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Research Article

Synergism Between Tramadol and Meloxicam in the Formalin Test Involves Both Opioidergic and Serotonergic Pathways

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT This study was designed to evaluate the antinociceptive interaction of the tramadol–meloxicam combination in different proportions (tramadol + meloxicam in 1:1, 1:3, and 3:1 ratios), as well as the role of nitric oxide, opioidergic, and serotonergic pathways in the antinociceptive effect of the combination. The effects of individual drugs and fixed-ratio combinations were assayed using the 3% formalin test in mice. Isobolographic analysis was employed to characterize the synergism produced by the combinations. Tramadol (3.16–10 mg/kg, i.m.), meloxicam (3.16–17.8 mg/kg, i.m.), and tramadol–meloxicam combinations produced a dose-dependent antinociceptive effect. ED_{30} values were estimated for the individual drugs, and isobolograms were constructed. The tramadol + meloxicam 1:1 and 1:3 ratio combinations showed synergistic interactions while the 3:1 ratio produced additive effects. Naloxone (1 mg/kg, i.m.) or methiothepin (0.1 mg/kg, i.m.), but not L-NAME (3 mg/kg, i.m.), prevented the antinociceptive effects of the combination. These data suggest that (1) the tramadol–meloxicam combination produces a functional synergistic interaction that involves both opioid and serotonin receptors, and (2) this combination may be a promising tool in pain management. Drug Dev Res 73: 43–50, 2012.

Key words: tramadol; meloxicam; synergism; opioid receptors; serotonin receptors

INTRODUCTION

Opioids remain the most effective therapy available for the treatment of moderate to severe pain in humans. However, the problems arising from unwanted side effects persist. Thus, combinations of opioids and other analgesic drugs are commonly used to control postoperative pain. The potential advantage of using combination therapy is that the analgesic effects can be maximized, whereas the incidence of side effects could be minimized. In addition, the multiplicity of mechanisms involved in pain suggests

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that combination therapy can improve pain management [Raffa, 2001].

Tramadol is a synthetic, centrally acting, analgesic agent widely used for pain relief in children and adults [Scott and Perry, 2000]. It is effective in moderate to severe postoperative pain with an overall efficacy similar to that of morphine or alfentanil. Previous clinical studies have shown that co-administration of magnesium, ketamine [Unlügenç et al., 2002], ketorolac [Pieri et al., 2002], or acetyl salicylate [Pang et al., 2000] and tramadol improves analgesia and patient comfort and decreases the amount of tramadol required for pain management. Animal studies supporting these interactions are lacking. Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) of the enolic acid class of oxicam derivatives indicated for the treatment of rheumatoid arthritis, osteoarthritis and other joint diseases [Engelhardt, 1996; Euller-Ziegler et al., 2001]. It acts mainly through inhibition of cyclooxygenase-2 [Laird et al., 1997; Pairet et al., 1998]. The present study was designed to assess the possible synergistic interaction between tramadol and meloxicam after intramuscular administration by isobolographic analyses. In addition, the possible role of nitrergic, opioidergic, and serotonergic pathways in the synergy induced by the tramadol–meloxicam combination was also assessed.

MATERIALS AND METHODS

Animals

Male Balb/c mice aged 8–9 weeks and weighing 20–25 g were used. The mice were housed at 22°C with a 12-h/12-h light/dark cycle. Animals had free access to food and tap water up to the time of the experiment. All experiments were conducted in accordance with the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [Zimmerman, 1983]. In addition, the study was approved by our local Ethics Committee.

Drugs

Tramadol was obtained from Grünenthal de México, S.A. de C.V. (Mexico City, México), and meloxicam was a gift of Senosiain, S.A. de C.V. (Celaya, México). L-NG-nitroarginine methyl ester (L-NAME), naloxone, and methiothepin were obtained from Sigma (St. Louis, MO). All drugs were dissolved in sterile saline.

Measurement of Nociceptive Activity

Nociception was assessed using the formalin test. Mice were placed in clear plastic chambers with a mirror placed at a 45-degree angle to allow an unobstructed view of the paw. The injection was made into the plantar surface of the right hindpaw with 30 µl of dilute 3% formalin using a 30-gauge needle. Animals were then returned to the chambers; nociceptive behavior was observed immediately after formalin injection. Nociceptive behavior was quantified as the licking time on the injected paw. Mice were sacrificed in a CO_2 chamber at the end of the experiment.

Experimental Design

Different groups were used to characterize the dose-response curve of the various drugs. Increasing doses of tramadol (3.16, 5.6, 7.5, and 10 mg/kg) or meloxicam (3.16, 5.6, 10, and 17.8 mg/kg) were given i.m. 20 min before s.c. administration of 3% formalin. Controls were administered saline solution. Once the dose-response curve of each drug was obtained, an experimental ED_{30} value was determined for each drug. The tramadol–meloxicam combination was evaluated in different proportions (tramadol + meloxicam in 1:1, 1:3, and 3:1 ratios). To assess the possible mechanism(s) of action for the combination, L-NAME (3 mg/kg), naloxone (1 mg/kg), methiothepin (0.1 mg/kg), or vehicle were administered i.p. 10 min before the tramadol+meloxicam combination (ED_{30} value); 50 min later, formalin was injected.

Data Analysis

Data are presented as mean ± SEM for ≥ 6 animals per group. The total time of licking corresponding to the second phase of the assay was determined from 15–45 min with regard to formalin administration. Dose-response data are presented as the percentage antinociception of the total licking time on the second phase of the formalin test. The percentage antinociception was calculated according to the following equation [Argüelles et al., 2002]:

$$[(\text{Vehicle} - \text{postcompound})/\text{vehicle}] \times 100.$$

Dose-response curves were constructed and the experimental points fitted using least-squares linear regression. The SE estimate was calculated as described by Tallarida [2000].

Isobolographic analysis is a convenient tool for evaluating the interaction between analgesic drugs [Argüelles et al., 2002; Tallarida, 2000]. In the present study, we used this technique to determine the nature of interactions between tramadol and meloxicam. Isobolographic analysis assumes that the combination of drugs is made from equipotent doses of the individual drugs. Thus, from the dose-response curves of each individual agent, the dose resulting in 50% of the effect (ED_{50} value) can be determined. However, considering that a maximal effect of 100% as the total suppression of formalin-induced licking and that meloxicam was unable to achieve a 50% response, the calculation of an ED_{50} value was not feasible.

Therefore, the ED_{30} value was estimated instead of the ED_{50} value. Subsequently, a dose-response curve was obtained by concurrent delivery of the two drugs in a constant dose ratio (fixed-ratio) based on the ED_{30} values of each individual agent. The ED_{30} value was evaluated for three combinations (tramadol+meloxicam in 1:1, 1:3, and 3:1 ratios). From the resulting dose-response curve of the combination, the experimental ED_{30} value was then calculated.

To determine whether the interaction between two drugs given in combination was synergistic, additive, or antagonistic, the theoretical additive ED_{50} value (Z_{add}) was estimated from the dose-response curves of each drug administered individually, considering that the observed effect with the combination results of the sum of the individual effects of each component. This theoretical ED_{30} value was then compared with the experimental ED_{30} value (Z_{exp}) to determine whether there is a statistically significant difference [Tallarida et al., 1999; Tallarida, 2002].

The theoretical and experimental ED_{30} values of the studied combinations were also contrasted by calculating the interaction index (γ) as follows:

$$\gamma = ED_{30} \text{ value of combination (experimental)} / ED_{30} \text{ value of combination (theoretical)}.$$

The interaction index indicates the portion of the ED_{30} value of individual drugs that accounts for the corresponding ED_{30} value in the combination. Values of ~ 1 correspond to an additive interaction, values of > 1 imply an antagonistic interaction, and values of < 1 indicate a synergistic interaction.

Statistical Analysis

Dose-response data were analyzed by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test for post hoc comparison. The theoretical additive ED_{30} and the experimentally derived ED_{30} values were evaluated using Student's *t*-test. An experimental ED_{30} value significantly lower than the theoretical additive ED_{30} value was considered to indicate a synergistic interaction between tramadol and meloxicam. Mechanisms of action (control group compared with the antagonist group) were evaluated by one-way ANOVA followed by the Student–Newman–Keuls test. Statistical significance was considered to be achieved when $P < 0.05$.

RESULTS

Antinociceptive Effects of Tramadol, Meloxicam, and Tramadol + Meloxicam Combinations

Tramadol and meloxicam significantly reduced formalin-induced licking in mice (Fig. 1). Figures 2A,B shows dose-response curves for these drugs as well as their combinations during the second phase of the formalin test. The individual drugs and the combinations decreased the nociceptive behavior in a dose-dependent manner, reaching a maximal effect of ~80.8%, 52.12%, and 57.23% for tramadol, meloxicam, and the tramadol + meloxicam 1:3 combination, respectively).

Isobolographic Analysis

The maximum effect reached by the greatest dose of the tramadol + meloxicam combinations in the 1:1, 3:1, and 1:3 ratios were approximately 53%, 46%, and 57%, respectively. Of note, the sum of the individual effects (ED_{30} value of each drug) suggests that the tramadol doses would contribute 30% of its maximum effect (80.8%), i.e., 24.2%. Likewise, if it is assumed

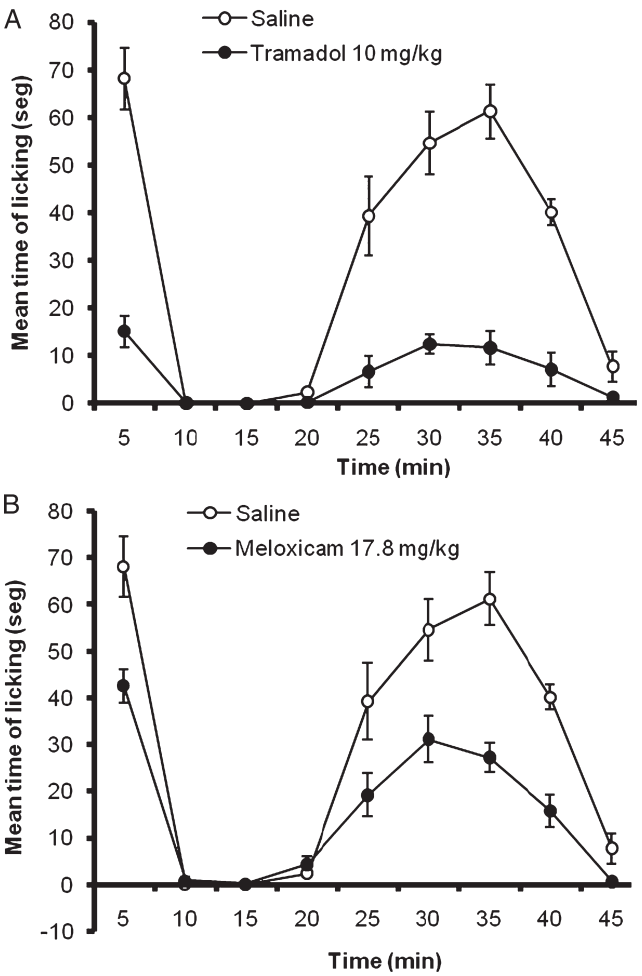

Fig. 1. Time course of the antinociceptive effect of tramamol (10 mg/kg, i.m., **A**) or meloxicam (17.8 mg/kg, i.m., **B**) in mice submitted to the 3% formalin test. Data are expressed as the mean time of licking ± SEM of ≥ 6 animals.

that the maximum effect of meloxicam was 52.1%, the ED_{30} value of this drug would contribute 15.63% of the effect of the combination according to the experimental test. The algebraic sum of such effects would be around 39.8%, which is less than the maximum effect of each combination. Thus, the tramadol + meloxicam combination in 1:1 and 1:3, but not 3:1, ratios produced the greatest effect (Fig. 3A,C). Accordingly, the experimental ED_{30} values of the tramadol + meloxicam combinations in 1:1 and 1:3 ratios were lower compared with the theoretical additive ED_{30} value of the combination (Fig. 3A and C, Table 1). Furthermore, analysis of the interaction index showed an increase in potency for the tramadol+meloxicam combinations in 1:1 ($\gamma = 0.61$) and 1:3 ($\gamma = 0.55$), but not 3:1 ($\gamma = 0.89$) ratio.

Mechanism of Action

L-NAME was unable to reverse the antinociceptive effect of the combination (Fig. 4A). In contrast, naloxone and methiothepin significantly reduced the antinociceptive effect of the tramadol–meloxicam combination (Fig. 4B,C).

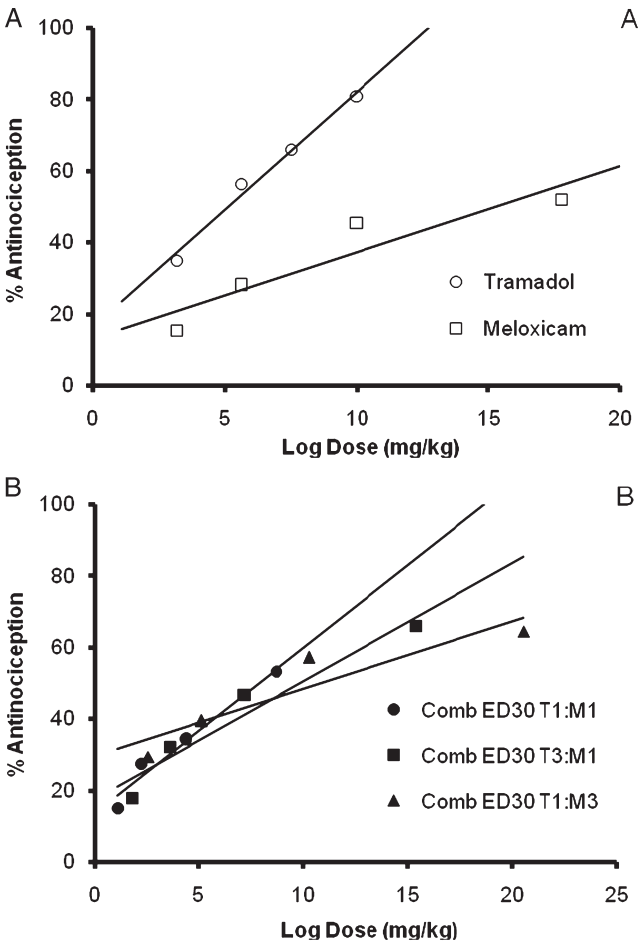

Fig. 2. Comparative dose-response curves for the antinociceptive effect of tramadol and meloxicam alone (**A**) or combined (**B**) during the second phase of the formalin test. Doses of tramadol (○) were 3.1, 5.6, 7.5, and 10 mg/kg (i.m.), whereas those of meloxicam (□) were 3.1, 5.6, 10 and 17.8 mg/kg. Doses of the tramadol+meloxicam combination in 1:1 ratio (●) were 2.8, 1.4, 0.7, and 0.4+5.9, 2.9, 1.5, and 0.7 mg/kg, respectively. Doses of the tramadol+meloxicam combination in 3:1 ratio (■) were 4.2, 2.1, 1.1 and 0.5+2.9, 1.5, 0.7, and 0.4 mg/kg, respectively. Doses of the tramadol+meloxicam combination in 1:3 ratio (▲) were 1.4, 0.7, 0.4, and 0.2+8.8, 4.4, 2.2 and 1.1 mg/kg, respectively.

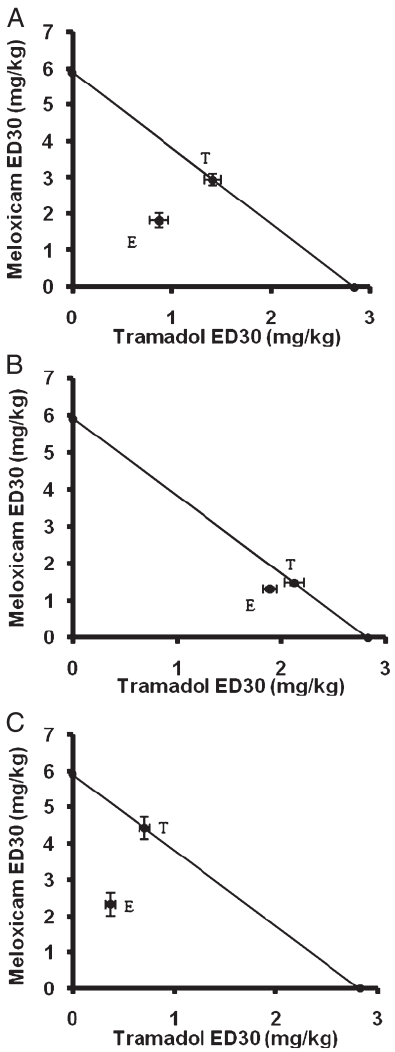

Fig. 3. Isobolograms showing the interaction between tramadol and meloxicam (1:1 [**A**], 3:1 [**B**], and 1:3 [**C**] ratio) in the mice formalin test. Horizontal and vertical bars indicate SEM. The oblique line between the *x* and *y* axes are the theoretical additive line. The point in the middle of this line, indicated by T, is the theoretical additive point calculated from the individual drug ED_{30} values. The point indicated by E is the actually observed ED_{30} value with the combination. In all cases, the experimental ED_{30} value point is situated below the additive line, being significantly different for the theoretical ED_{30} value, indicating a significant synergism ($P<0.05$).

TABLE 1. Theoretical (Z_{add}) and Experimental (Z_{exp}) ED_{30} Values ± SEM for the Tramadol (T)/Meloxicam (M) Combination in Different Proportions.

	T+M, 1:1 ratio ED_{30} values	T+M, 3:1 ratio ED_{30} values	T+M, 1:3 ratio ED_{30} values
Z_{add} (mg/kg)	4.36 ± 0.25	3.6 ± 0.16	5.13 ± 0.36
Z_{exp} (mg/kg)	2.69 ± 0.27*	3.2 ± 0.02	2.85 ± 0.05*
Interaction index	0.61	0.89	0.55

*$P < 0.05$ vs Z_{add}, by the Student's *t*-test.

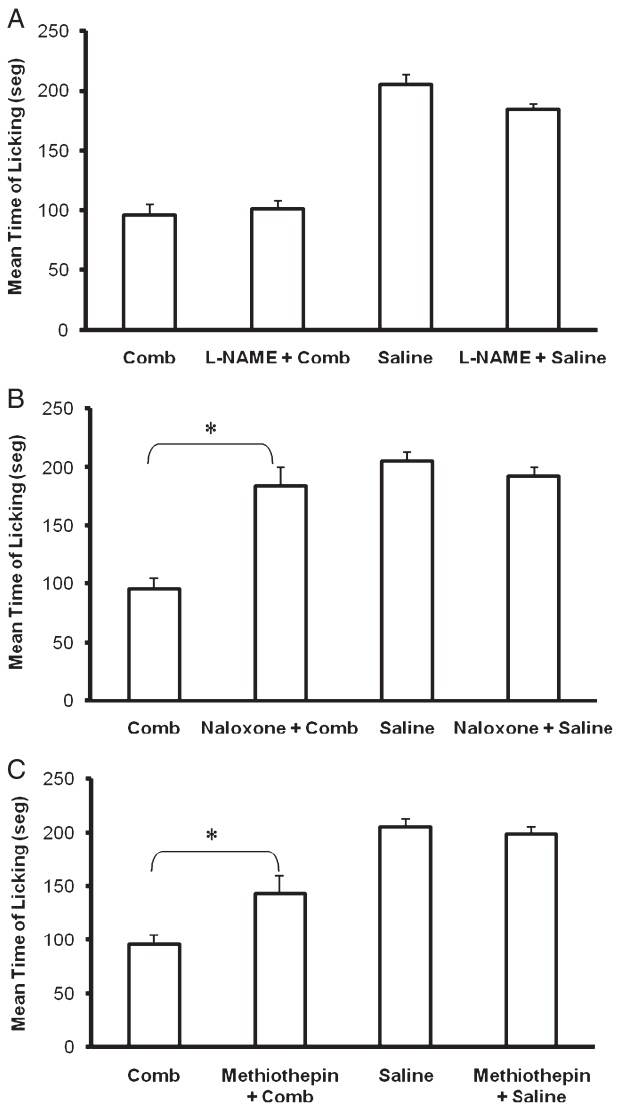

Fig. 4. Effect of L-NAME (**A**), naloxone (**B**), and methiothepin (**C**) on the antinociceptive effect of the tramadol–meloxicam combination (1:1 ratio). Bars are the mean ± SEM for at least 6 animals. *Significantly different ($P < 0.05$) from the combination (Comb), by one-way ANOVA followed the Student–Newman–Keuls test.

DISCUSSION

The current study demonstrates that tramadol produces dose-dependent antinociception in the formalin test. The antinociceptive effect of tramadol has been shown in several pain animal models, including the formalin test. Therefore, our results are in agreement with previous observations indicating that tramadol produces antinociception after systemic administration [Chen et al., 2002; Granados-Soto and Argüelles, 2005; Pozos-Guillen et al., 2006]. Systemic administration of meloxicam produced a dose-related antinociceptive effect during the second phase of the assay. Our results agree with previous studies showing that systemic meloxicam is able to reduce nociception in several pain animal models [Engelhardt et al., 1995; Laird et al., 1997; Santos et al., 1998; Pinardi et al., 2003; Dudhgaonkar et al., 2008]. The findings of the study also confirm that opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) show different profiles of antinociceptive activity, as tramadol exhibited greater antinociceptive potency and efficacy (Fig. 2A).

The present study focused on the nature of the interaction between tramadol and meloxicam in different proportions. Previous studies have shown that tramadol is able to increase the effect of adrenergic and serotonergic drugs [Pinardi et al., 1998], ketamine [Chen et al., 2002], metamizol [Poveda et al., 2003], naproxen [Satyanarayana et al., 2004], gabapentin [Granados-Soto and Argüelles, 2005], rofecoxib [García-Hernández et al., 2007], and deskétoprofen [Miranda and Pinardi, 2009]. However, to our knowledge, this is the first report regarding a synergistic interaction between tramadol and meloxicam. Our results confirm several observations showing that co-administration of opioids and NSAIDs leads to a synergistic interaction in inflammatory [Fletcher et al., 1997; Jiménez-Andrade et al., 2003; Poveda et al., 2003] and acute [Chen et al., 2002; Miranda et al., 2007] pain models in mice and rats as well as to an opioid-sparing effect in humans [Silvanto et al., 2002].

Particularly, in the present study, isobolographic analyses demonstrated a significant synergistic interaction between tramadol and meloxicam for the proportions 1:1, 1:3, but not for 3:1, which resulted in an additive effect. It is interesting to note that, according to the proportion of the drug in the combination, a synergistic interaction can became additive. This fact suggests that the proportion of drugs is an important

feature for the synergistic effect of the combination as previously shown [Berenbaum, 1989; Chou, 2006; Miranda and Pinardi, 2009] and strongly supports the need to assess different drug ratios when evaluating drug interactions.

The synergism observed between tramadol and meloxicam supports the general premise of interactions between analgesic drugs that act through different mechanisms of action [Berenbaum, 1989; Chou, 2006]. Tramadol is a weak opioid that also inhibits norepinephrine and serotonin reuptake [Driessen et al., 1993; Bamigbade et al., 1997; Oliva et al., 2002], whereas meloxicam is a cyclooxygenase-2 preferring inhibitor [Laird et al., 1997; Pairet et al., 1998]. Moreover, there is evidence that other mechanisms of action participate in the antinociceptive effects of these drugs. For instance, μ-opioid receptor agonists inhibit activation of adenylyl cyclase [Ingram and Williams, 1996] and release of substance P and calcitonin gene-related peptide from primary afferent neurons [Yaksh, 1988]; they open K^+ channels leading to hyperpolarization, reduction in firing of the primary afferent neuron, and antinociception [Yoshimura and North, 1983; Rodrigues and Duarte, 2000]. Meloxicam activates the nitric oxide–cyclic GMP pathway [Aguirre-Bañuelos and Granados-Soto, 2000], Ca^{2+}-activated K^+ channels [Ortiz et al., 2005], and the cholinergic inhibitory descendent system [Miranda et al., 2003] in the formalin test. The fact that naloxone (an opioid antagonist) and methiothepin (a 5-$HT_{1/2/6/7}$ receptor antagonist [Hoyer et al., 1994]) reduce the antinociceptive effect of the combination strongly suggests that at least some of these mechanisms participate in the observed synergy with tramadol and meloxicam.

Nitric oxide, opioid, and serotonergic mechanisms were analyzed by testing the effects of L-NAME, naloxone, and methiothepin on tramadol/meloxicam-induced antinociception. The local antinociceptive effect of the combination was unaffected by the nitric oxide synthesis inhibitor L-NAME [Gibson et al., 1990], thus precluding the involvement of the nitric oxide pathway in the effect of the combination. The lack of effect could not be attributed to the dose of L-NAME used, as this dose has been shown to reduce tolerance to morphine-induced antinociception [Homayoun et al., 2003]. This result seems surprising, as the peripheral antinociceptive of meloxicam is diminished by L-NAME in rats submitted to the formalin test [Aguirre-Bañuelos and Granados-Soto, 2000]. This difference could be attributable to the administration route. In contrast, systemic naloxone diminished the antinociceptive activity of the tramadol–meloxicam combination. Our data are in line with several observations indicating that tramadol, but not meloxicam, activates μ opioid as well as α_2 adrenoceptors [Raffa et al., 1992; Kayser et al., 1992; Ide et al., 2006]. In addition, systemic administration of methiothepin significantly reduced combination-induced antinociception. These data suggest that tramadol [Bamigbade et al., 1997; Oliva et al., 2002] and meloxicam, as is the case for other NSAIDs [Björkman, 1995; Pini et al., 1995, 1996], may interact with the spinal serotonergic system by inhibiting the reuptake or increasing release of spinal 5-HT. 5-HT could target specific 5-HT receptors in the spinal cord. Since methiothepin is a high-affinity 5-$HT_{1/2/6/7}$ receptor antagonist [Hoyer et al., 1994], the present data suggest that these receptors could be involved in combination-induced antinociception in the formalin test. More specifically, the candidate spinal receptor could be either 5-$HT_{1/2}$ receptors, linked to spinal antinociception [Oyama et al., 1996; Sasaki et al., 2001], but not 5-$HT_{6/7}$ receptors, as their spinal activation is associated with pronociception [Rocha-González et al., 2005; Castañeda-Corral et al., 2009]. However, on the basis of this experiment, the possible participation of other types of spinal 5-HT receptors cannot be ruled out. Together, these data suggest that the tramadol–meloxicam combination activates opioid and serotonergic receptors to produce antinociception in the formalin test.

In conclusion, the present study demonstrated that tramadol and meloxicam produce antinociception in the formalin test after i.m. administration. Moreover, the data indicate the presence of a functional synergistic interaction between tramadol and meloxicam that involves the opioid and serotonergic system.

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