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RESEARCH ARTICLE

Participation of ATP-sensitive K+ channels and μ-opioid receptors in the antinociceptive synergism of the paracetamol–tapentadol co-administration in the formalin-induced pain assay in mice

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Abstract

The purpose of this study was to assess the interaction and mechanisms of action of the paracetamol-tapentadol combination in the formalin-induced pain model in mice. Paracetamol (56.23–562.3 mg/kg, i.p.) or tapentadol (1–10 mg/kg, i.p.) were administered 15 min prior the intraplantar injection of formalin. The ED_{50} value of each drug was determined through the dose–response curves. The ED_{50} values were used to calculate the combinations in three fixed proportions (1:1, 1:3, and 3:1). Naloxone (1 and 5 mg/kg, i.p.), L-NAME (3 mg/kg, i.p.), or glibenclamide (10 mg/kg, i.p.) were administered before the combination of drugs to evaluate the antinociceptive mechanisms of action. The results showed that the combination 1:1 and paracetamol3-tapenadol1 ratios produced additive effects, whereas the paracetamol1-tapentadol3 proportion showed an antinociceptive synergistic interaction. Moreover, naloxone and glibenclamide reversed the antinociceptive activity of the paracetamol-tapentadol mixture. Our results indicate that the paracetamol-tapentadol combination produces an antinociceptive synergistic interaction with the possible participation of ATP-sensitive K+ channels and μ-opioid receptors in the second phase of the formalin-induced pain model in mice.

KEYWORDS

antinociceptive synergism, ATP-sensitive K^+ channels, formalin test, paracetamol, tapentadol

1 | INTRODUCTION

Many patients hospitalized after surgery have some degree of pain despite receiving pharmacological treatment which delays and extends their recovery. On the other hand, poor pain management (overdose) can cause a large number of complications and adverse effects. Therefore, the continuous search for new drugs and techniques to treat pain is a priority (Argoff, 2014).

Current evidence suggests that the co-administration of different types of analgesics is the best way to treat acute pain because they act through different antinociceptive mechanisms of action, being particularly interesting the use of opioid analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs). The combination of analgesics has some advantages such as: the increase of the effectiveness and the decrease of adverse effects. Several organizations around the world recommend the use of multiple drugs therapy for patients with special characteristics (elderly patients) and those who need pharmacological treatment for extended periods. The above is indicated to treat pain and other diseases (Raffa, 2001).

Recently, our research group has demonstrated that the co-administration of tapentadol and NSAIDs—such as ketorolac and diclofenac—produces a potentiation of the antinociceptive activity in comparison to the individual effect of each drug in animal pain models (Barreras-Espinoza et al., 2017; Zapata-Morales et al., 2016; Zapata-Morales, Alonso-Castro, Granados-Soto, Sánchez-Enriquez, & Isiordia-Espinoza, 2018). For instance, the ketorolac–tapentadol combination produced antinociception by the participation of opioid receptors and ATP-sensitive K^+ channels in the formalin-induced trigeminal pain model in mice (Barreras-Espinoza et al., 2017). Moreover, the diclofenac–tapentadol combination showed a synergistic antinociceptive action in the acetic acid-induced visceral pain model with a gastric injury similar to tapentadol alone but with lower gastric injury compared to diclofenac alone (Zapata-Morales et al., 2018). Following this research line, the objective of this study was to assess the antinociceptive activity and the mechanism of action of the paracetamol–tapentadol combination in the second phase of the formalin test in mice.

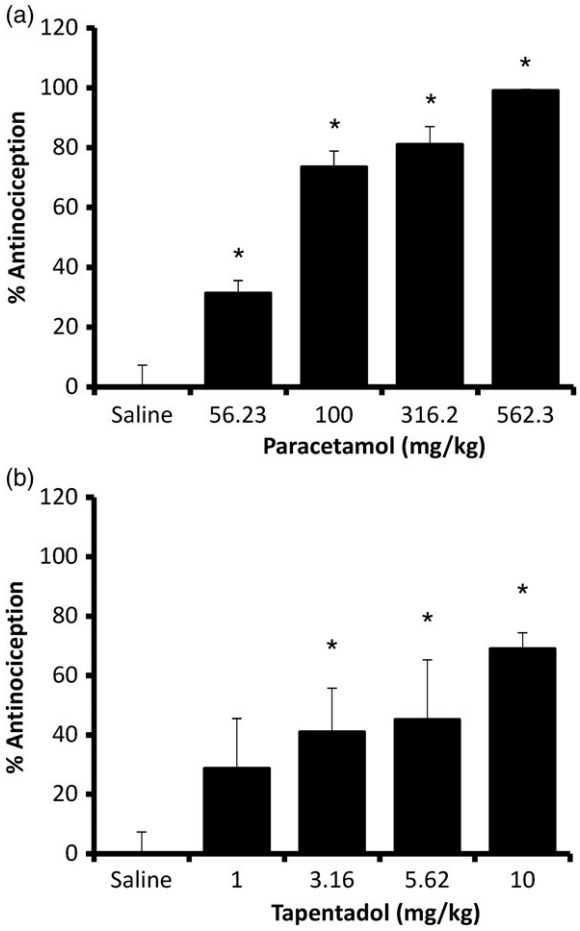

FIGURE 1 Antinociceptive activity of paracetamol (a) and tapentadol (b) in the second phase of the formalin assay. Data are means ± SEM at least for six animals. *Statistical difference by one-way ANOVA and Student–Newman–Keuls test

2 | MATERIALS AND METHODS

2.1 | Animals

CD-1 male mice weighing 25–30 g from the Universidad Autónoma Metropolitana-Xochimilco animal facility were housed in isolated cages at 24 °C under a light–dark cycle of 12:12. The animals were supplied with food (LabDiet, St. Louis, MO) and water ad libitum. The experiments were carried out according to the National Institute of Health Guide for Treatment and Care for Laboratory Animals and following the International Pain Guidelines in animals (Zimmerman, 1983) and the Official Mexican Norm NOM 062-ZOO-1999 (Technical specifications for the production, care and use of laboratory animals). All procedures were approved by the Research Bioethics Committee of Universidad Autónoma Metropolitana-Xochimilco.

2.2 | Drugs

Paracetamol (P0300000), naloxone (N7758), glibenclamide (G0639), and L-NG-nitroarginine methyl-ester (L-NAME; N5751) were purchased from Sigma Aldrich (St. Louis, MO). Tapentadol tablets (Palexia 50 mg, Grünenthal, Mexico) were bought in a commercial pharmacy from San Luis Potosí, México. All drugs (salts) were prepared in saline for their administration to rodents.

2.3 | Formalin test

The formalin assay was carried out with minimal modifications. Each animal was housed separately for 1 hr in observation chambers for 3 days as well as 1-hr prior the experiment. Each mouse was injected into the plantar surface of the right hind paw with 30 μl of 3% formalin using a 30-gauge needle. Thereafter, each animal was returned to the chamber and the nociceptive behavior was estimated as the licking time on the injected paw using mirrors at 45° to allow better vision and observation of the paw. The results were recorded every 5 min during 45 min (Abbott, Franklin, & Westbrook, 1995; Hunskaar, Fasmer, & Hole, 1985; Tjølsen, Berge, Hunskaar, Rosland, & Hole, 1992). A CO_2 chamber was used for the euthanasia of the mice.

2.4 | Experimental design

Paracetamol (56.23, 100, 316.2, or 562.3 mg/kg, i.p.) or tapentadol (1, 3.16, 5.62, or 10 mg/kg, i.p.) was administered 15 min prior the intraplantar formalin injection. A control group administered with sterile saline (i.p.) was used. The antinociceptive action (%) was evaluated with the following equation: [(vehicle-post compound)/vehicle] x 100. The 50% of the effect (ED_{50} value) of each drug was determined through the dose–response curves. Then, the ED_{50} values were employed to calculate the combination in three fixed proportions (1:1,

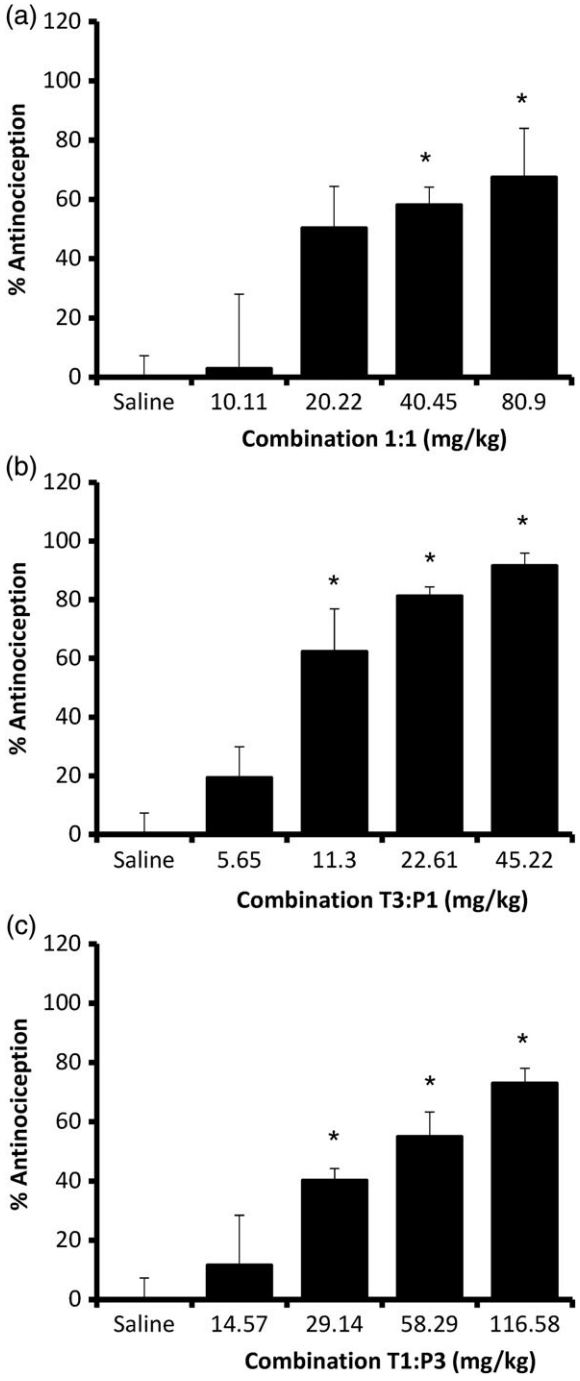

FIGURE 2 Antinociceptive effect of the paracetamol-tapentadol mixture (1:1 [a], 1:3 [b], and 3:1 [c] proportions) in the second phase of the formalin pain model. Data are means ± SEM at least for six animals. *Statistical difference by one-way ANOVA followed the Student–Newman–Keuls test

1:3, and 3:1). Each ratio of the combination was evaluated using four mice groups.

2.5 | Mechanisms of action

Naloxone (1 and 5 mg/kg), L-NAME (3 mg/kg), or glibenclamide (10 mg/kg) were administered i.p. 15 min before paracetamol, tapentadol, or their combination. The formalin was injected to each mouse 30 min after the drugs mixture.

2.6 | Statistical analysis

One-way analysis of variance and the Student–Newman–Keuls test were utilized to compare the antinociceptive activity between groups. Isobolograms and interaction index were employed to determinate

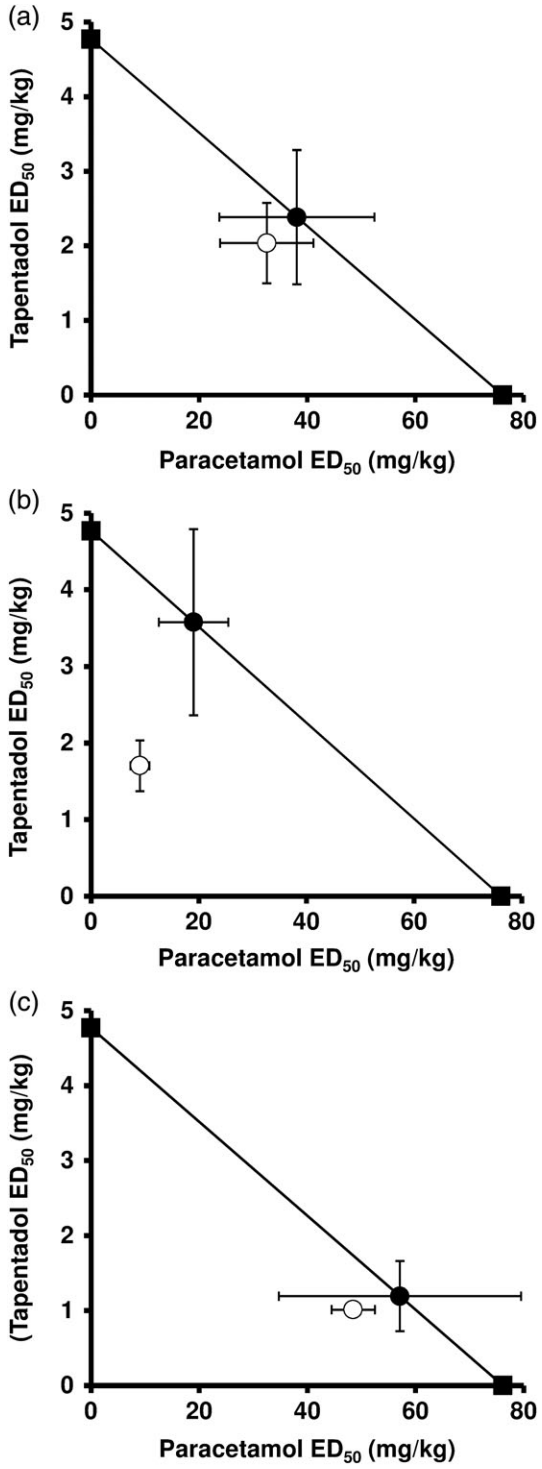

FIGURE 3 Graphs showing the antinociceptive interaction of paracetamol and tapentadol in the second phase of the formalin-induced pain in male CD-1 mice (1:1 [a], 1:3 [b], and 3:1 [c] ratios). The points on the lines represent the theoretical additive activity of each proportion (●). The experimental ED_{50} values are observed like open circles (○)

TABLE 1 Theoretical and experimental ED_{50} values and interaction index of the paracetamol–tapentadol mixture

	Combinations		
	1:1	P1:T3	P3:T1
Theoretical ED_{50} values (mg/kg)	40.45 ± 15.25	22.61 ± 7.68	58.29 ± 22.86
Experimental ED_{50} values (mg/kg)	34.55 ± 0.12	10.76 ± 0.08*	49.48 ± 0.04
Interaction index	0.85	0.47	0.84

Note. Values are means ± SEM. *$p < .05$ Theoretical versus Experimental ED_{50} values by the Student's *t*-test. P = paracetamol; T = Tapentadol.

the interaction among paracetamol and tapentadol (Tallarida, 2000, 2002). A $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Antinociception

Each individual drug diminished the nociception induced with formalin in a dose-dependent manner. Paracetamol 562.3 mg/kg and tapentadol 10 mg/kg produced the maximum antinociceptive effect—99 and 69%, respectively—of each individual drug (Figure 1). The antinociceptive action of the different proportions of the drugs mixture produced a dose-dependent response observing the maximum effect (91%) with the paracetamol1-tapentadol3 proportion (Figure 2a,c).

3.2 | Isobolograms and interaction index

According to the graphic representation of the results, the 1:1 and the paracetamol3:tapentadol1 ratios induced an interaction of additive type. In both cases, the experimental ED_{50} value point is placed inside the error bars of the additive lines (Figure 3a,c, respectively) which was confirmed by the interaction index (Table 1). Moreover, the experimental ED_{50} value point of the paracetamol1-tapentadol3 proportion is located bellow of the additive line indicating an antinociceptive synergistic effect according to the isobologram (Figure 3b) and supported by the interaction index (Table 1).

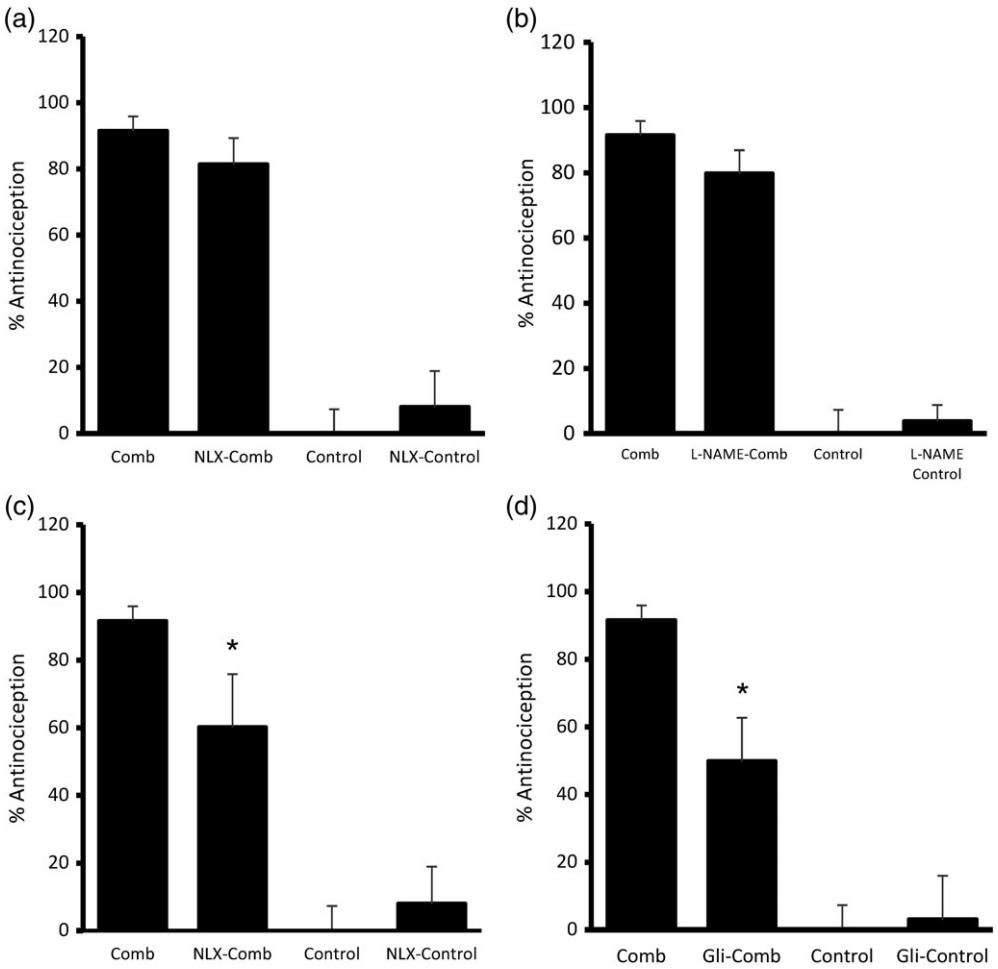

FIGURE 4 Activity of naloxone (a), L-NAME (b), and glibenclamide (c) on the antinociceptive effect of the paracetamol-tapentadol combination in the second phase of the mouse model of the formalin. NLX: Naloxone. *Statistical difference using one-way ANOVA and the Student–Newman–Keuls test

3.3 | Mechanisms of action

Naloxone (5 mg/kg) and glibenclamiden (10 mg/kg) partially reversed the antinociceptive activity of the paracetamol1-tapentadol3 combination (Figure 4b,d, respectively). However, Naloxone (1 mg/kg) and L-NAME (3 mg/kg) did not antagonize the antinociceptive action of the drugs mixture (Figure 4a,c, respectively). Moreover, naloxone (5 mg/kg) reversed the antinociceptive activity of tapentadol, but did not antagonize the analgesic effect of paracetamol (Figure 5a,b).

4 | DISCUSSION

This is the first study reporting the effect of the paracetamol-tapentadol combination in the formalin test in mice. The 1:1 and paracetamol3-tapentadol1 ratios produced additive action in the second phase of the formalin-induced pain assay. Moreover, the paracetamol1-tapentadol3 ratio induced an antinociceptive synergistic interaction in the second phase of the formalin test. All these findings were confirmed using both isobolographic analysis and interaction index. Similar results have been observed when other NSAIDs and tapentadol were used in somatic and visceral animal pain models (Barreras-Espinoza et al., 2017; Zapata-Morales et al., 2016, 2018) as well as when paracetamol has been used in combination with other opioid analgesics in the formalin and acetic acid assays in rodents (Fernández-Dueñas, Poveda, Sánchez, & Ciruela, 2012; Janovsky and Krsiak, 2011; Miranda, Puig, Dursteler, Prieto, & Pinardi, 2007; Miranda, Noriega, & Prieto, 2012).

This study explored three ratios of the paracetamol–tapentadol combination to determine which of these fixed proportions could be the better option to treat acute pain in mice. It has been shown that the effect of a two-analgesic combination depends on the proportions in which this combination is tested, obtaining synergistic effects, additives as well as antagonisms. This kind of studies using several proportions of an analgesic combination are essential, particularly when human clinical trials will be carried out, the information of animal models could be the only guide to define the experimental doses (Barreras-Espinoza et al., 2017; Maves, Pechman, Meller, & Gebhart, 1994; Zapata-Morales et al., 2016, 2018).

According to the pharmacokinetic findings of tapentadol, the drug–drug interactions are unlikely (Hartrick & Rozek, 2011; Kneip, Terlinden, Beier, & Chen, 2008; Smit et al., 2010; Terlinden, Kogel, Englberger, & Tzschentke, 2010). A study evaluated the pharmacokinetic parameters of tapentadol in humans and indicated that there were not relevant changes in the plasma levels of this drug given alone or co-administered with paracetamol (Smit et al., 2010). Furthermore, the difference in the pharmacokinetic parameters of the NSAIDs could not be attributed to the antinociceptive synergy with tapentadol due to their highly lipophilic properties (Dembo, Park, & Kharasch, 2005; Kuehl, Lampe, Potter, & Bigler, 2005; Mehanna, 2003). For the above, we believe that the interaction between paracetamol and tapentadol was of pharmacodynamic kind.

The antinociceptive synergistic interaction of the paracetamol–tapentadol combination surely involved the block several pain

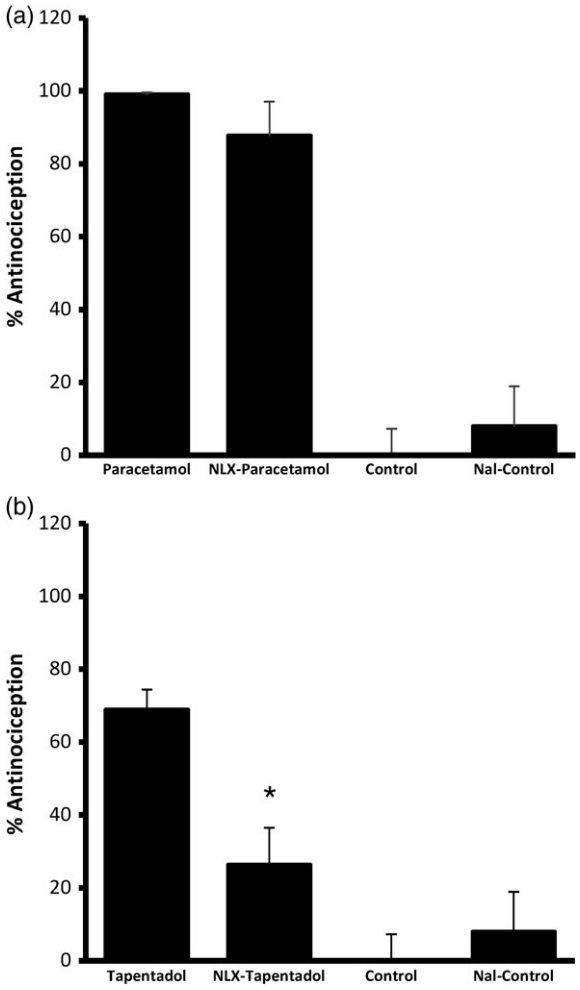

FIGURE 5 Effect of naloxone on the antinociception of paracetamol (a) and tapentadol (b) in the second phase of the formalin-induced pain assay. NLX: Naloxone. *Statistical difference by one-way ANOVA and the Student–Newman–Keuls test

pathways, which supports the overall principle of drugs interaction with different antinociceptive mechanisms (Berenbaum, 1989; Chou, 2006). The binding to peripheral, spinal, and supraspinal μ-opioid receptors as well as the inhibition of noradrenaline reuptake are the antinociceptive mechanisms of action of tapentadol (Barreras-Espinoza et al., 2017; Schröder, Vry, Tzschentke, Jahnel, & Christoph, 2010). Furthermore, paracetamol produced its analgesic effect through different mechanisms of action such as the inhibition of the cyclooxygenase enzyme as well as the involvement of the nitric oxide synthase (Bujalska, 2003), the indirect modulation of the opioidergic system (Bujalska, 2004a, 2004b; Pini, Vitale, Ottani, & Sandrini, 1997), and its binding to spinal adenosine A_1 and CB_1 cannabinoid receptors (Sawynok & Reid, 2012). In this study, naloxone and glibenclamide reverted the antinociceptive activity of the paracetamol1-tapentadol3 combination which suggest the possible involvement μ-opioid receptors and the activation of ATP-sensitive K^+ channels. Previously, our research group reported that the tapentadol-ketorolac combination produces also the activation of both antinociceptive pathways (Barreras-Espinoza et al., 2017).

In conclusion, this study indicates that the paracetamol1-tapentadol3 combination induces a synergistic antinociceptive effect

in the second phase of the formalin-induced pain test with the possible participation of ATP-sensitive K+ channels and μ-opioid receptors. Moreover, the 1:1 and paracetamol3-tapentadol1 proportions induce an additive antinociceptive action in the second phase of the formalin model in mice.

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