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Antinociception, Tolerance, and Physical Dependence Comparison Between Morphine and Tramadol

H. F. MIRANDA AND G. PINARDI

Department of Pharmacology, Faculty of Medicine, Universidad de Chile, Santiago, Chile

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MIRANDA, H. F. AND G. PINARDI. *Antinociception, tolerance, and physical dependence comparison between morphine and tramadol.* PHARMACOL BIOCHEM BEHAV **61**(4) 357–360, 1998.—The mechanism of action of tramadol includes the activation of opioid receptors, and the potential ability of the drug to induce tolerance and physical dependence has been evaluated in different animal species and humans. This work was designed to study the involvement of opioid receptors in the antinociceptive activity and the potential ability to develop tolerance, crosstolerance, and/or physical dependence of tramadol. The writhes induced by acetic acid administration was used as algesiometric test. After chronic administration of tramadol, tolerance was evaluated by measuring the antinociceptive activity, and physical dependence was measured by naloxone administration. Morphine was used as drug of comparison. The IP administration of tramadol produced a dose-dependent antinociception with an ED_{50} value of 7.82 ± 1.16 mg/kg, which was unchanged after chronic administration of either tramadol (39.1 or 100 mg/kg) or morphine (1.05 or 100 mg/kg). By contrast, the ED_{50} for morphine (0.21 ± 0.08 mg/kg) was significantly reduced only by chronic pretreatment with both doses of morphine (tolerance). Physical dependence was developed only in mice pretreated with morphine, as evidenced by the presence of jumps, wet-dog shakes, tachypnea, piloerection, seizures, diarrhea, and urination after the administration of naloxone (1 mg/kg). These findings suggest that the antinociceptive activity of tramadol in mice is due to activation of opioid and nonopioid mechanisms, and as opposed to morphine, is not likely to induce tolerance and physical dependence. © 1998 Elsevier Science Inc.

Tramadol Morphine Naloxone Antinociception Tolerance physical dependence

TRAMADOL, a compound derived from cyclohexanol HCl, is an effective and safe analgesic for the management of pain that can be administered either orally, intramuscularly, intravenously, or by patient-controlled analgesia (1,13,27). Tramadol in experimental and clinical trials exhibited a good analgesic efficacy and a potency comparable to codeine (8,13,27). In acute postoperative pain its efficacy was similar to that of morphine and, in addition, a long-term tramadol treatment in patients with nocigenic, neurogenic, or sympathogenic pain was as effective as sustained release of morphine and significantly better than either amitriptyline or carbamazepine (1,30).

The mechanism of action of tramadol includes the activation of opioid receptors (22), and even if tolerance and dependence were not described after repeated administration of tramadol in humans (1,21,27,29), the potential ability of the drug to induce dependence has been preclinically evaluated in different animal species (6,18,33). Tramadol has not been associated with significant opioid side effects such as respiratory depression, constipation, or sedation (13,29); however, tramadol administration results in some undesired effects, such as nausea and vomiting, which are typically observed with opioids (8,30). In addition, the use of naloxone to measure the opioid involvement in the antinociceptive activity of tramadol has produced conflictive results (2,4,12,21).

This work was designed to study the involvement of opioid receptors in the antinociceptive activity and the potential ability to develop tolerance and/or physical dependence of tramadol, as compared to morphine.

METHOD

Male CF-1 mice, weighing 20–25 g, from the colony of the Department of Pharmacology were used throughout the experimental work. They had free access to food and water in a regulated environment (22 ± 1°C) with light/dark cycles of 12 L:12 D. The animals were acclimatized to the laboratory environment

Requests for reprints should be addressed to H. F. Miranda, Department of Pharmacology, Box 70.000, Santiago 7, Chile.

for at least 2 h before being used; the ethical standards guidelines were followed as previously described (25). Each animal was used only once and received only one dose of the drugs tested. All drugs were freshly prepared by dissolving them in normal saline and doses were calculated on the basis of the drug salts.

All observations during the assay were performed by the authors in a randomized and blind manner. Evaluation of antinociceptive activity was accomplished as previously reported (17). The antinociceptive activity of tramadol was measured at several time periods after drug administration (10, 20, 30, 40, and 60 min) to determine the time of peak effect. Thirty minutes after intraperitoneal (IP) administration of drugs or saline (10 ml/kg), mice were injected IP with 10 ml/kg of 0.6% acetic acid. The number of writhes was counted during a 5-min period, starting 5 min after the administration of the acetic acid solution. A writhe was defined as a contraction of the abdominal muscles accompanied by an elongation of the body and extension of the forelimbs. Control animals (saline) were run interspersed concurrently with the drug-treated animals, which prevented all the controls being run on a single group of mice at one time during the course of the investigation.

A dose–response curve, determined at the time of peak effect (30 min), was constructed to assess the antinociceptive action of tramadol. The dose of tramadol that produced 50% of antinociception (ED_{50}: 50% reduction of control writhes) was calculated using standard linear regression analysis of the log dose–response curve. Antinociceptive activity was expressed as percent inhibition of the usual number of writhes observed in saline control animals (27.2 ± 1.0, $n = 30$).

A modification of the method of Song and Takemori (26) was used to study tolerance and dependence. Mice were made chronically tolerant by subcutaneous (SC) injections of tramadol (100 mg/kg) or morphine (100 mg/kg), three times daily during 5 days (0900, 1300, and 1700 h). A similar schedule was applied for the induction of tolerance using equieffective doses of tramadol or morphine, corresponding to five times the ED_{50} (39.1 and 1.05 mg/kg, respectively). The degree of tolerance was tested by measuring the antinociception induced by the ED_{50} of either morphine or tramadol in the writhing test. The physical dependence was evaluated by the ocurrence of different signs (seizures, wet-dog shakes, urination, diarrhea, piloerection, jumps, and tachypnea) induced by the IP administration of naloxone (1 mg/kg). The intensity of the withdrawal syndrome (IWS) was arbitrarily evaluated by assigning a number to the group (morphine or tramadol), which resulted from the ratio: number of animals presenting signs in a 20-min observation period/total number of animals in the test (20).

The drugs used were: tramadol hydrochloride, a gift of Grunenthal Chilena, naloxone hydrochloride, obtained from Sigma Chemical Co., St. Louis, MO, and morphine hydrochloride from Merck Chemical Co., Darmstadt, Germany.

Results are presented as mean values ± SEM and were examined by Student's *t*-test for unpaired data. The lines obtained by linear regression analysis of the log dose–response curves were analyzed for parallelism according to Tallarida and Murray (28). Statistical significance was accepted at the 0.05 level.

RESULTS

Antinociceptive Activity

The IP administration of tramadol produced a dose-dependent antinociceptive activity measured by the acetic acid writhing test, with an ED_{50} value of 7.82 ± 1.16 mg/kg. The comparison drug was morphine. Morphine was administered IP and displayed a dose–response curve with an ED_{50} value of 0.21 ± 0.08 mg/kg. The statistical analysis of the dose–response curves of tramadol and morphine demonstrates that the curves are not parallel ($p > 0.05$) (Fig. 1).

Tolerance/Dependence Studies

In mice made chronically tolerant by SC administration three times daily during 5 days of equieffective doses of tramadol or morphine (1.05 and 39.1 mg/kg, respectively) or with 100 mg/kg of tramadol or morphine, the antinociceptive activity induced by the ED_{50} of tramadol in both pretreatment was not changed. However, the effect of morphine ED50 was significantly lower only in the both groups pretreated chronically with morphine (Table 1), with a tendency for the reduction of morphine antinociception in tramadol-dependent mice. Physical dependence revealed by the administration of 1 mg/kg of naloxone was developed only in mice pretreated with morphine, as evidenced by the presence of jumps, wet-dog shakes, tachypnea, and piloerection in every animal of the group (six out of six) and seizures, diarrhea, and urination in five out of six animals pretreated with the schedule of 100 mg/kg of morphine. Naloxone administration to mice pretreated three times daily for 5 days with 1.05 mg/kg of morphine SC induced a lower intensity withdrawal syndrome consistent in wet-dog shakes, piloerection, and tachypnea in three out of six animals. In contrast, mice pretreated with 100 mg/kg of tramadol challenged with naloxone showed only diarrhea, urination, and piloerection in one out of six animals. Mice rendered tolerant by the administration of 39.1 mg/kg of tramadol injected with naloxone showed only wet-dog shakes and piloerection in one out of six animals. These results, expressed as IWS appear in Fig. 2.

DISCUSSION

Tramadol is an antinociceptive agent with low affinity for opioid receptors, because it is approximately 10-fold less potent than codeine, 1000-fold weaker than methadone, and 6000-fold weaker than morphine in the displacement of [^{3}H]DAMGO from opioid binding sites in rat brain prepara-

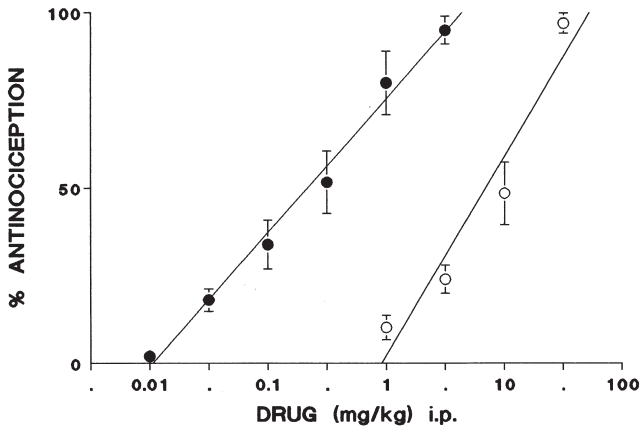

FIG. 1. Dose–response curves for the antinociceptive activity displayed by the IP administration of morphine (●) and tramadol (○) in the mouse writhing test. Each point represents the mean ± SEM of 12 mice for tramadol and 18 mice for morphine.

TABLE 1

ANTINOCICEPTIVE ACTIVITY OF THE ED_{50} OF TRAMADOL AND MORPHINE IN MICE PRETREATED THREE TIMES DAILY FOR 5 DAYS WITH S.C. TRAMADOL (39.1 OR 100 MG/KG) AND MORPHINE (1.05 OR 100 MG/KG)

	% Antinociception		
	Tramadol (mg/kg)		
	Control	39.1	100
Tramadol (7.82 mg/kg)	51.5 ± 5.2	50.2 ± 8.0	45.6 ± 2.6
Morphine (0.21 mg/kg)	50.1 ± 5.0	45.3 ± 3.6	37.5 ± 2.0
	Morphine (mg/kg)		
	Control	1.05	100
Morphine (0.21 mg/kg)	50.1 ± 5.0	8.1 ± 1.6*	15.4 ± 1.1*
Tramadol (7.82 mg/kg)	51.5 ± 5.2	49.7 ± 3.8	48.5 ± 4.0

Values represent means ± SEM of eight animals per group.
*Significant difference from corresponding control (Student's *t*-test: $p < 0.05$).

tions (10,22). This low range of affinity for opioid receptors appears as insufficient to explain its antinociceptive activity, and it has been proposed that inhibition of neuronal uptake of noradrenaline and serotonin may contribute to the antinociceptive efficacy (5,9,22).

The ability of tramadol to induce antinociceptive activity by only partially activating opioid receptors through O-desmethyltramadol (7) is reflected in the fact that the dose–response curve is not parallel with the dose–response curve

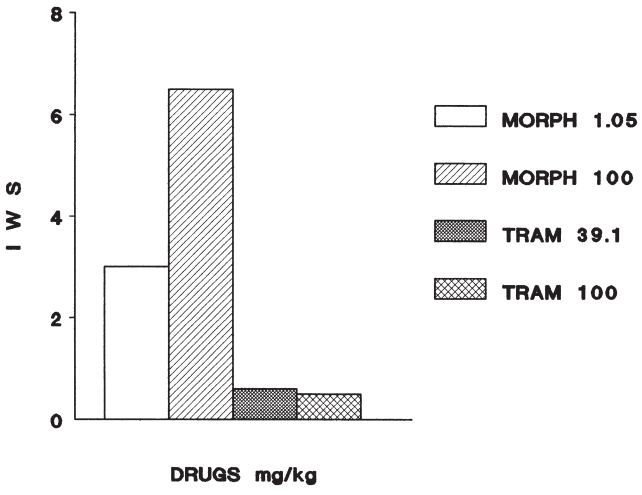

FIG. 2. Intensity of the withdrawal syndrome (IWS) induced by the IP administration of naloxone (1 mg/kg) in mice chronically pretreated with 1.05 or 100 mg/kg of morphine (MORPH) and 39.1 or 100 mg/kg of tramadol (TRAM) three times daily for 5 days. Data are values obtained from six mice in each group, as described in the Method section.

displayed by morphine; furthermore, the different degree of relative potency (0.21 mg/kg morphine vs. 7.82 mg/kg of tramadol; 39.4-fold) suggests a different pharmacological antinociceptive profile of tramadol with respect to morphine. Mattia et al. (16), using ICR mice and warm-water tail flick, reported that morphine is threefold more potent than tramadol, a difference that might be due to the nociceptive stimulus (chemical vs. thermal) used in the algesiometric test and the different mouse strain. The schedule of administration of either tramadol or morphine for the induction of tolerance might not be directly related to the pharmacokinetic properties of both drugs, because morphine has a long half-life active metabolite, morphine-6β-glucuronide (19), and tramadol in mice supposedly activates opioid receptors through an active metabolite of high affinity to the μ subtype (O-desmethyltramadol) with a possibly shorter half-life (7,24). However, the three times daily schedule of injections of tramadol or morphine, in contrast with once or twice daily injections (14,15) is appropriate, because a sustained activation of μ receptors, which have been mainly inplicated in the development of tolerance and dependence, should occur (19,31).

It has been demonstrated that chronic treatment of animals with opioid agents leads to tolerance, in which an increasingly larger amount of drug is required to produce the same acute effect, which can be measured by determining the ED_{50} for antinociception. In the present work, tolerance was developed only in the case of morphine (Table 1) using both schedules, because the ED_{50} for morphine antinociception was reduced by 70–84%. Crosstolerance between morphine and tramadol was not observed, as shown by the unchanged ED_{50} for the antinociceptive activity of tramadol in the morphine-pretreated animals, in agreement with the observation of Kayser et al. (11), who reported that repeated administration of low doses of tramadol in rats (1 mg/kg IV twice daily for 4 days) did not induce tolerance or crosstolerance to morphine. Nevertheless, even if the results are not significant, Table 1 shows a tendency for the reduction of morphine antinociception in tramadol-dependent mice. Similarly, Mattia et al. (16) using a different protocol to induce tolerance, reported a minimal development of tolerance to tramadol antinociception, with minimal crosstolerance with morphine, and suggested that these drugs may act through different mechanisms. As discussed previously, the difference in protocols and in nociceptive stimuli may explain these results. These findings reinforce the different pharmacological profile between morphine and tramadol.

It is known that the chronic administration of some drugs, i.e., diazepam, alcohol, opioids, etc., induces a neuronal-dependent state in which the discontinuation of the treatment is followed by the development of a time-limited withdrawal syndrome, as a reflection of physical dependence, which might be considered as the summation of a number of separate actions mediated through activation of different subtypes of opioid receptors (32). A similar syndrome can be precipitated by the administration of a specific antagonist of the agonist inductor of the dependence. For instance, naloxone is generally used to demonstrate morphine dependence (3,14). The results of the present work confirm the development of physical dependence only in mice chronically pretreated with morphine, as tramadol pretreated animals did not show withdrawal signs. Furthermore, using different protocols in mice, rats, and monkeys, the development of tolerance and dependence was always less pronounced with tramadol than with codeine, pentazocine, nalbuphine, buprenorphine, and morphine (7,11,18, 33). In addition, in a multicenter study in humans tramadol

did not induce dependence, as shown by the failure of naloxone to elicit withdrawal symptoms (23).

In conclusion, the results of the present study suggest that, in mice, the antinociceptive activity of tramadol is due to activation of opioid receptor and nonopioid mechanisms. Tramadol, as opposed to morphine, is not likely to induce tolerance and physical dependence.

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