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TRAMADOL INDUCES ANTIDEPRESSANT-TYPE EFFECTS IN MICE

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Abstract. Tramadol is a clinically-effective, centrally-acting analgesic. This drug is a racemic mixture of two enantiomers, each one displaying different mechanisms: (+)tramadol displays opioid agonist properties and inhibits serotonin reuptake while (-)tramadol inhibit preferentially noradrenaline reuptake. The action of tramadol on the monoaminergic reuptake is similar to that of antidepressant drugs. Therefore, we have examined the effects of (±)tramadol, (+)tramadol and (-)tramadol in a test predictive of antidepressant activity, the forced swimming test in mice. Both (±)tramadol and its (-) enantiomer displayed a dose-dependent reduction on immobility; while the effect induced by the (+)enantiomer was not significant. Inhibition of noradrenaline synthesis, but not of serotonin synthesis, was capable of blocking the effect of (±)tramadol. The alpha-adrenoceptor antagonist phentolamine, as well as the alpha$_2$-adrenergic antagonist yohimbine, and the beta-adrenoceptor blocker propranolol, countered the immobility-reducing action of (±)tramadol. Moreover, neither the serotoninergic blocker methysergide nor the opioid antagonist naloxone antagonized the effect of (±)tramadol. Our results show that (±)tramadol and (-)tramadol have antidepressant-like effect in mice, probably mediated by the noradrenergic system rather than the serotoninergic or opioidergic ones.

Key Words: tramadol, antidepressant, serotonin, noradrenaline, opioids

Introduction

Tramadol hydrochloride, (1*RS*,2*RS*)-2-{(dimethyl-amino)methyl}-1-(3-methoxyphenyl)-cyclohexanol HCl, is a synthetic centrally-acting analgesic used mainly for the treatment of moderate or severe pain (1). Clinical experience with tramadol indicates that it produces different effects compared with traditional, centrally-acting analgesics (1, 18).

Tramadol has a relatively weak opioid receptor affinity, with a K_i in the micromolar range (2). This compound is also able to inhibit the reuptake of monoamines (3), a mechanism similar to that of antidepressant drugs. However, this drug is a racemic mixture of two enantiomers, each one displaying different affinities for various types of receptors. The (+)tramadol enantiomer is a selective agonist for μ receptors wich preferentially inhibits serotonin reuptake and enhances serotonin efflux in the brain, whereas the (-)enantiomer mainly inhibits noradrenaline reuptake (3-4). In addition, the active M1 metabolite of tramadol, produced by *O*-demethylation (M1= *O*-demethyltramadol), shows a higher

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affinity for opioid receptors than the parent compound; this M1 metabolite shows a weaker action in the inhibition of monoamine reuptake and in facilitating the serotonin efflux (1). Thus, tramadol causes the activation of both systems mainly involved in the inhibition of pain: the opioid and the descending monoaminergic systems.

In addition to its role in pain processes, opioids have been largely implicated in depressive disorders. The high concentration of opioid peptides and receptors in limbic areas involved in the regulation of mood and behavior support this hypothesis (5). Clinically, β–endorphins have been associated with specific clinical symptoms of depression (6). Moreover, it has been suggested that the hypoalgesic and mood-elevating action of antidepressants might be mediated by the opioid system in the central nervous system (CNS) (7). In this respect, some reports demonstrate that antidepressant drugs have an influence on opioid systems (8). In addition, recent studies by our laboratory show that inhibition of the enkephalin-degrading enzymes have antidepressant-like effects (9). Finally, buprenorphine has shown antidepressant properties in clinical studies (10).

Given the implication of both opioid and monoaminergic systems in depressive disorders, and the dual mechanism of action of tramadol, the testing of this drug and its enantiomers in a model of depression in mice, the forced swimming test (11), is considered to be of interest. This test was developed to predict the antidepressant action of drugs, and there is a significant correlation between clinical potency and potency of antidepressants in this test. In addition, we have evaluated the participation of noradrenergic, serotoninergic and opioid mechanisms.

Methods

Animals: Albino Swiss male mice (25-30 g) obtained from the Central Animal Service of the University of Cádiz were used. The animals were maintained under standard conditions: 12-h light-dark schedule (light on at 8 h 00 min a.m.) with *ad libitum* food and water and at constant temperature (21 ± 1 °C). Animals were housed in the test room 24 h before starting the experiments. The experimental protocol was approved by the Local Committee for Animal Experimentation of the Faculty of Medicine of the University of Cádiz (Licence number 079604). Experiments were carried out blind between 10 h 00 min and 13 h 00 min, and 10 animals were used per group.

The forced swimming test: The procedure was that described by Porsolt et al. in 1977 (11). Naive mice were dropped individually into glass cylinders (height= 25 cm, diameter= 10 cm) containing water 6 cm deep at 22 ± 1 °C, and left there for 6 min. The total duration of immobility during the last 4 min was recorded. Reduction of immobility in this test was considered to indicate antidepressant activity. A mouse was judged to be immobile when it remained floating in the water making only the movements necessary to keep its head above the water.

Drugs and injections: Racemic tramadol hydrochloride, (+)tramadol hydrochloride and (-)tramadol hydrochloride were donated by Grünenthal-Germany, Andrómaco-Spain. Other drugs, imipramine HCl, D,L-α-methyl-*p*-tyrosine (AMPT), *p*-chlorophenylalanine methylester hydrochloride (PCPA), phentolamine HCl, yohimbine HCl, D,L-propranolol HCl, methysegide maleate and naloxone HCl were obtained by a commercial source, Sigma. All drugs were dissolved in saline, tramadol and its enantiomers, or their vehicle, were i.p. injected 60 minutes before the test. The inhibitors of the monoaminergic synthesis (AMPT and PCPA) and the noradrenergic, serotoninergic and opioid antagonists, were associated with racemic tramadol. Antagonists, or their saline control injection, were i.p. injected 30 min before the test, AMPT (200 mg/Kg), or saline, was given in two i.p. injections, 24 and 2 hours before the swimming test. PCPA, or saline, was i.p. injected twice (300 mg/Kg each dose)

36 and 12 hours before the experiment. Control animals received the saline vehicle only. The injections were given in a volume of 0.1ml/10g body wt.

Statistics: The results are expressed as percentages of the mean value of control (saline treated) animals. Statistical analysis was performed on the raw data. Differences between groups were analyzed using a Student-Newman-Keuls test following significant main effect of treatments by one-way ANOVA. A p value of < 0.05 was considered to be significant.

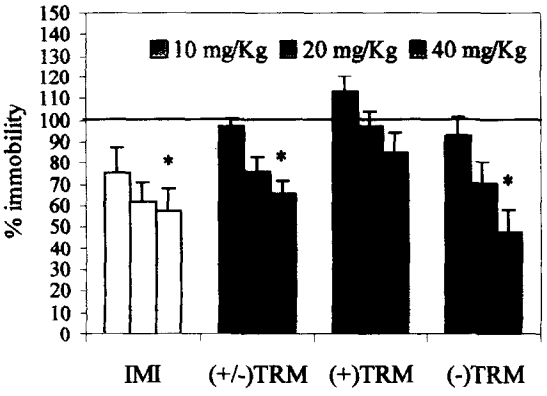

Fig. 1
Dose-effect relationship for imipramine (IMI: 5, 10, 20 mg/Kg, i.p.), tramadol and its enantiomers (TRM= tramadol) in the forced swimming test. * $p< 0.05$ vs Saline.

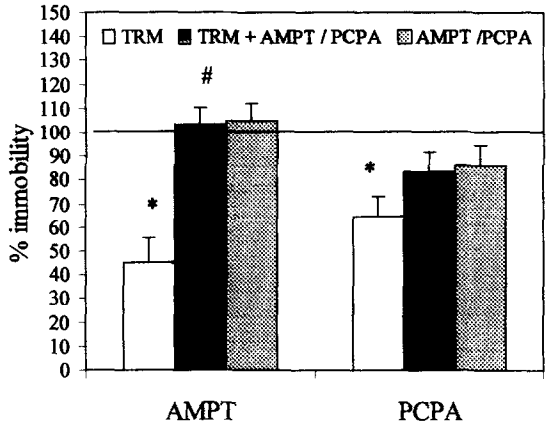

Fig. 2
Effect of pretreatment with AMPT and PCPA on the immobility-reducing action of racemic tramadol (TRM: 40 mg/Kg, i.p.). * $p< 0.05$ vs Saline, # $p< 0.05$ vs TRM.

Results

As shown in Fig. 1, racemic tramadol and (-)tramadol demonstrated antidepressant-like activity by reducing the immobility time dose-dependently in the forced swimming test ($F_{(3,36)}= 8.3444$, $p< 0.001$ and $F_{(3,34)}= 7.6176$, $p< 0.001$, repectively). This effect was similar to that of imipramine ($F_{(3,33)}= 4.2710$, $p< 0.05$). Although a slight tendency towards inhibition was seen with (+)tramadol, its effect was not statistically significant ($F_{(3,36)}= 1.8330$, $p= 0.1586$).

The effects of AMPT and PCPA administrations on the immobility-reducing action of racemic tramadol is shown in Fig. 2. The inhibition of noradrenaline synthesis by AMPT (2 x 100 mg/Kg, i.p.) had no effect on immobility time when compared to saline. However, pretreatment with AMPT antagonized the immobility-reducing action of tramadol (103.12 ± 7.14% vs 44.96 ± 10.71%, $p< 0.0001$). On the other hand, administration of PCPA (2 x 300 mg/Kg, i.p.) prior to drug treatment did not antagonize the reduction in immobility time produced by tramadol (83.53 ± 8.15% vs 64.74 ± 8.43%, $p> 0.05$). PCPA alone caused no significant changes in immobility.

Fig. 3 summarizes the effects on the antidepressant-type action of tramadol produced by the blocking of the noradrenergic, serotoninergic and opioid recptors. None of the antagonists induced any significant effect on the immobility. Thus, the variation of

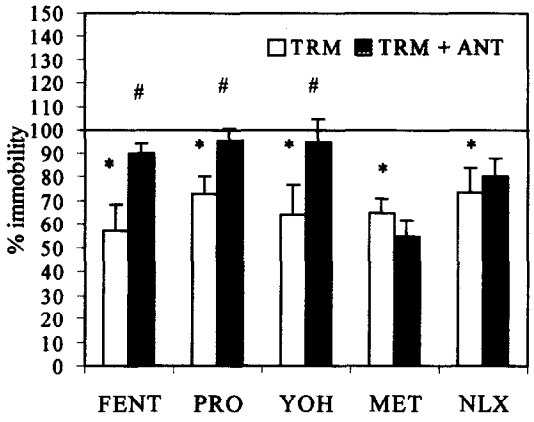

Fig. 3
Counter effects of different antagonists (ANT) on the imobility time reduction, induced by racemic tramadol (TRM: 40 mg/Kg, i.p.). FENT = phentolamine, PRO= propranolol, YOH= yohimbine, MET= methysergide, NLX= naloxone. * $p< 0.05$ vs Saline, # $p< 0.05$ vs TRM.

immobility time induced by phentolamine, propranolol, yohimbine, methysergide and naloxone administered alone were, respecively: -10.89% (n.s.), -15.23% (n.s.), +0.63% (n.s.), -12.16% (n.s.) and +5.89% (n.s.) versus control group. The reduction in immobility induced by tramadol was reversed by both the non-specific alpha-antagonist phentolamine (89.72 ± 4.41% vs 57.05 ± 11.36%, $p< 0.05$) and by the specific alpha$_2$ blocking agent yohimbine (94.93 ± 9.90% vs 63.96 ± 12.39%, $p< 0.05$). The non-specific beta-adrenergic antagonist propranolol reversed the action of tramadol at 2 mg/Kg (95.64 ± 4.94% vs 72.96 ± 7.15%, $p< 0.05$).

In relation to the serotoninergic system, blocking of 5-HT receptors by methysergide (2 mg/Kg, i.p.) did not significantly modify the effect of racemic tramadol (54.67 ± 7.09% vs 64.79 ± 6.26%, $p> 0.05$). Neither the opioid antagonist naloxone (2 mg/Kg, i.p.) reverse this effect of racemic tramadol (80.29 ± 7.49% vs 73.28 ± 10.26%, $p> 0.05$).

Discussion

In this study, the antidepressant-like effect of tramadol and its enantiomers was investigated in the forced swimming test (11), an animal model predictive of antidepressant activity.

In our experimental conditions, only the racemic and the (-) enantiomer of tramadol showed a clear inhibition of immobility latencies (antidepressant-type effect). This effect was similar to that obtained with the tricyclic antidepressant imipramine. The (+) enantiomer of tramadol, which preferentially inhibits serotonin reuptake, was ineffective in this test, although a tendency towards inhibition was seen at high doses.

In accordance with these results, AMPT (inhibitor of noradrenaline synthesis, 12), phentolamine (non-specific alpha-adrenergic receptor antagonist), yohimbine (alpha$_2$-adrenergic receptor antagonist) and propranolol (non-specific beta-adrenergic receptor antagonist) were all capable of reducing the effect of (±) tramadol, while PCPA (inhibitor of serotonin synthesis, 13) and methysergide (non-specific 5HT-receptor antagonist) were not. This indicated that the noradrenergic mechanisms were those responsible for the antidepressant-like effect observed with the racemic form of tramadol. However, in the forced swimming test, the role of serotonin seems minimal, since serotoninergic drugs showed no effect in this test (14). In fact, clomipramine which preferentially inhibits the serotonin reuptake, similar to the (+) tramadol enantiomer, has been suggested to show an effect in the forced swimming test in mice, but rather through involvement of desmethylclomipramine, which preferentially acts on noradrenergic neurons (15). Conversely, in rats in which clomipramine is poorly metabolized (16), it is inactive in the forced swimming test (17). In view of this, the implication of serotonin needs to be explored in other

depression tests, in order to reach precise conclusions. Nevertheless, the analgesic effect of tramadol seems to be clearly related to serotonin and noradrenaline in several pain tests (2).

Examination of the neurochemical profile of tramadol reveals that it binds to opioid receptors in the same concentration range in which it inhibits the uptake of noradrenaline and serotonin . Naloxone partially inhibits the analgesic effect of tramadol in animals (2), although in humans, a clear antagonism has been reported (18). Similarly, in our experiments using the swimming test paradigm, the antidepressant-like effect of tramadol was not antagonized by the opiate antagonist. These results, at first, rule out an opioid component. However, taken into account that similar doses of naloxone reversed the effect of antidepressant drugs in the same test (19) as well as in nociceptive test (20), and that (+) tramadol, wich displays higher opioid properties than the racemate (3-4), shows a slight tendence; the implication of an opioid component in the effect induced by (±)tramadol is needed to be further explored. Indeed, the antidepressant effects of opioids in several depression test have been reported (9). In humans, opiates has been proved be useful in treating some forms of refractory depression (10).

Finally, several studies have documented two components in the efficacy of antidepressants as an adjuvant therapy for chronic pain. One of them is the increase in mood level, frequently decreased in chronic pain patients; and the other is a proper antinociceptive effect. In fact, monoamines and opioid pathways are implicated both in pain and mood. In this respect, it could be inferred from our experimental studies that tramadol might add an affective (positive emotional) component to its analgesic effect. Further preclinical studies are needed to explore the effect of different administration regimes and the efficacy of tramadol in other types of depression tests.

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