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Opioid and monoamine systems mediate the discriminative stimulus of tramadol in rats

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Abstract

We analyzed the ability of the mu opioid peptide receptor ligands morphine and naloxone and several antidepressant drugs that are serotonin (fluoxetine), noradrenaline (reboxetine), mixed serotonin and noradrenaline (milnacipram and venlafaxine), dopamine (nomifensine) reuptake inhibitors, as well as roxindole (a nonselective drug) to substitute for, or alter, tramadol discrimination. Male Wistar rats were trained to discriminate tramadol (20 mg/kg) from saline in a two-choice water-reinforced paradigm. Out of the drugs studied, only morphine substituted for tramadol. In combination experiments, naloxone (0.1–1 mg/kg) attenuated the stimulus effects of tramadol (20 mg/kg) and the substitution evoked by morphine (2 mg/kg). Milnacipram (10 mg/kg) or reboxetine (10 mg/kg) enhanced the effects of tramadol (2.5–10 mg/kg); the other antidepressant drugs used failed to modulate tramadol discrimination. Our results indicate that tramadol can be used as a stimulus cue in rats, and that mu opioid peptide mechanisms are involved in its effects, while noradrenergic uptake inhibitors can enhance tramadol discrimination.

Keywords: Tramadol; Morphine; Naloxone; Antidepressant drug; Discrimination

1. Introduction

Tramadol, a 4-phenylpiperidine analogue of codeine, belongs to a group of the centrally acting and clinically approved drugs used to treat pain disorders (Shipton, 2000; Attal, 2001). In contrast to such typical opioid receptor agonists as morphine, tramadol binds weakly to mu opioid peptide receptors (a K_i value ≈ μmol), and its affinity for these receptors in in vitro assays is ca. 3600–6000 times weaker than that of morphine (Raffa et al., 1992; Driessen et al., 1993; Gillen et al., 2000; cf. McClellan and Scott, 2003). Tramadol forms the antinociceptive active metabolite *o*-demethyltramadol (M_1) which shows higher affinity for opioid peptide receptors, yet still ca. 10-fold lower than does morphine (Frink et al., 1996). The analgesic action of tramadol is only partly inhibited by the mu opioid peptide receptor antagonist naloxone (Franceschini et al., 1999), which may also suggest its nonopioid-dependent mechanisms of action. On the other hand, involvement of opioidergic systems in the behavioral effects of tramadol is indicated by a drug discrimination analysis showing that tramadol fully substitutes for morphine in rats trained to recognize morphine from saline, this effect being blocked by the selective mu opioid peptide receptor antagonist naltrexone (Ren and Zheng, 2000).

Tramadol exists in the form of a racemic mixture of two active enantiomers (Frink et al., 1996), either displaying different binding and pharmacological profile. The (+)-enantiomer and its metabolite [(+)-M_1] are selective agonists of mu opioid peptide receptors and serve as preferential inhibitors of serotonin reuptake, while the (−)-enantiomer and the (−)-M_1 metabolite inhibit mainly noradrenaline

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reuptake (Matthiesen et al., 1998). The interaction with the monoamine neurotransmission of (+)-enantiomer, (−)-enantiomer or (±)-tramadol closely resembles that of antidepressant drugs, being either selective serotonin reuptake inhibiors (SSRIs), noradrenaline reuptake inhibitors (NARIs) or both serotonin and noradrenaline reuptake inhibitors (SNRIs), respectively. In fact, tramadol has been found to be active in the forced swimming test (Rojas-Corrales et al., 1998; Hopwood et al., 2001; Singh et al., 2004) and the learned helplessness model (Rojas-Corrales et al., 2002), which are commonly used for screening antidepressant-like activity in rodents. Moreover, in clinical trials, tramadol is described as an effective therapeutic agent in treating obsessive–compulsive disorders (Goldsmith et al., 1999).

In the present study, we used the drug discrimination technique as a rodent model to study the mechanism of action of tramadol (Goudie and Leathley, 1993). To find out whether opioid and nonopioid mechanisms may participate in the discriminative stimulus effects of tramadol, we analyzed the ability of the mu opioid receptor peptide agonist morphine and of several antidepressants drugs to substitute for tramadol in rats. Moreover, we propounded the hypothesis that combined treatment with the mu opioid peptide receptor antagonist naloxone or a number of antidepressant drugs alters the interoceptive state generated by tramadol administration. We employed several clinically approved antidepressant drugs showing distinct pharmacological mechanisms. Fluoxetine has been found to be an SSRI (Jacobs and Fornal, 1991; Stokes, 1993), reboxetine is an NARI (Riva et al., 1989; Wong et al., 1997), milnacipram (Moret et al., 1985) and venlafaxine (Muth et al., 1986; Yardley et al., 1990) have been found to be SNRIs, nomifensine is an inhibitor of dopamine reuptake (DARI; Meiergerd and Schenk, 1994; Wieczorek and Kruk, 1994), while roxindole has been found to be a nonselective drug with high affinity (K_i <150 nm) for 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, α_1- and α_2-adrenoceptors, dopamine D1-like and D2-like, and histamine H_1 receptors (Newman-Tancredi et al., 1999; Millan et al., 2002). Fluoxetine, milnacipram, nomifensine, reboxetine, roxindole and venlafaxine were used at a dose range and pretreatment time which were effective in acute rodent experiments measuring potential antidepressant activity (Stenger et al., 1987; Yardley et al., 1990; Jordan et al., 1994; Ossowska et al., 1996; Rogóż et al., 1998, 1999a,b; Malatynska et al., 2002; Harkin et al., 2004).

2. Materials and methods

2.1. Animals

Male Wistar rats (n=16, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland) weighing 280–300 g at the beginning of the experiment were used. The rats were housed in groups of 2/cage (38×25×15 cm) in a colony room maintained at 21±1 °C and 40–50% humidity on a 12-h light–dark cycle (the lights on at 0600 h). Rodent chow was available ad libitum; the amount of water that an animal received was restricted to that given during daily training sessions, after test sessions (10–15 min) and on weekends (36 h). All the experiments were conducted during the light phase of the light–dark cycle (between 0800 and 1400 h), in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the approval of the Bioethics Commission (compliant with the Polish Law of 21 August 1997).

2.2. Drugs

The following drugs were used (supplier): fluoxetine hydrochloride (Farmacon, Kraków, Poland), milnacipram hydrochloride (Centre de Recherche Pierre Fabre, France), morphine hydrochloride (Polfa Kutno, Poland), naloxone hydrochloride (Sigma, St. Louis, USA), nomifensine maleate (Sigma), reboxetine hydrochloride (Pharmacia&Upjohn, Kalamazoo, USA), roxindole hydrochloride (Merck, Darmstadt, Germany), tramadol hydrochloride (Pliva, Kraków, Poland) and venlafaxine hydrochloride (Wyeth–Ayerst Research, Princeton, USA). Fluoxetine, milnacipram, reboxetine, roxindol and venlafaxine were given at 60 min, naloxone at 45 min, and morphine, nomifensine and tramadol at 30 min before tests. All the drugs were dissolved in a 0.9% NaCl, except for fluoxetine and roxindol which were diluted in distilled water, and nomifensine which was dissolved in 2–3 drops of ethanol and diluted as required in distilled water. All those drugs were administered intraperitoneally (i.p.) in a volume of 1 ml/kg. The drug doses refer to the weight of the respective salts.

2.3. Apparatus

Commercially available, two-lever operant chambers (MedAssociates, St. Albans, USA) were used. Each chamber was equipped with a water-filled dispenser mounted equidistantly between two response levers on one wall and was housed in a light- and soundproof cubicle (MedAssociates). Illumination came from a 28-V house light; ventilation and masking noise were supplied with a blower. A computer with MedState software was used to program and record all the experimental events.

2.4. Discrimination procedure

Standard two-lever, water-reinforced drug discrimination procedures were utilized (Filip and Cunningham, 2002, 2003). Two groups of rats (n=8 rats/group) were trained to discriminate tramadol (20 mg/kg i.p.) from saline (1 ml/kg). The training dose of tramadol (20 mg/kg) was chosen on the basis of the literature showing that the tramadol potency in substitution tests in rats is ca. eightfold lower in the

morphine (4 mg/kg i.p.) discrimination (Ren and Zheng, 2000). In addition, our own unpublished data on tramadol locomotor scoring indicated that 30–40 mg/kg of tramadol potently decreased basal activity in rats (Filip et al., unpublished data). Daily sessions lasted 30 min and were conducted on Mondays through Fridays. In the initial "errorless training" phase, only the stimulus-appropriate (drug or saline) lever was present. Training began under a fixed ratio 1 (FR 1) schedule of water reinforcement and the FR requirement was incremented until all the animals were responding reliably under the FR 20 schedule for each experimental condition. For half of the rats, left-lever responses were reinforced after tramadol (20 mg/kg) administration, whereas right-lever responses were reinforced after saline administration; the conditions were reversed for the remaining rats. During that phase of training, tramadol and saline were administered irregularly with the restriction that neither condition prevailed for more than three consecutive sessions. After the responding was stabilized, discrimination training was initiated and both levers were presented simultaneously during 15-min sessions. The rats were made to respond on the stimulus-appropriate (correct) lever in order to obtain water reinforcement, and there were no programmed consequences of responding on the incorrect lever. That phase of training continued until the performance of all the trained rats met the criterion (defined as mean accuracies of at least 80% correct for 10 consecutive sessions).

When the rats achieved the criterion of accuracy, test sessions were initiated and training sessions were run on the intervening days to maintain discrimination accuracy. The rats were required to maintain accuracies of at least 80% correct for the saline and cocaine maintenance sessions which immediately preceded a test. During test sessions, the animals were placed in the chambers and, upon completion of 20 responses on either lever, a single reinforcer was delivered and the house lights were turned off. The sessions were terminated after 15 min if the rats did not complete 20 responses on either lever. Then the rats were removed from the chamber, returned to the colony and allowed free access to water for 10–15 min beginning 15–30 min after the end of each test. Several pharmacological manipulations were performed during the test sessions.

In substitution (generalization) tests, the rats were examined for lever responses after various doses of the training drug, morphine, naloxone, or a few antidepressant drugs. In combination (antagonism or potentiation) tests, doses of the antidepressant drugs or naloxone were administered prior to the different doses of tramadol (2.5–20 mg/kg).

2.5. Data analysis

During training sessions, accuracy was defined as the percentage of correct responses to total responses before the delivery of the first reinforcer; during test sessions, performance was expressed as the percentage of tramadol-appropriate responses to total responses before the delivery of the first reinforcer. Response rates (responses per minute) were also evaluated during training and test sessions as a measure of behavioral disruption. For training sessions, the response rate was calculated as the total number of responses emitted on either lever before completion of the first FR 20 divided by the number of minutes taken to complete that FR 20. During test sessions, the response rate was calculated as the total number of responses before the completion of 20 responses on either lever divided by the number of minutes necessary to complete the FR 20. Only the data from animals that completed the FR 20 during test sessions were used.

A drug was considered to fully substitute for tramadol if at least 80% of responses occurred on the drug-appropriate lever after a dose of that drug; while a complete antagonism was claimed to occur when no more than 20% drug-lever responding occurred after pretreatment with a dose a potential antagonist given in combination with tramadol (20 mg/kg). Student's *t*-test for repeated measures was used to compare the percentage of drug-lever responding and response rate during test sessions with the corresponding values for either the previous drug session (substitution tests), or the training dose tested alone (combination tests). A two-way analysis of variance (ANOVA) for repeated measures was used to find out whether the percentage of tramadol-lever responding and response rates observed for several doses of tramadol differed in the presence vs. absence of a fixed dose of the test drug (combination tests); post hoc comparisons for each dose of tramadol with and without the test drug were made using Student's *t*-test. Log-probit analyses were used to estimate the dose of tramadol predicted to elicit 50% drug-appropriate responding (ED_{50}) and 95% confidence limits (CL) for each treatment combination (Tallarida and Murray, 1987). All comparisons were made with an experimentwise type I error rate (alpha) set at 0.05.

3. Results

3.1. Tramadol (20 mg/kg)–saline discrimination

Acquisition of the tramadol (20 mg/kg) vs. saline discrimination was met in an average of 26 sessions (range: 21–29; Fig. 1). Administration of tramadol (2.5–20 mg/kg) to rats produced a dose-dependent increase in the tramadol-appropriate responding, whereas saline administration resulted in 2% tramadol-lever responding (Fig. 2). The drug-lever responding after 2.5, 5 and 10 mg/kg of tramadol was significantly different from the preceding tramadol training session ($P<0.05$). The dose of tramadol predicted to elicit 50% tramadol-lever responding (ED_{50}) in rats was 8.38 mg/kg (95% CL 6.07–11.56 mg/kg). Response rates for all the test doses of tramadol and saline did not differ

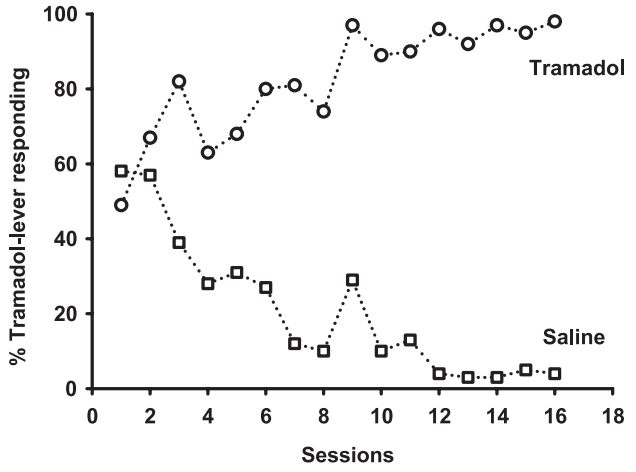

Fig. 1. Acqusition of the tramadol cue. The percentage responding on the tramadol lever as a function of the training session is shown. Data points are the mean from 16 rats and are expressed as a percentage of responses on the tramadol lever to complete the first FR 20 during which no lever presses were reinforced.

from those obtained during the immediately preceding tramadol maintenance session ($P>0.05$; Fig. 2).

3.2. Substitution studies

The preferential mu opioid peptide receptor agonist morphine (1–3 mg/kg) caused full substitution for tramadol (a maximum of 86% drug-lever responding at a dose of 2 mg/kg); the drug-lever responding at 1 mg/kg, but not 2 or 3 mg/kg, of morphine was significantly different from the preceding tramadol maintenance session ($P<0.05$). Any dose of morphine did not affect response rates ($P>0.05$; Fig. 2).

The preferential mu opioid peptide receptor antagonist naloxone (0.03–1 mg/kg; data not shown), milnacipram (Fig. 4, left), fluoxetine, venlafaxine (Fig. 5), nomifensine or roxindol (Fig. 6) at the doses tested evoked more than ca. 20% drug-lever responding when given alone; reboxetine elicited ca. 33% drug-lever responding when given alone (Fig. 4). None of the tested drugs alone altered the response rates (Figs. 4–6; for naloxone data not shown).

3.3. Combination studies

Pretreatment with naloxone (0.1–1 mg/kg) caused a dose-dependent attenuation of the discriminability of tramadol, 20 mg/kg, which alone elicited ca. 97% drug-lever responding; a statistically significant reduction in drug-lever responding was observed for a combination of naloxone (0.3 and 1 mg/kg) and tramadol ($P<0.05$). The effects of naloxone, 1 mg/kg, plus tramadol did not differ from the saline maintenance session ($P>0.05$). No dose of naloxone affected the response rates after tramadol, 20 mg/kg ($P>0.05$; Fig. 3).

Pretreatment with naloxone (0.1–1 mg/kg) significantly reduced the discriminability of morphine, 2 mg/kg, which alone elicited full substitution (ca. 97% drug-lever responding) for tramadol ($P>0.05$). The response rates after any dose of naloxone plus morphine were not altered ($P>0.05$; Fig. 3).

Administration of a fixed dose of milnacipram (10 mg/kg) in combination with various doses of tramadol (2.5–10 mg/kg) altered the tramadol-lever responding [$F(1,12)=22.93$, $P=0.0004$]; a significant increase in the tramadol-lever responding at all the tested doses of tramadol compared to tramadol given alone ($P<0.05$), as well as a leftward shift in the tramadol dose–response curve were observed (Fig. 4). The discriminability of tramadol was potently increased after milnacipram, as reflected by a significant decrease in the dose of tramadol predicted to elicit 50% drug-lever responding (ED_{50}) in animals pre-

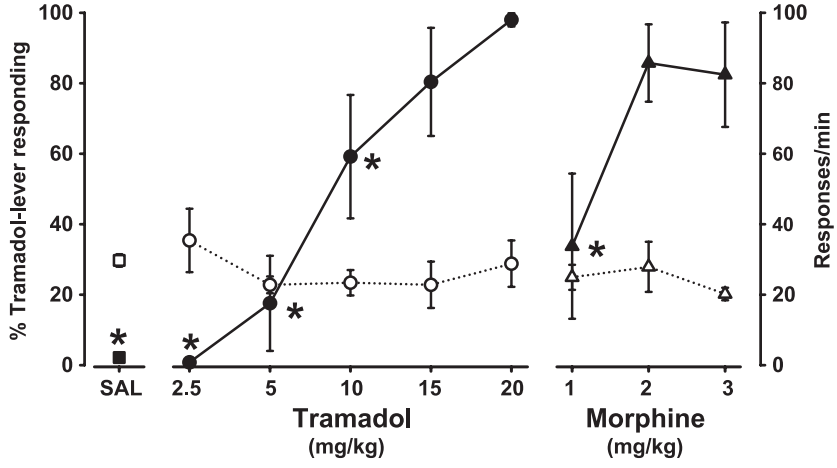

Fig. 2. Substitution studies with tramadol and morphine in rats trained to discriminate tramadol (20 mg/kg) from saline. Symbols show the mean percentage of tramadol-lever responses (±S.E.M.; closed symbols) and the mean number of responses/min (±S.E.M.; open symbols). Performance is shown after injection of saline (SAL; 1 ml/kg; squares), tramadol (circles) or morphine (triangles). All the data points represent the means of data from 6 to 8/8 rats [n/N, number of rats (n) completing the FR 20 on either lever out of the number of rats tested (N)]. Asterisks (*) denote performance during test sessions which was significantly different from that observed after the preceding maintenance training drug session ($P<0.05$).

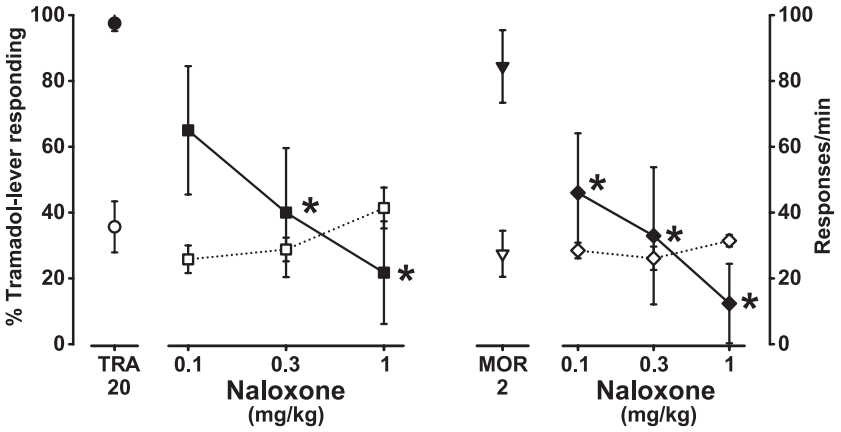

Fig. 3. Antagonism studies with naloxone in rats trained to discriminate tramadol (20 mg/kg) from saline. Symbols show the mean percentage of tramadol-lever responses (±S.E.M.; closed symbols) and the mean number of responses/min (±S.E.M.; open symbols). Left panels: performance is shown after injection of tramadol alone (TRA; 20 mg/kg; circles) or preceded by injection of naloxone (0.1–1 mg/kg; squares). Right panels: performance is shown after injection of morphine alone (MOR; 2 mg/kg; triangles) or preceded by injection of naloxone (0.1–1 mg/kg; diamonds). All the data points represent the means of data from 7 to 8/8 rats. Asterisks (*) denote performance during test sessions which was significantly different ($P<0.05$) from that observed after tramadol, 20 mg/kg, or morphine, 2 mg/kg.

treated with milnacipram, 10 mg/kg [ED_{50}=2.85 mg/kg (95% CL 1.47–5.53 mg/kg); Table 1]. The response rates after milnacipram, 10 mg/kg, plus tramadol (2.5–10 mg/kg) were not altered [$F(1,12)=0.04$, $P=0.84$; Fig. 4].

The fixed dose of reboxetine (10 mg/kg) altered the tramadol-lever responding [$F(1,14)=5.4$, $P=0.036$]; a significant increase in the tramadol-lever responding was observed at all the tested doses of tramadol compared to tramadol given alone ($P<0.05$); a leftward shift in the tramadol dose–response curve took place (Fig. 4). The dose of tramadol predicted to elicit 50% drug-lever responding (ED_{50}) in rats after reboxetine, 10 mg/kg [ED_{50}=4.51 mg/kg (95% CL 2.37–8.57 mg/kg); Table 1], was decreased. The response rates after reboxetine (10 mg/kg) plus tramadol (2.5–10 mg/kg) were not changed [$F(1,14)=0.58$, $P=0.46$; Fig. 4].

Pretreatment with fluoxetine (10 mg/kg) or venlafaxine (10 mg/kg) did not alter the tramadol-lever responding [$F(1,12)=0.6$, $P=0.45$ and $F(1,12)=0.02$, $P=0.89$, respectively; Fig. 5], nor did it significantly change the ED_{50} value for tramadol (Table 1). The response rates were not significantly altered after a combination of fluoxetine [$F(1,12)=1.94$, $P=0.19$] or venlafaxine [$F(1,12)=1.89$, $P=0.2$] and tramadol (Fig. 5).

Administration of the fixed dose of roxindole (1 mg/kg) in combination with tramadol (2.5–20 mg/kg), or of nomifensine (1 mg/kg) in combination with tramadol (5–20 mg/kg), did not alter the tramadol-lever responding

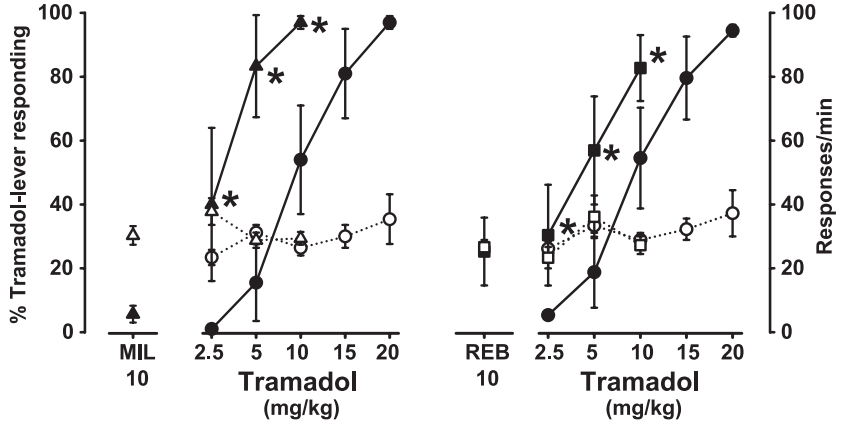

Fig. 4. Substitution and combination studies with the SNRI milnacipram and the NARI reboxetine in rats trained to discriminate tramadol (20 mg/kg) from saline. Symbols show the mean percentage of tramadol-lever responses (±S.E.M.; closed symbols) and the mean number of responses/min (±S.E.M.; open symbols). Left panels: performance is shown after injection of milnacipram alone (MIL; 10 mg/kg; triangles) or after combination of milnacipram+tramadol (2.5–10 mg/kg; triangles). Right panels: performance is shown after injection of reboxetine alone (REB; 10 mg/kg; squares) or after combination of milnacipram+tramadol (2.5–10 mg/kg; squares). For comparison, performance after tramadol alone is shown (circles). All the data points represent the means of data from 6 to 8/8 rats. Asterisks (*) denote performance during test sessions which was significantly different from that observed after the appropriate dose of tramadol ($P<0.05$).

Table 1
ED_{50} values for tramadol obtained in rats trained to discriminate tramadol (20 mg/kg) from saline and pretreated with antidepressants

Pretreatment dose (mg/kg)	ED_{50} (mg/kg)
–	8.38
Fluoxetine (10)	7.04
Milnacipram (10)	2.85
Nomifensine (1)	11.12
Reboxetine (10)	4.51
Roxindole (1)	10.68
Venlafaxine (10)	8.09

[$F(1,11)=0.32$, $P=0.33$ and $F(1,11)=1.23$, $P=0.29$, respectively; Fig. 6], nor did it significantly change the ED_{50} value for tramadol (Table 1). The response rates were not significantly altered after a combination of roxindole or nomifensine and tramadol [$F(1,11)=2.54$, $P=0.14$ and $F(1,11)=4.07$, $P=0.69$, respectively; Fig. 6]. It should be added here that doses of nomifensine or roxindole higher than 1 mg/kg (i.e., 3, 5 or 10 mg/kg) evoked behavioral disruption in animals (only 1 or 2 out of the 7–8 animals used in the tests were able to complete FR 20 on either lever; data not shown).

4. Discussion

The present study indicates that tramadol can be used as a stimulus cue in rats. In fact, in an average of 26 training sessions, all the trained animals were able to recognize the interoceptive state evoked by administration of tramadol as compared to the vehicle. Moreover, pharmacological analyses using mu opioid peptide receptor ligands and different monoamine reuptake inhibitors provided an insight into the underlying mechanisms of the discriminative stimulus effect of tramadol.

In the first place, we have found that the mu opioid peptide system plays an important role in tramadol discrimination, because morphine (a preferential mu opioid peptide receptor agonist; Leslie et al., 1980) fully substitutes for tramadol, while naloxone (a preferential mu opioid peptide receptor antagonist; Leslie et al., 1980; Goldberg et al., 1998) reduces both the tramadol discrimination and the morphine substitution for tramadol. The above effects of morphine and naloxone are specific, because no changes in the animals' response rates have been observed. The relationship between tramadol and morphine is symmetric, because other authors have shown the full substitution of tramadol for morphine in rats trained to recognize morphine from saline, this effect being blocked by the selective mu opioid peptide receptor antagonist naltrexone (Ren and Zheng, 2000). As regards opioidergic mechanisms, it should be stressed that tramadol binds weakly to mu opioid peptide receptors (Raffa et al., 1992; Driessen et al., 1993; McClellan and Scott, 2003). However, the metabolite of tramadol M_1 shows a considerable higher affinity for mu opioid peptide receptors than the parent drug (Frink et al., 1996). We did not ascertain in the present study whether M_1 participated in the discriminative stimulus effects of tramadol; however, in discrimination sessions, the pretreatment time of tramadol was 30 min, while its half-life is about 5 h (Dayer et al., 1997; McClellan and Scott, 2003), which seems to exclude a role of the metabolite.

Tramadol exists as a racemic mixture of two enantiomers (Frink et al., 1996), being either a serotonin [(+)-enanatiomer] or a noradrenaline [(−)-enantiomer] reuptake inhibitor (Matthiesen et al., 1998), which gives support to the concept that monoaminergic component(s) may also play some role in tramadol discrimination. The choice of reuptake inhibitors in the present study was influenced by their selectivity for noradrenaline (reboxetine; Riva et al., 1989; Wong et al., 1997), serotonin (fluoxetine; Jacobs and Fornal,

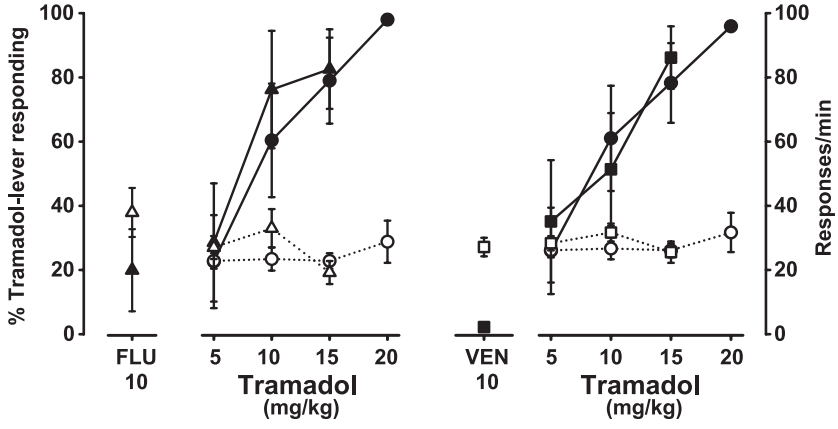

Fig. 5. Substitution and combination studies with the SSRI fluoxetine and the SNRI venlafaxine in rats trained to discriminate tramadol (20 mg/kg) from saline. Symbols show the mean percentage of tramadol-lever responses (±S.E.M.; closed symbols) and the mean number of responses/min (±S.E.M.; open symbols). Left panels: performance is shown after injection of fluoxetine alone (FLU; 10 mg/kg; triangles) or after combination of fluoxetine+tramadol (5–15 mg/kg; triangles). Right panels: performance is shown after injection of venlafaxine alone (VEN; 10 mg/kg; squares) or after combination of venlafaxine+tramadol (5–15 mg/kg; squares). For comparison, performance after tramadol alone is shown (circles). All the data points represent the means of data from 6 to 8/8 rats.

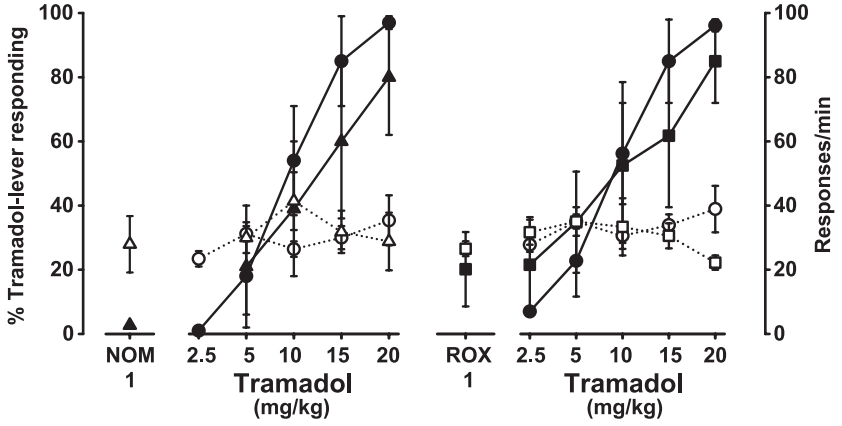

Fig. 6. Substitution and combination studies with the DARI nomifensine and the nonselective antidepressant roxindole in rats trained to discriminate tramadol (20 mg/kg) from saline. Symbols show the mean percentage of tramadol-lever responses (±S.E.M.; closed symbols) and the mean number of responses/min (±S.E.M.; open symbols). Left panels: performance is shown after injection of nomifensine alone (NOM; 1 mg/kg; triangles) or after combination of nomifensine+tramadol (5–20 mg/kg; triangles). Right panels: performance is shown after injection of roxindole alone (ROX; 1 mg/kg; squares) or after combination of roxindole+tramadol (2.5–20 mg/kg; squares). For comparison, performance after tramadol alone is shown (circles). All the data points represent the means of data from 5 to 8/8 rats.

1991; Stokes, 1993), or both serotonin and noradrenaline transporter sites (milnacipram and venlafaxine; Moret et al., 1985; Muth et al., 1986; Yardley et al., 1990). In this study, we found that reboxetine and milnacipram enhanced two- to fourfold tramadol discrimination, whereas fluoxetine and venlafaxine were inactive in that respect.

An important key step forward in understanding the influence of the above-mentioned monoamine reuptake inhibitors on tramadol discrimination was to take account of their affinities for transporter sites. Thus reboxetine and milnacipram have been found to possess high affinity for noradrenaline transporter sites (K_i in a range of 13–100 nM; Moret et al., 1985; Riva et al., 1989; Wong et al., 1997), and their IC_{50} value for noradrenaline uptake inhibition in vitro is lower than 10 nM (Moret et al., 1985; Riva et al., 1989; Wong et al., 1997). Both milnacipram and reboxetine inhibit serotonin reuptake less potently than that of noradrenaline in vitro, the serotonin/noradrenaline IC_{50} values being 20 and 134, respectively (Moret et al., 1985; Riva et al., 1989; Wong et al., 1997). On the other hand, fluoxetine and venlafaxine are potent SSRIs (Muth et al., 1986; Yardley et al., 1990; Stokes, 1993). Venlafaxine (but not fluoxetine) inhibits noradrenaline reuptake, being a ca. threefold weaker serotonin reuptake blocker in in vitro assays (Muth et al., 1986; Stokes , 1993). The lack of the effect of venlafaxine and fluoxetine is surprising, the more so as both these drugs share a number of pharmacological features which may allow to extend and overlap therapeutic indications of the use of venlafaxine or fluoxetine and tramadol. For example, a combination of venlafaxine+tramadol or fluoxetine+tramadol was more effective in increasing the pain threshold in a rat model of neuropathic pain than was either of those drugs administered alone (Uyar et al., 2003; Singh et al., 2004). On the other hand, in partial support for our results, fluoxetine neither substituted for morphine discrimination nor altered morphine dose–response curve in rats (Hynes et al., 1985).

In our studies with nomifensine, we found that that DARI (Meiergerd and Schenk, 1994; Wieczorek and Kruk, 1994) was inactive towards tramadol discrimination; the latter observation, as well as the findings reported elsewhere in the paper, prove that noradrenergic uptake inhibitors can enhance tramadol discrimination; they also indicate smaller significance of the serotonin and dopamine systems for the modulation of tramadol stimulus in rats. It should be stressed here that the negative results obtained with fluoxetine may be due to the use of only one dose of the drug; moreover, the latter antidepressant and nomifensine were tested for one pretreatment time only. Our results obtained with another antidepressant, roxindole (a nonselective drug with high affinity for serotonin 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, α_1- and α_2-adrenoceptor, dopamine D1- and D2-like, and histamine H_1 receptor sites; Newman-Tancredi et al., 1999; Millan et al., 2002) and in particular the findings that none of the monoamine inhibitors used substituted for tramadol indicate that the stimulus properties of tramadol are complex and different classes of neurotransmitter systems and receptors may be involved. These findings seem interesting enough due to the pharmacological profile of tramadol resembling that of monoamine reuptake inhibitors (Matthiesen et al., 1998) and its antidepressant-like activity in rodents (Rojas-Corrales et al., 1998; Hopwood et al., 2001; Singh et al., 2004).

As regards the mechanisms of enhancement of tramadol discrimination by milnacipram and reboxetine, apart from the pharmacological factor (see above), a possible pharmacokinetic interaction should be taken into account. In fact, it was demonstrated that the metabolism of several antidepressant drugs and tramadol depended on cytochrome *P*-450 (Paar et al., 1997; Caccia, 1998; Alfaro et al., 2000; Subrahmanyam et al., 2001). While tramadol metabolism depends on cytochrome *P*-450 2D6 activity (Paar et al.,

1997; Subrahmanyam et al., 2001), both the antidepressant drugs that enhance tramadol discrimination differently modulate the cytochrome *P*-450 system: reboxetine preferentially inhibits mainly cytochrome *P*-450 3A4 isoenzyme (Caccia, 1998; Wienkers et al., 1999), whereas milnacipram does not interact with the cytochrome *P*-450 system (Caccia, 1998). Hence, an explanation based on a kinetic interaction between milnacipram or reboxetine and tramadol seems rather doubtful.

In conclusion, our results indicate that tramadol may serve as a compound stimulus in rats. Moreover, pharmacological analyses show that opioid mechanisms are involved in the discriminative stimulus effects of tramadol, while noradrenergic (but not serotonergic or dopaminergic) transmission may enhance tramadol discrimination.

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