, tramadol may have antidepressant and anxiolytic-like effects. This study investigates, through single-unit extracellular recording techniques, the in vivo effects of tramadol on locus coeruleus (LC) neurons and its possible effects on α_2-adrenoceptors, opioid receptors and the 5-HT system. Tramadol produced a dose-dependent and complete inhibition of LC activity ($ED_{50} = 2.1$ mg/kg). This inhibitory effect was prevented and reversed by the selective α_2-adrenoceptor antagonist, idazoxan, but not by the opioid receptor antagonist, naloxone. The inhibition of the synthesis of 5-HT by *p*-chlorophenylalanine and the pre-administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT at 40 μg/kg, caused a significant potentiation of the tramadol effect decreasing the ED_{50} by 53% and 67% respectively. Lower doses of 8-OH-DPAT, of 1 and 4 μg/kg, did not significantly modify the tramadol effect. In summary, the results indicate that tramadol elicits an inhibitory effect on LC neurons in vivo through α_2-adrenoceptors. Moreover, this effect is modulated by the 5-HT system and particularly by 5-HT_{1A} receptors.

Keywords: Tramadol; Locus coeruleus; α_2-Adrenoceptor; Opioid receptor; Serotonin; 5-HT_{1A} receptors

1. Introduction

Tramadol, (1RS,2RS)-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is a centrally-acting analgesic which is widely used in clinical practice. Tramadol is a synthetic opioid that binds weakly to μ-opioid receptors (Hennies et al., 1988). Nevertheless, a non-opioid mechanism is also involved in tramadol analgesia. This works by enhancing the extraneuronal concentration of noradrenaline and serotonin (5-HT) by interfering with both the reuptake and release mechanisms (Raffa et al., 1992; Bamigbade et al., 1997).

These monoaminergic properties, very similar to those of some tricyclics or non-tricyclic antidepressants (i.e venlafaxine, duloxetine or milnacipran), may also account for a broad spectrum of actions in other neuropsychiatric disorders besides pain. Tramadol has shown antidepressant-like effects in mice (Rojas-Corrales et al., 1998, 2004) and rats (Rojas-Corrales et al., 2002) and induces changes in the CNS similar to those induced with conventional antidepressants; 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), whereas it increases the binding of α_1-adrenoceptors and dopamine D_2/D_3 receptors (Faron-Gorecka et al., 2004b). In clinical practice, tramadol has been used successfully in several psychiatric disorders such as refractory major depression (Shapira et al., 2001), severe suicidal ideation (Spencer, 2000) and antidepressant potentiation (Fanelli and Montgomery, 1998). Tramadol has also been used with positive effects in anxiety and anxiety-like disorders such as obsessive-compulsive disorders and

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in the treatment of Tourette's Syndrome (Shapira et al., 1997). Considering all these preclinical and clinical data, it seems clear that tramadol, in addition to its analgesic effect, also demonstrates antidepressive and anxiolytic effects.

The locus coeruleus (LC) is a pontine nucleus clearly implicated in the regulation of pain (Hirata and Aston-Jones, 1994), depression (Harro and Oreland, 2001) and anxiety (Tanaka et al., 2000). Moreover, opiate analgesics (Ruiz-Durantez et al., 2003), antidepressants (Mateo et al., 2000; Szabo and Blier, 2001) and anxiolytics (Tanaka et al., 2000) are able to modify the neuronal activity of this nucleus in vivo. In the case of tramadol, several studies have shown its effect on LC neuronal activity but only in vitro where the preparation of the brain slices containing the LC disrupts the reciprocal interactions between 5-HT and noradrenaline neurons. Thus, both enantiomers of tramadol, (−)-tramadol and (+)-tramadol, inhibited the firing rate of LC neurons in pontine slices of rats (Sevcik et al., 1993). More recently and also in vitro, Halfpenny and colleages (Halfpenny et al., 1999) showed that tramadol significantly increased the stimulated noradrenaline efflux but not the magnitude of electrically-stimulated noradrenaline efflux or uptake in LC slices after chronic treatment (Hopwood et al., 2001). Regarding other nuclei implicated in pain transmission, tramadol modulates the 5-HT neurotransmitter system similarly to other well-known antidepressants (Hopwood et al., 2001). From these studies it seems clear that tramadol has some of the pre- and postsynaptic neurochemical features of a conventional antidepressant, as might be predicted from its pharmacology and broad spectrum of actions.

Taking into account the serotonergic inhibitory pathway from the dorsal raphe nucleus (DRN) to the LC that has been described (Segal, 1979), we proposed to study the effects in vivo of tramadol on LC neurons, with the object of elucidating the possible cooperative or non-cooperative role of these nuclei in the net effect displayed by tramadol. The participation of μ-opiate receptors and noradrenergic α_2-adrenoceptors was also investigated. In addition, considering the well-documented existence of a functional modulator role of 5-HT in the firing activity of LC neurons (Mateo et al., 2000), which may involve 5-HT_{1A} receptors (Aston-Jones et al., 1991; Ruiz-Ortega and Ugedo, 1997; Szabo and Blier, 2001) and given that these neural circuitries may be of importance for the mechanism of action of antidepressants and analgesics, we considered it would also be of interest to study the in vivo effects of the analgesic tramadol on LC neurons and their possible modulation by 5-HT, with the aim of contributing to a better understanding of its analgesic and antidepressant-like effects.

2. Methods

2.1. Animal treatments and surgical procedure

The experiments were performed using adult male Sprague–Dawley rats weighing 220–300 g. The animals were housed under standard laboratory conditions (22 °C, 12 h light/dark cycles, lights on at 08:00 AM, food and water ad libitum). Every effort was made to minimize animal suffering and to use the minimum possible number of animals. The experimental procedures were carried out in accordance with the U.K. Animals (Scientific Procedures) Act (1986) and associated guidelines, and with the European Community Council Directive of 24th November 1986 (86/609/EEC).

Rats were anesthetized with chloral hydrate (400 mg/kg i.p.); subsequently a cannula was inserted into the trachea and the right jugular vein was cannulated for systemic (i.v.) injections of anaesthetic and all drugs. Supplemental doses of anaesthetic were given to maintain constant anesthesia and to prevent any nociceptive reaction. Body temperature was maintained at 37 °C with a heating pad. The rat was placed in a stereotaxic frame with the head oriented at a 15° angle to the horizontal plane (nose down). To approach the LC, the skull was exposed, and a hole (approximately 3 mm diameter) was drilled for the insertion of the recording electrode at 1.1 mm lateral to the midline and 3.7 mm posterior to the lamboid fontanel over the cerebellum. The dura over the cerebellum was carefully removed.

To study the acute effect of tramadol on LC neurons in vivo, dose-effect curves were performed for tramadol, which was injected at 2–3 min intervals, in doubling doses, until maximal effect was reached. This experimental group will be referred as the "control group". Subsequent injection of idazoxan (1 mg/kg, i.v.), an α_2-adrenoceptor antagonist, or naloxone (5 mg/kg, i.v.), an opioid receptor antagonist, was administered in order to reverse the tramadol effect. If the inhibitory effect of tramadol was not totally recovered after the administration of naloxone (5 mg/kg, i.v.), idazoxan (1 mg/kg, i.v.) was administered subsequently. In another set of experiments, idazoxan (100 μg/kg, i.v.) or naloxone (5 mg/kg, i.v.) were administrated 3–5 min before dose-response curves for tramadol were performed. Secondly, to study the role of 5-HT in the effect of tramadol, 5-HT was depleted by the intraperitoneal administration of the 5-HT synthesis inhibitor, *p*-chlorophenylalanine (PCPA, 400 mg/kg, 24 h before the experiment) in another group of rats. This protocol produces a dramatic depletion of 5-HT and 5-hydroxyindole acetic acid (5-HIAA) content in the LC (Reader et al., 1986; Mateo et al., 2000). Following this, dose-response curves were performed for tramadol. Thirdly, the role of 5-HT_{1A} receptors in the effect displayed by tramadol was evaluated by pre-treating animals with the selective agonist of 5-HT_{1A} receptors, 8-OH-DPAT, 4–6 min before dose-response curves for tramadol were obtained.

2.2. Extracellular recordings of LC neurons in vivo

Single-unit extracellular recordings of LC neurons were performed as described previously (Ugedo et al., 1998). The recording electrode was an Omegadot single-barrel glass micropipette filled with a 2% solution of Pontamine Sky Blue in 0.5% sodium acetate and broken back to a tip diameter of 1–2.5 μm. The extracellular signal from the electrode was amplified, discriminated and monitored on an oscilloscope and with an audio monitor too. Discriminated spikes were fed into a PC and processed using computer software (CED micro 1401 interface and Spike2 software, Cambridge Electronic Design, U.K.). LC neurons were encountered 5.5–6.0 mm below the dural surface, just ventral to a zone of relative silence (corresponding to the IVth ventricle), and medial to neurons of the mesencephalic nucleus of the Vth cranial nerve (which could be activated by depression of the mandible). LC neurons were identified by standard criteria that included: long duration action potential (>2 ms), spontaneous firing at a regular rhythm, a slow firing rate between 0.5–5 Hz and characteristic spikes with a long-lasting positive-negative waveform. The basal firing rate was recorded at least 2 min prior to any drug administration. Only one noradrenergic LC cell was pharmacologically studied in each animal.

At the end of the experiment, a 5 μA cathodal current was passed through the recording electrode to leave a blue spot at the recording site. The brain was fixed and removed, cut into sections of 50 μm and stained with neutral red. The site of recording was examined microscopically. Only measurements from cells within the LC were included in this study.

2.3. Analysis of data and statistical analyses

Changes in firing rate are expressed as percentages of the baseline firing rate. Dose-concentration-effect curves were analyzed for the best non-linear fit to a logistic three-parameter equation (Parker and Waud, 1971): $E = E_{max}[A]^n/(ED_{50}{}^n + [A]^n)$, where $[A]$ is the i.v. dose of tramadol and E is the effect on the firing rate induced by A; E_{max} is the maximal percentage change at "infinite" dose (100%); ED_{50} is the effective dose for eliciting 50% of E_{max}; n is the slope factor of the dose-response curve. Experimental data were analyzed

by using the computer program Graphd Prism (v. 3.0; GraphPad Software, Inc.). In the experiments where the role of 5-HT_{1A} receptors in the inhibitory effect displayed by tramadol was evaluated, the baseline firing rate after the injection of 8-OH-DPAT was taken as 100%. Statistical significance was assessed by means of two-way repeated measures analysis of variance (ANOVA; with tramadol doses and pre-treatment as main factors) followed by the Bonferroni test. For comparisons between more than two groups, one-way repeated measures or a one-way ANOVA followed by the Newman–Keuls test were chosen. For comparisons between two groups and for the analysis of the firing rate before and after drug administration unpaired and paired Student *t*-test were used respectively. The level of significance was considered as $P < 0.05$. Data are reported as mean ± S.E.M.

2.4. Drugs

The following drugs were used: (±)-tramadol (courtesy of Grünenthal-Andrómaco, Spain); 8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT), *p*-chlorophenylalanine (PCPA), idazoxan hydrochloride and naloxone hydrochloride (provided by Sigma Chemicals Co. Spain). All drugs solutions were prepared immediately before each trial and dissolved in saline (0.9% NaCl).

3. Results

3.1. Effect of tramadol on the firing activity of locus coeruleus neurons and its interaction with α_2-adrenoceptors and opioid receptors

Administration of tramadol depressed the spontaneous activity of LC neurons in a dose-dependent manner ($P < 0.001$, one-way repeated measures ANOVA, $n = 5$; Figs. 1A and 2). Complete inhibition was achieved in all cells tested and the mean ED_{50} value estimated from the dose-effect curves was 2.1 ± 0.2 mg/kg ($n = 5$; Table 1). Next, the role of α_2-adrenoceptors and opioid receptors in the effect of tramadol on LC neurons was explored through two approaches: first, after the complete inhibition of the LC neurons was reached due to the tramadol administration, the recovery effect of idazoxan (1 mg/kg, i.v.), an α_2-adrenoceptor antagonist, and naloxone (5 mg/kg, i.v.), an opioid receptor antagonist, was studied. Second, dose-response curves for the tramadol-induced inhibition of the firing rate were obtained from rats pre-treated with idazoxan (100 µg/kg, i.v.) or naloxone (5 mg/kg, i.v.).

The first approach showed that the tramadol inhibitory effect was rapidly and completely reversed by the subsequent administration of idazoxan (final mean firing rate was 123 ± 45% of initial basal firing rate, $P > 0.05$, paired Student *t*-test, $n = 4$, see Fig. 1A). In contrast, naloxone failed to reverse the tramadol inhibition (mean firing rate was 4 ± 2% of initial basal firing rate, $P < 0.01$, paired Student *t*-test, $n = 5$). However, and similarly to what happened before, subsequent idazoxan (1 mg/kg, i.v.) administration produced a recovery of the firing activity (final mean firing rate was a 100 ± 20% of initial basal firing rate, $P > 0.05$, paired Student *t*-test, $n = 4$; Fig. 1B). In addition, idazoxan at 1 mg/kg

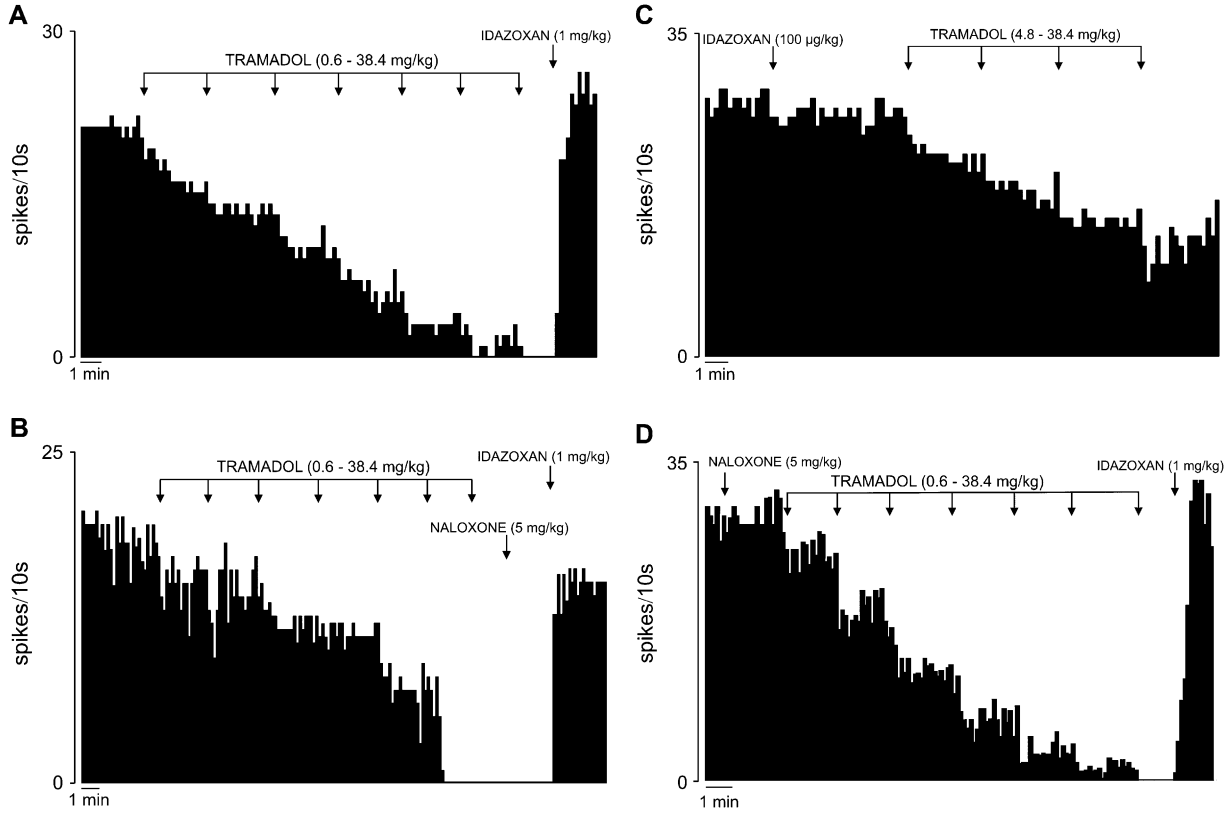

Fig. 1. Representative example of firing rate recording from locus coeruleus neurons showing the dose-dependent inhibitory effect of tramadol and its involvement with α_2-adrenoceptors (A, C) and opioid receptors (B, D). Note that the dose-dependent inhibitory effect of tramadol was reversed by the subsequent administration of the antagonist of α_2-adrenoceptors, idazoxan (1 mg/kg, i.v.) (A). In contrast, the opioid receptor antagonist, naloxone (5 mg/kg, i.v.), failed to reverse the tramadol inhibition (B). Moreover, the pre-administration of idazoxan (100 µg/kg, i.v.) counteracted the tramadol-induced inhibition (C) and the pre-treatment with naloxone (5 mg/kg, i.v.) did not modify the tramadol-induced inhibition of the firing rate, compared to the effect displayed by tramadol alone (D).

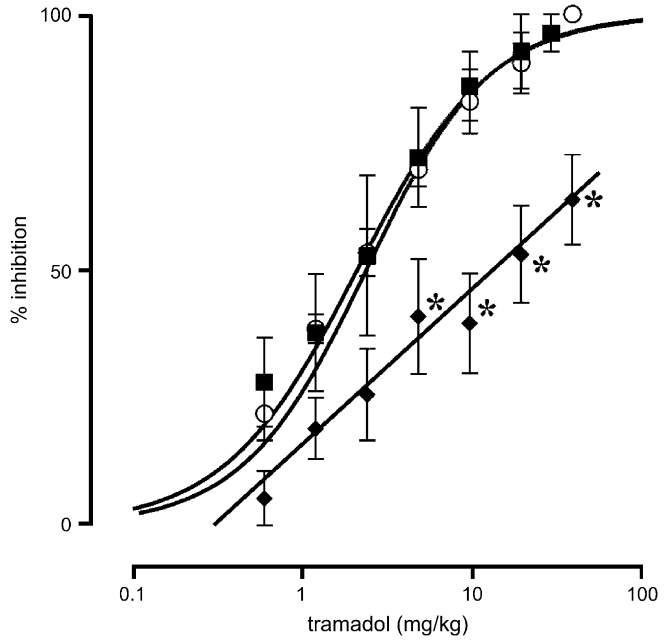

Fig. 2. Dose-effect curves illustrating the inhibitory effect of tramadol on locus coeruleus neuron firing rate after the administration of idazoxan, an antagonist of α_2-adrenoceptors, or naloxone, an opioid receptor antagonist. Symbols represent mean ± S.E.M. of the percentage of reduction from basal firing rate in the control (○, $n = 5$) and experimental groups, 3–5 min after administration of idazoxan (◆, 100 μg/kg, i.v., $n = 6$) or naloxone (■, 5 mg/kg, i.v., $n = 5$). The horizontal axis represents the cumulative doses of tramadol administered i.v. at 3 min intervals. Note the pre-administration of idazoxan blocked the tramadol-induced inhibition; however naloxone did not modify the inhibitory effect displayed by tramadol alone. *$P < 0.05$ (two-way repeated measures ANOVA, followed by Bonferroni test) when compared to the tramadol group (control group).

administered alone, enhanced the firing activity (mean firing rate was 54 ± 7% of initial basal firing rate, $P < 0.05$, paired Student t-test, $n = 5$; Table 1) in agreement with previous studies (Freedman and Aghajanian, 1984; Szabo and Blier, 2001) and naloxone (5 mg/kg, i.v.) did not modify the basal firing rate ($P > 0.05$, paired Student t-test, $n = 5$; Table 1) as previously shown Ruiz-Durantez and colleagues (Ruiz-Durantez et al., 2003).

The second approach showed that pre-treatment with idazoxan (100 μg/kg, i.v.) counteracted the tramadol-induced inhibition of LC neurons significantly ($P < 0.0001$, two-way repeated measures ANOVA, $n = 6$). The maximal inhibition reached was 64 ± 9% ($P < 0.01$, Bonferroni test, $n = 5$, with respect to the inhibition achieved in animals treated with tramadol (control group) at the highest dose (38.4 mg/kg); Figs. 1C and 2). In contrast, as shown in Figs. 1D and 2, the pre-treatment with naloxone (5 mg/kg, i.v.) did not significantly modify the tramadol-induced inhibition of the firing rate on LC neurons compared to the effect displayed by tramadol (control group) ($P > 0.05$, two-way repeated measures ANOVA, Table 1). Following idazoxan (1 mg/kg, i.v.) administration, the firing activity recovered (final mean firing rate was 135 ± 46% of initial basal firing rate, $P > 0.05$, paired Student t-test, $n = 3$; Fig. 1D). In addition, as previously shown neither idazoxan at 100 μg/kg (Freedman and Aghajanian, 1984) nor naloxone at 5 mg/kg modified the basal firing rate ($P > 0.05$, paired Student t-test, $n = 5$ and $n = 5$ respectively; Table 1).

3.2. Effect of acute administration of tramadol on the firing activity of locus coeruleus neurons in PCPA-treated rats

In order to evaluate the contribution of the 5-HT system to the inhibitory effect displayed by tramadol on LC neurons, the synthesis of 5-HT was inhibited by PCPA (400 mg/kg, i.p.) administered 24 h before the experiment. After PCPA treatment, LC neurons fired at the same frequency as LC neurons from non-treated animals (Table 1). However, the depletion of 5-HT significantly shifted the dose-response curve to the left ($P < 0.01$, two-way repeated measures ANOVA, $n = 4$) and decreased the ED_{50} by 53% ($n = 4$) when compared with that obtained in non-PCPA-treated rats (control group) ($P < 0.01$, unpaired Student t-test, Fig. 3A). In addition, as exemplified in Fig. 3B,

Table 1
Spontaneous firing activity of locus coeruleus neurons before and after a single intravenous dose of idazoxan, naloxone or 8-OH-DPAT

Drug	Before drug		After drug			ED_{50}
	Firing activity	Range of firing	Firing activity	Range of firing		
Control group	2.4 ± 0.4	1.4–3.7			[5]	2.1 ± 0.2
Idazoxan (1 mg/kg)	1.1 ± 0.2	0.8–1.8	1.7 ± 0.3	1.2–2.9	[5]	
Idazoxan (100 μg/kg)	2.0 ± 0.5	0.5–3.3	2.1 ± 0.6	0.5–3.2	[6]	NE
Naloxone (5 mg/kg)	1.7 ± 0.5	0.6–3.6	1.8 ± 0.6	0.8–4.0	[5]	2.4 ± 0.8
PCPA (400 mg/kg)	1.5 ± 0.4	0.9–3.0			[6]	0.9 ± 0.2*
8-OH-DPAT (1 μg/kg)	2.5 ± 0.6	0.7–4.3	2.6 ± 0.6	0.7–4.6	[6]	1.5 ± 0.3
8-OH-DPAT (4 μg/kg)	2.3 ± 0.2	1.7–2.7	2.9 ± 0.2	2.6–3.3	[4]	2.6 ± 0.7
8-OH-DPAT (40 μg/kg)	1.7 ± 0.3	0.5–4.3	2.0 ± 0.3	0.6–4.5	[22]	0.7 ± 0.1*

Parameters of locus coeruleus neurons were obtained from 3 to 5 interspike time interval histogram samples before and after drug administration. ED_{50} is estimated from the dose–response curve for tramadol administration in each treatment group using the Parker and Waud equation when possible. Each value represents the mean ± S.E.M. of n cells per group. The value between brackets represents the administered dose of each compound. The value between square brackets represents the number of cells recorded. PCPA: the synthesis of 5-HT was inhibited by PCPA (*p*-chlorophenylalanine; 400 mg/kg, i.p.) administered 24 h before the experiment. NE: ED_{50} not estimated. *$P < 0.05$ (unpaired t-test or one-way ANOVA, followed by Newman–Keuls test) when compared to the ED_{50} value of the control group.

this inhibitory effect was rapidly and completely reversed by idazoxan (1 mg/kg, i.v.) (final mean firing rate was 123 ± 25% of initial basal firing rate, $P > 0.05$, paired Student t-test, $n = 4$).

3.3. Effect of the pre-treatment of 8-OH-DPAT, agonist of 5-HT$_{1A}$ receptor, on the inhibitory effect of tramadol on locus coeruleus neurons

We next examined the involvement of the 5-HT$_{1A}$ receptor in the observed modulation by 5-HT of the effect of tramadol on LC neurons. The well-established agonist of the 5-HT$_{1A}$ receptor, 8-OH-DPAT, was administrated at doses of 1, 4 and 40 μg/kg. After drug application, there was a slight increase in the firing rate but by the time the tramadol dose-response curves were performed, when the cell was stabilized, the mean firing rate values were no different from previous mean basal firing rate values (see Table 1). The pre-treatment with the low doses of 8-OH-DPAT (1 and 4 μg/kg, i.v.) did not significantly modify the ED_{50} value from that of the control group ($P > 0.05$, one-way ANOVA, $n = 5$ and $n = 4$ respectively, Table 1 and Fig. 4A). However, the treatment with the highest dose of 8-OH-DPAT (40 μg/kg, i.v.) caused a significant potentiation of the tramadol-induced inhibition of LC neurons ($P < 0.01$, two-way repeated measures ANOVA, $n = 5$) so that the dose-response curve showed a shift to the left and the ED_{50} value was reduced by 67% compared to the control group ($P < 0.05$, one-way ANOVA followed by Newman–Keuls test, $n = 5$, Table 1, Figs. 4B). As previously found, this inhibitory effect was rapidly and completely reversed by idazoxan (1 mg/kg, i.v.) (final mean firing rate was 176 ± 39% of initial basal firing rate, $P > 0.05$, paired Student's t-test, $n = 4$).

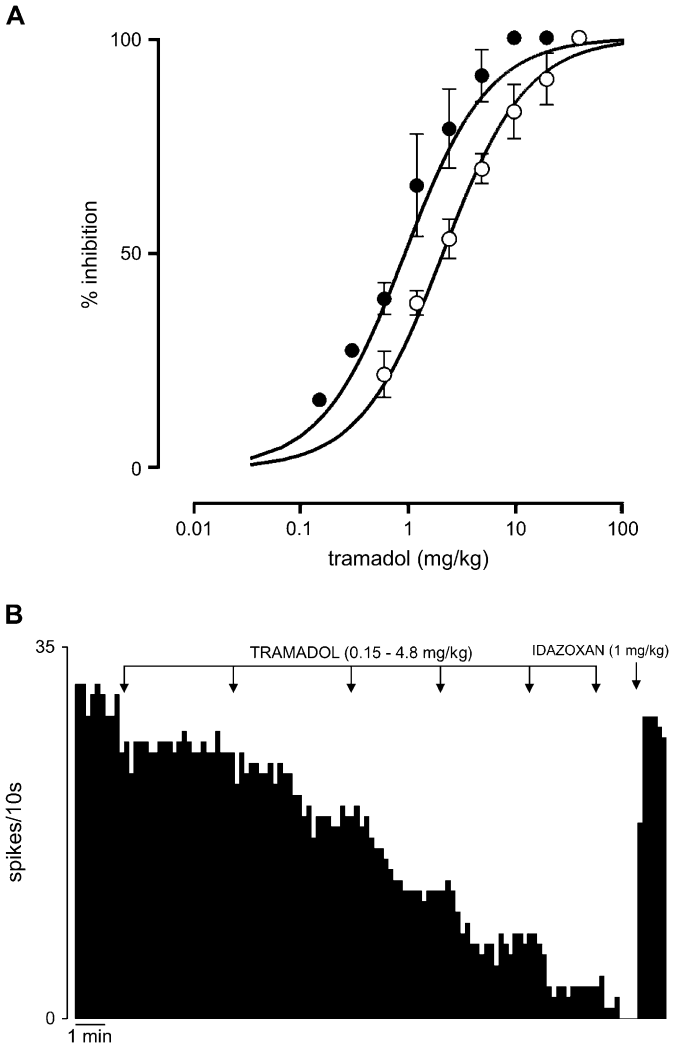

Fig. 3. Dose-effect curves illustrating the inhibitory effect of tramadol on locus coeruleus neuron firing rate in non-treated (control group) and PCPA (*p*-chlorophenylalanine) treated rats (A). Symbols represent mean ± S.E.M. of the percentage of reduction from basal firing rate in the control (○, $n = 5$) and experimental group (●, $n = 4$), 24 h after administration of inhibitor of the synthesis of serotonin, PCPA (400 mg/kg, i.p.). The horizontal axis represents the cumulative doses of tramadol administered i.v. at 3 min intervals. Note that the dose-response curve for PCPA-treated rats was shifted to the left and the ED_{50} decreased by 53% when compared with in non-treated rats (control group). Representative example of firing rate recording from locus coeruleus neurons showing the effect of cumulative doses of tramadol in PCPA-treated rat (B). Note that the subsequent injection of idazoxan (1 mg/kg, i.v.) completely reversed the tramadol effect.

4. Discussion

The present study demonstrates that tramadol depresses, in a dose-dependent manner, the firing rate of rat LC neurons in vivo. This effect seems to be mediated mainly by α_2-adrenoceptors and not by opioid receptors. Furthermore, the results indicate that the 5-HT system, and particularly, 5-HT$_{1A}$ receptors, may modulate the acute responses of tramadol in the LC; both the 5-HT depletion and the activation of 5-HT$_{1A}$ receptors potentiated tramadol inhibition.

In vitro, previous studies have shown that both enantiomers of tramadol, (−)-tramadol and (+)-tramadol, inhibited the firing rate of LC neurons in pontine slices of rats (Sevcik et al., 1993). In agreement with this, our study has shown that tramadol inhibits, in a dose dependent way, the firing activity of LC neurons in vivo. In addition, our study clearly shows that the effect displayed by tramadol is mediated by α_2-adrenoceptors. There are α_2-adrenoceptors located on the cell bodies and terminals of noradrenaline neurons and their activation induces an inhibitory action on neuron firing activity (Svensson et al., 1975). In fact, in the presence of a noradrenaline reuptake blocker, such as the antidepressant desipramine, α_2-adrenoreceptors are activated via increased concentrations of endogeous noradrenaline and thus attenuate the firing activity of LC neurons (Egan et al., 1983). The actions of tramadol in rodents and humans include the modulation of α_2-adrenoceptor activation (Raffa et al., 1992; Desmeules et al., 1996; Rojas-Corrales et al., 1998; Yalcin et al., 2005). Tramadol enhances the extraneuronal concentration of the monoamine neurotransmitters noradrenaline and 5-HT by interfering with the uptake and release mechanisms (Raffa et al., 1992; Bamigbade et al., 1997) and significantly increases stimulated noradrenaline efflux in LC brain slices (Halfpenny et al., 1999). In addition, LC activity is highly responsive to cardiovascular changes due to it receiving

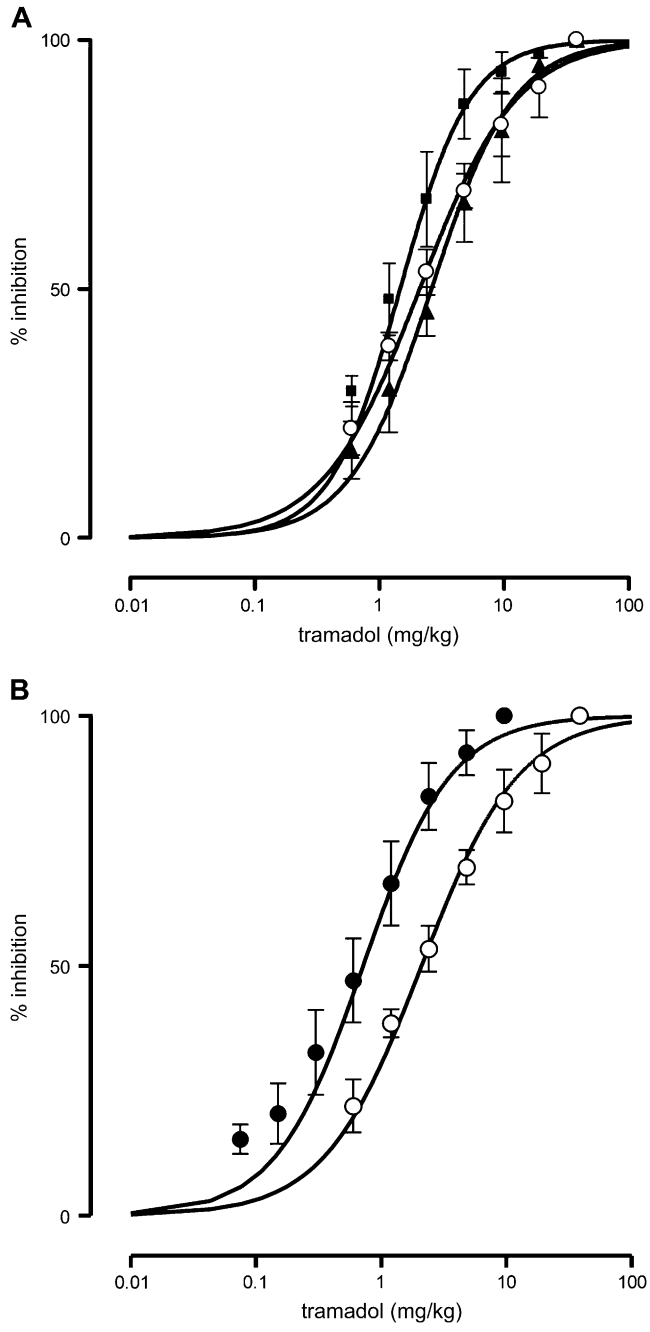

Fig. 4. Dose-effect curves illustrating the inhibitory effect of tramadol on locus coeruleus neuron firing rate after the administration of 8-OH-DPAT. Symbols represent means ± S.E.M. of the percentage of reduction from basal firing rate in the control (○, $n = 5$) and experimental groups, 3–5 min after the intravenous administration of 8-OH-DPAT at 1 μg/kg (■, $n = 5$, A), 4 μg/kg (▲, $n = 3$, A) and 40 μg/kg (●, $n = 5$, B). The horizontal axis represents the cumulative doses of tramadol administered i.v. at 3 min intervals. Note that the pre-treatment with 8-OH-DPAT, an agonist of 5-HT_{1A} receptor, at low doses (1 and 4 μg/kg, i.v.) did not significantly modify the ED_{50} value from that of the control group (A). However, at the higher dose (40 μg/kg, i.v.), it shifted the dose-response curve to the left and the ED_{50} value decreased by 67% with respect to the control group (B).

impulses from arterial baroreceptors and cardiopulmonary receptors which in turn influence the activity of noradrenergic neurons. Haemodynamic changes, such as blood loss, a reduction in blood pressure or an increase in pCO2, caused marked and long-lasting increases in LC activity (Svensson, 1987). In the current study tramadol decreases the firing rate and it occasionally produces hypotension after intravenous administration (Raimundo et al., 2006), so, an increase in the firing rate in the LC and not a decrease would be expected because of blood pressure changes. Therefore, as with other antidepressant drugs, it seems that tramadol inhibits the firing activity of LC neurons by increasing the extraneuronal concentrations of noradrenaline, and subsequently activating the α_2-adrenoreceptors.

Tramadol is an analgesic with several targets of action and it binds weakly but effectively to μ-opioid receptors (Hennies et al., 1988). Since LC neurons are endowed with opioids receptors (Pert et al., 1975) and it has been repeatedly shown that opiates acting via μ-opioid receptors inhibit LC activity (Aghajanian, 1978), it could be expected that the effects of tramadol are mediated by opiate receptors. However, in the present study the effect of tramadol was not reversed by the opioid receptor antagonist naloxone, which indicates that μ-opioid receptors were not involved. This is similar to the findings from certain behavioural studies, where naloxone does not reverse the antidepressant-like effect of tramadol in the forced swimming test in mice (Rojas-Corrales et al., 1998). In addition, antinociception studies have demonstrated that tramadol-induced antinociception is exerted through both opioid and non-opioid mechanisms, since the antinociception is not fully blocked by naloxone (Raffa et al., 1992).

Our study has shown that both the depletion of 5-HT and the activation of 5-HT_{1A} receptors potentiate the tramadol-induced inhibition on LC neurons. There are several lines of evidence supporting the notion that the 5-HT system also influences brain noradrenaline neuron responses mediated through α_2-adrenoceptors. In fact, lesion of 5-HT neurons prevents the effect of clonidine, an agonist of α_2-adrenoceptors, and induces suppression of avoidance behaviour and of locomotion activity in rats (Kostowski et al., 1981). Destruction of 5-HT neurons or administration of 5-HT depletion reduces the release of growth hormone induced by clonidine in rats (Soderpalm et al., 1987). On the other hand, as mentioned above, tramadol inhibits 5-HT uptake (Driessen and Reimann, 1992; Raffa et al., 1992) thereby increasing 5-HT levels. The LC is densely innervated by serotoninergic fibers and terminals, which arise from the DRN (Segal, 1979) and pericoerulear 5-HT neurons (Aston-Jones et al., 1991). Moreover, the same dose of PCPA as used in the present study decreases the 5-HT and 5-HIAA content in the LC (Reader et al., 1986; Mateo et al., 2000). Our results suggest that endogenous 5-HT modulates tramadol response. In addition, idazoxan, an antagonist of α_2-adrenoceptors, completely reversed the tramadol effect as in the control conditions, suggesting that α_2-adrenoceptors are not directly implicated in the PCPA modulation. The modulation shown with PCPA is similar to that observed with the pre-administration of 8-OH-DPAT, an agonist of 5-HT_{1A} receptors. Nevertheless, only the higher dose of 8-OH-DPAT (40 μg/kg), and not the lower doses (1 and 4 μg/kg), was able to potentiate the inhibition effect of tramadol. Moreover, considering that 8-OH-DPAT, even at these

lower doses, shuts down the firing of DRN (Martin-Ruiz and Ugedo, 2001), this suggests to us that the location of the 5-HT_{1A} receptors which control LC neuron firing is probably not on the cell body of 5-HT neurons. These 5-HT_{1A} receptors could be located on a neuron projection feeding onto a 5-HT terminal (Szabo et al., 2000).

In summary, the present results demonstrate that tramadol elicits an inhibitory effect on LC neurons through a similar mechanism to antidepressant drugs; this provides new data about its proposed antidepressant action. Tramadol is a good analgesic that, in contrast to morphine, is not likely to induce tolerant and physical dependence. It has not been associated with significant opioid side effects, such as respiratory depression, constipation or sedation (Lehmann et al., 1990; Franceschini et al., 1999). Given the close association between chronic pain and depression and the importance of the role that the LC plays in both, the results of the present study could be helpful in the development of new therapeutic strategies to treat and prevent threatening complications in these conditions. Nevertheless, further studies are necessary in the context of the analgesic, antidepressant-like and anxiolytic effects of tramadol in man and animals.

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