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Research Article

Serotoninergic and Noradrenergic Systems, But Not Dopaminergic, Mediate Tramadol-Induced Peripheral Antinociception in Rats

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Abstract

We have recently demonstrated that tramadol acts peripherally to induce antinociceptive action through nitric oxide and potassium channels but not opioid and cannabinoid receptors in rats. However, since its actions in the periphery have not been elucidated fully, the noradrenergic, serotonergic, and dopaminergic systems were evaluated in the peripheral antinociception of tramadol against prostaglandin-induced hyperalgesia E_2. The method of removing rat paws subjected to compression was used to measure the nociceptive threshold. The non-selective α_2-adrenergic yohimbine receptor antagonist (20, 40 µg) partially reversed tramadol analgesia, while the selective α_{2C}-adrenergic receptor antagonist rauwolscine (10, 15, 20 µg) totally antagonized the analgesia. On the contrary, antagonists of the other α_2-adrenergic receptor subtypes, α_{2A}, α_{2B} and α_{2D}, BRL44480, imiloxane and RX821002 (20 µg), respectively, did not alter antinociception. As for the serotonergic system, the 5-HT1B, 5-HT1D, and 5-HT3 receptor antagonists, isamoltane, BRL 15572, and ondansetron (100 ng, 1 and 10 µg), respectively, managed to block tramadol antinociception, but the antagonists of tramadol the other evaluated receptors, 5-HT2A and 5-HT7, ketanserin and SB269970 (10 µg), respectively, were not able to exert the same effect. D2, D3, and D4 receptor antagonists, remoxipride (4.20 µg), U99194 (32 µg), and L-745.870 (32 µg), respectively, did not change the tramadol-induced antinociception, suggesting that dopaminergic signaling may not be implicated in this antinociception. Our data suggest the peripheral antinociceptive action induced by tramadol when administered in a model of PGE_2-induced hyperalgesia and that this effect should involve the α_{2C}-adrenergic receptors, the $5\text{-}HT_{1B}$, $5\text{-}HT_{1D}$ and $5\text{-}HT_3$ serotonergic receptors. was unrelated to the activation of dopaminergic receptors

Keywords: *Tramadol; Norepinephrine; Serotonin; Antinociception; Monoamines*

Abbreviations

PGE2: Prostaglandin E2; NA: Noradrenaline; IPl: Intraplantar

Introduction

Tramadol ((2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol) is a centrally acting "weak opioid" drug that was developed in Germany and marketed globally in 1977 [1-3]. It is effective in the treatment of moderate to severe pain, such as postoperative pain, chronic pain, renal colic, acute trauma, and neuropathic pain, and it is even part of some multimodal anesthesia protocols [1,4]. The

mechanism of action of tramadol in antinociception is due to the central modulation of pain [5], not only by the opioid pathway, having a lower affinity for receptors compared to codeine and morphine [6], but also by its action on the serotonergic and noradrenergic systems [7,8]. Research reveals that tramadol can increase the influx of serotonin and norepinephrine into the central nervous system, although the specific mechanisms still need further investigation [9-11].

In addition to well-recognized systemic actions of tramadol, several preclinical studies report antinociception following spinal [12,13] and local peripheral administration [14-16]. In basic research, several local mechanisms for Tramadol have been reported, such as its weak peripheral agonism over μ opioid receptor [17], activation of adrenergic receptors [18], lowering of the activation of vanilloid receptor 1 (TRPV-1), blocking N-methyl-D-aspartate (NMDA) receptors [20], favoring the opening of nonspecific voltage-dependent potassium channels and acting in the nitric oxide pathway [21] or by direct blocking of sodium channels [22], and such actions also may contribute to antinociception, particularly when tramadol is given locally, and higher local tissue concentrations occur.

Experimentally, this drug has been successful in controlling peripheral pain in animal nociception models [23]. It is also effective in humans as an anesthetic adjuvant for brachial plexus block [24] or compared with several local anesthetics when applied subcutaneously [25].

The use of tramadol at the local level has been studied weakly, with few studies in the literature on the subject [26]. Subcutaneous or intradermal administration of opioids in humans is generally not recommended, so it does not seem to be the target of many studies. The pathways by which tramadol exerts local antinociception are not yet well elucidated, so the present work was dedicated to studying the possible monoaminergic pathways that tramadol could exert local antinociception in an animal model, using a model of mechanical hyperalgesia in rats.

Materials and Methods

Animals

Male Wistar rats weighing 170 ± 30 g were used at the Bioterism Center of the Federal University of Minas Gerais (CEBIO-ICB/UFMG). The animals were kept at a controlled temperature (23 ± 2°C), in a light/dark cycle of 12h (6:00-18:00h), and had free access to food and water. After the tests, the animals were euthanized by high-dose intraperitoneal injection of anesthetic (300 mg/kg ketamine hydrochloride and 15 mg/kg of xylazine hydrochloride, both Sigma-Aldrich, USA). The ethics committee on animal experimentation approved the project under protocol 138/2019. All experimental animal procedures followed the guide for the care and use of laboratory animals.

Drugs and solvents

The drugs were administered to the paw in a volume of 100 μL.

Tramadol

Tramadol (Teuto, Brazil) was dissolved and diluted in a saline solution (0.9% NaCl).

Hyperalgesic stimulus

Prostaglandin E_2 (PGE_2) (Sigma, USA) was dissolved in ethanol, and before injections, it was diluted in a saline solution (0.9% NaCl), obtaining a 2% ethanol concentration in saline.

Aminergic receptors antagonists

All antagonists were dissolved and diluted in saline solution (0.9% NaCl). Yohimbine, BRL15572, and SB269970 were purchased from Sigma (USA). The other noradrenergic pathway antagonists, dopaminergic antagonists, and isamoltan were purchased at Tocris (USA). Ketanserin is from Biotrend (Switzerland) and ondansetron is from Cristália (Brazil).

Noradrenergic

Yohimbine (non-selective α2 receptor antagonist), BRL44480 (selective α2A receptor antagonist), imiloxan (selective α2B receptor antagonist), rauwolscine (selective α2C receptor antagonist), and RX821002 (selective α2D receptor antagonist).

Serotoninergic

Selective antagonists of 5-HT receptors were isamoltan (5-HT1B), BRL15572 (5-HT1D), ketanserin (5-HT2A), ondansetron (5-HT3) and SB269970 (5-HT7).

Dopaminergic

The selective antagonists were remoxipride (D2), U99194 (D3), and L-754.870 (D4).

Experimental procedure

Paw pressure test

Sensitivity to mechanical nociceptive stimuli was measured using a modification of a classic paw pressure test [27]. Briefly, progressively increasing pressure was applied to the plantar surface of the rat's hind paws with an analgesimeter (Ugo Basile, Italy). The nociceptive threshold was defined as the force (g) that the rat attempted to withdraw its paw. Basal pain thresholds were set to 190 - 210g with a cut-off value of 300g to avoid any tissue damage. Considering hyperalgesia as a reduction of the nociceptive threshold, the other experiments had their results expressed as a function of the difference (Δ) of the basal threshold Changes in pain thresholds and that measured at the 3rd hour when there is a peak of PGE2 action.

Nociceptive threshold measurement, hyperalgesia, and antinociception

The nociceptive threshold is the pressure exerted by the device at which the animal removes its paw (nociceptive reflex). Before administering any drug, the baseline measurement of the nociceptive threshold is made, that is, the threshold at which no substance acts on the animal's tissue or the zero moment. This measurement was performed three times, with 10 s between each measurement, and the final value is the mean between them.

To assess the duration of the tramadol effect, nociceptive threshold values were monitored over time, from the third hour, when PGE2 peaks, to the sixth hour, when the hyperalgesic agent no longer acts.

Considering hyperalgesia as a reduction of the nociceptive threshold, the results of the other experiments were expressed as a function of the difference (Δ) between the baseline threshold and that measured in the third hour, when PGE2 reaches its peak. If a drug is administered before or during hyperalgesia and has an antinociceptive effect, there will be a reduction in hyperalgesia with the consequent reduction in Δ. On the contrary, if any injected drug has an action that interrupts the antinociception of tramadol, there will be a restoration of hyperalgesia and, likewise, an increase in Δ values close to those obtained when only the hyperalgesic agent is administered.

Experimental design

The protocols concerning the dose and the injection time of each drug used in this study were obtained through pilot experiments and literature data from our laboratory [28-30]. The differences in the drug injection times are according to the peak of action of each one to coincide with the same peak of action of all of them at the time of assessment of the nociceptive threshold (which is three hours after PGE_2 injection in the hind paw). Experimental protocols are described below, and the timeline of drug injections is indicated above each figure. Briefly, tramadol or its vehicles were injected 5 minutes before the third hour after PGE2 injection, the moment of maximal hyperalgesia caused by it. Yohimbine was injected 40 minutes prior to tramadol administration, and selective noradrenergic antagonists were injected

30 minutes prior to tramadol. The serotonergic and dopaminergic antagonists were injected 15 minutes before tramadol, except for remoxipride, administered 25 minutes before.

Statistical analysis

The GraphPad Prism 8.00 program was used for statistical analysis of the data. The results were expressed by means ± standard error (E.P.M.) for each experimental protocol. The data were submitted to one-way variance analysis (for temporal development analysis) or two-way followed by Bonferroni post-test for multiple comparations. Only p-values lower than 0.05 were considered statistically significant.

Results and Discussion

Participation of the noradrenergic system in the peripheral antinociception of tramadol

Effect of the non-selective α_2-noradrenergic receptor antagonist yohimbine on the peripheral antinociception of tramadol

When intraplantar administration of the non-selective noradrenergic antagonist yohimbine, there was partial reversal (20 µg/paw) of the antinociceptive effect of tramadol (80 µg/paw), and total reversal occurred when administering a higher dose (40 µg/paw) of the antagonist (Figure 1).

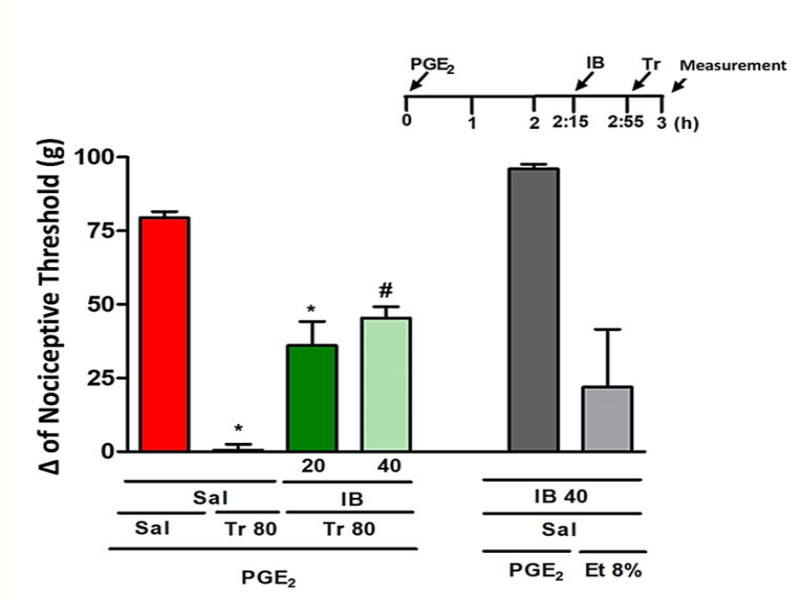

Figure 1: *Reversal of the peripheral antinociceptive effect of tramadol (Tr, 80 µg/paw) by intraplantar administration of yohimbine (IB, 20 or 40 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. n = 5. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). # indicates a statistical difference in relation to the antinociception control group (PGE2 + Sal + Tr). P<0.05, ANOVA + post-hoc Bonferroni.*

Effect of selective α_2-noradrenergic receptor antagonists on the peripheral antinociception of tramadol

To verify which α2 noradrenergic receptor subtypes participate in the peripheral antinociception of tramadol (80 µg/paw), selective α2A, α2B, α2C and α 2D noradrenergic receptor antagonists BRL 44480, imiloxone, rauwolscine, and 821002 RX, respectively, were ad-

ministered i.pl. Only rauwolscine (10, 15, 20 µg/paw) was able to reverse the peripheral antinociception of tramadol in a dose-dependent manner (Figure 2), but not the other antagonists, 20 µg/paw (Figure 3-5).

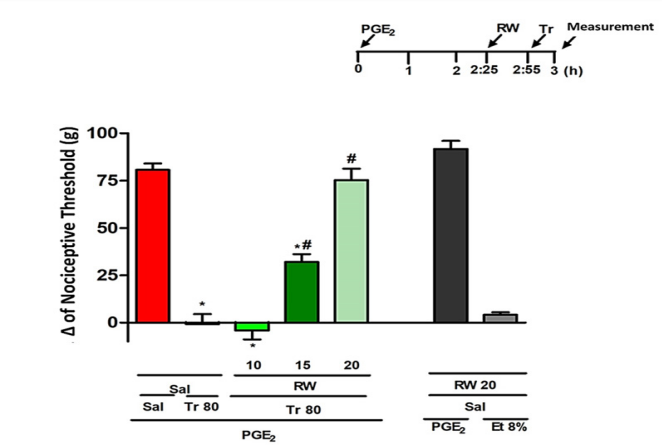

Figure 2: *Antagonism of the antinociceptive effect of tramadol (80 µg/paw) by the administration of rauwolscine (RW, 10, 15, 20 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. n=5. * iIndicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). # indicates a statistical difference in relation to the antinociception control group (PGE2 + Sal + Tr). P<0.05, ANOVA + post-hoc Bonferroni.*

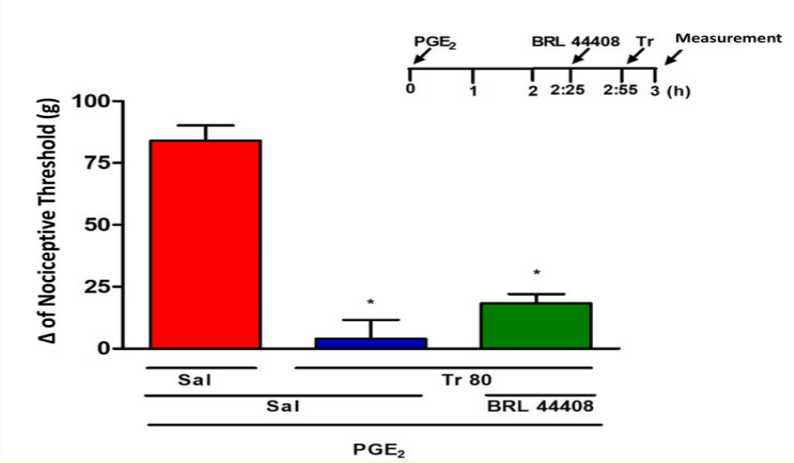

Figure 3: *Effect of BR 44408 injection (20 µg/paw) on the peripheral antinociception of tramadol (Tr, 80 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Salt). P<0.05, ANOVA + post-hoc Bonferroni.*

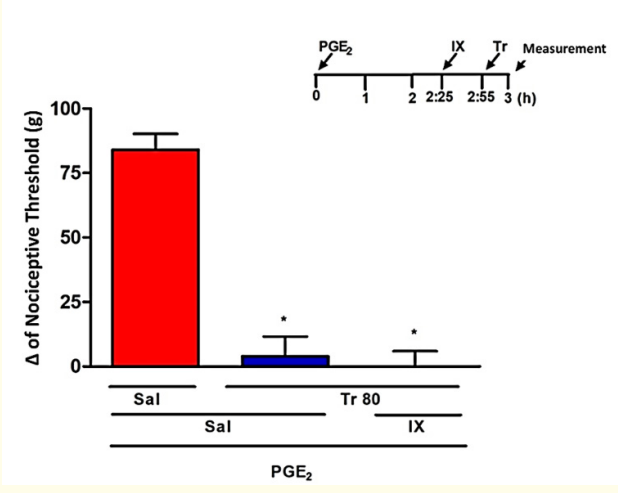

Figure 4: *Effect of the injection of Imiloxan (IX, 20 µg/paw) on the peripheral antinociception of tramadol (Tr, 80 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). P<0.05, ANOVA + post-hoc Bonferroni.*

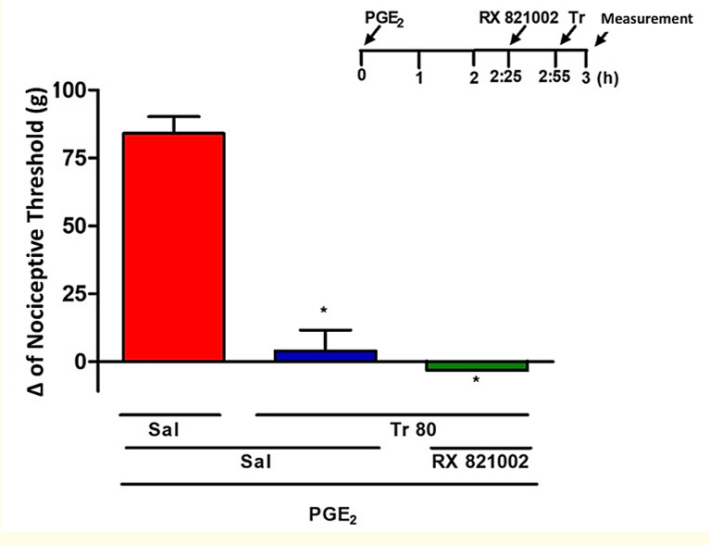

Figure 5: *Effect of RX 821002 injection (20 µg/paw) on the peripheral antinociception of tramadol (Tr, 80 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). P<0.05, ANOVA + post-hoc Bonferroni.*

Participation of the serotonergic system in the peripheral antinociception of tramadol

Effect of 5-HT1 receptor antagonists on tramadol-induced antinociception

To evaluate the participation of 5-HT1 receptors in the peripheral antinociception of tramadol, the selective antagonists isamoltane

and BRL 15572 (0.1, 1, 10 µg/paw), 5-HT1B and 5-HT1D receptors, respectively, were injected intraplantally. Both drugs produced a total reversal of the peripheral antinociception of tramadol (80 µg/paw) in a dose-dependent manner (Figure 6 and 7).

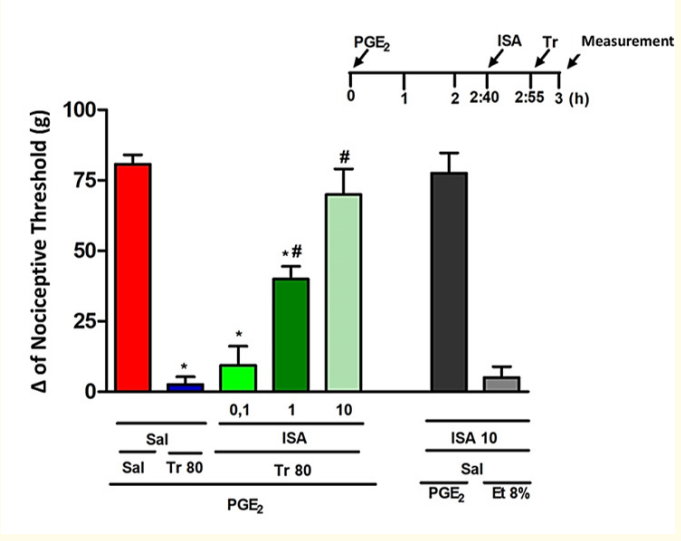

Figure 6: *Antagonism of the antinociceptive effect of tramadol (Tr, 80 µg/paw) by the administration of isamoltan (ISA, 0.1,1.10 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. n=5. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). # indicates a statistical difference in relation to the antinociception control group (PGE2 + Sal + Tr). P<0.05, ANOVA + post-hoc Bonferroni.*

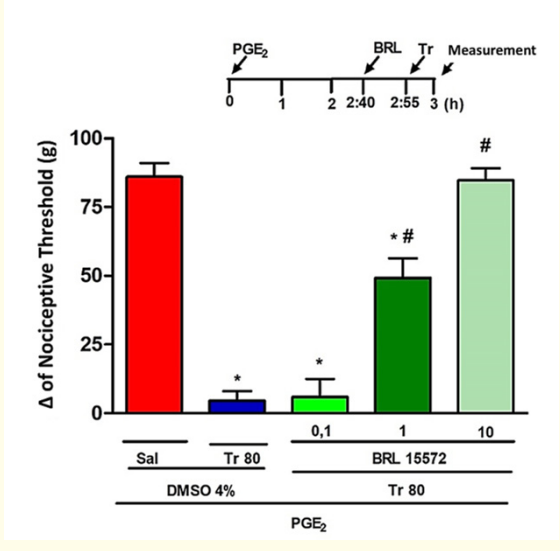

Figure 7: *Antagonism of the antinociceptive effect of tramadol (Tr, 80 µg/paw) by the administration of BRL 15572 (0.1, 1, 10 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. n=5. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + DMSO 4% + Sal). # indicates a statistical difference in relation to the antinociception control group (PGE2 + DMSO 4% + Tr). P<0.05, ANOVA + post-hoc Bonferroni.*

Effect of 5-HT2A receptor antagonist on tramadol-induced antinociception

Figure 8 shows that the administration i.pl. of ketanserin (10 µg/paw), a selective antagonist of 5-HT2A receptors, was not able to reverse the peripheral antinociception of tramadol (80 µg/paw).

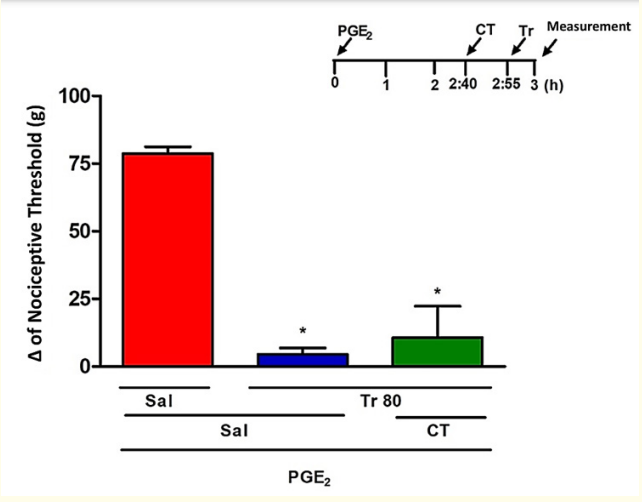

Figure 8: *Effect of ketanserin injection (CT, 10 µg/paw) on the peripheral antinociception of tramadol (Tr, 80 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). P<0.05, ANOVA + post-hoc Bonferroni.*

Effect of 5-HT3 receptor antagonist on tramadol-induced antinociception

Figure 9 shows that the administration i.pl. of ondansetron (0.1, 1, 10 µg/paw), a selective antagonist of 5-HT3 receptors, was able to reverse the peripheral antinociception of tramadol (80 µg/paw) in a dose-dependent manner.

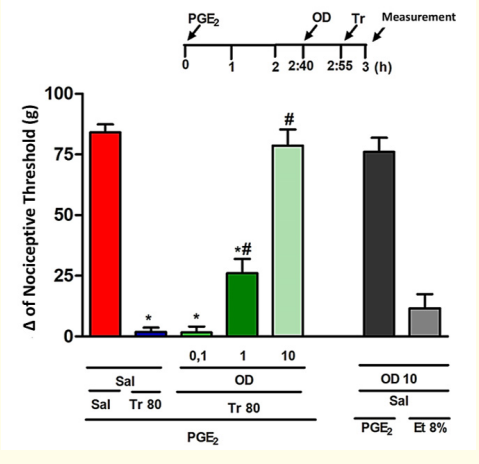

Figure 9: *Antagonism of the antinociceptive effect of tramadol (Tr, 80 µg/paw) by the administration of ondansetron (OD, 0.1, 1, 10 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. n=5. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + DMSO 4% + Sal). # indicates a statistical difference in relation to the antinociception control group (PGE2 + DMSO 4% + Tr). P<0.05, ANOVA + post-hoc Bonferroni.*

Effect of 5-HT7 receptor antagonist on tramadol-induced antinociception

Figure 10 shows that the administration i.pl. of SB269970 (10µg/paw), a selective antagonist of 5-HT7 receptors, could not reverse the peripheral antinociception of tramadol (80 µg/paw).

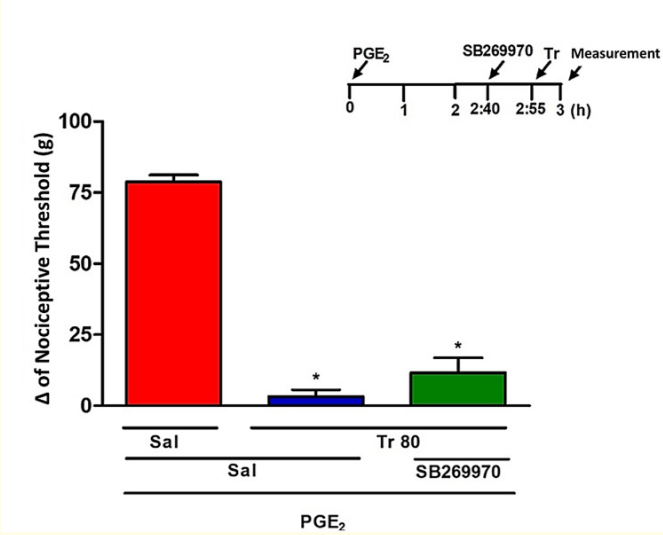

Figure 10: *Effect of SB269970 injection (10 µg/paw) on the peripheral antinociception of tramadol (Tr, 80 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). P<0.05, ANOVA + post-hoc Bonferroni.*

Participation of the dopaminergic system in the peripheral antinociception of tramadol

To study the participation of dopaminergic receptors in tramadol-induced peripheral antinociception, the selective D2, D3, and D4 receptor antagonists remoxipride (20 µg/paw) (Figure 11), U99194 (32 µg/paw) (Figure 12), and L-745.870 (32 µg/paw) (Figure 13) were injected i.pl., respectively. There was no reversal of the antinociceptive effect of tramadol (80 µg/paw) in any test performed with these antagonists.

Discussion

Studies on tramadol remain largely focused on its systemic mechanism of action. Little is known about the pathways by which its peripheral antinociception occurs. In veterinary medicine, tramadol is the opioid analgesic most prescribed for moderate to severe pain in Brazil and the United States [31-33]. Since the adverse effects, at least in some species, are little evidenced, being restricted to the reduction of intestinal motility and respiratory rate, emesis, and excitation, especially in cats, this drug seems to be a safe option for the treatment of several painful pathologies [33,34].

In animals, the local effect of tramadol in a plantar incision model was evaluated. Peripheral nociception in an incisional model and mechanical hyperalgesia was described by [35], in which naloxone could not reverse antinociception. This reinforces the theory that the peripheral antinociception of tramadol is not due exclusively to the opioid pathway. In addition, it has been shown that the opioid pathway

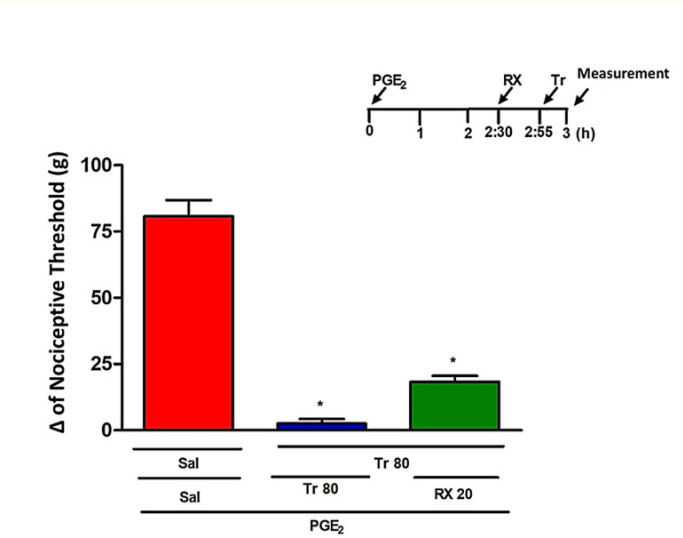

Figure 11: *Effect of remoxipride injection (RX, 20 µg/paw) on the peripheral antinociception of tramadol (Tr, 80 µg/paw). The drugs were administered at the times indicated in the diagram depicted in the upper right corner of the graph. Each bar represents the mean ± E.P.M. n = 5. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). P<0.05, ANOVA + post-hoc Bonferroni.*

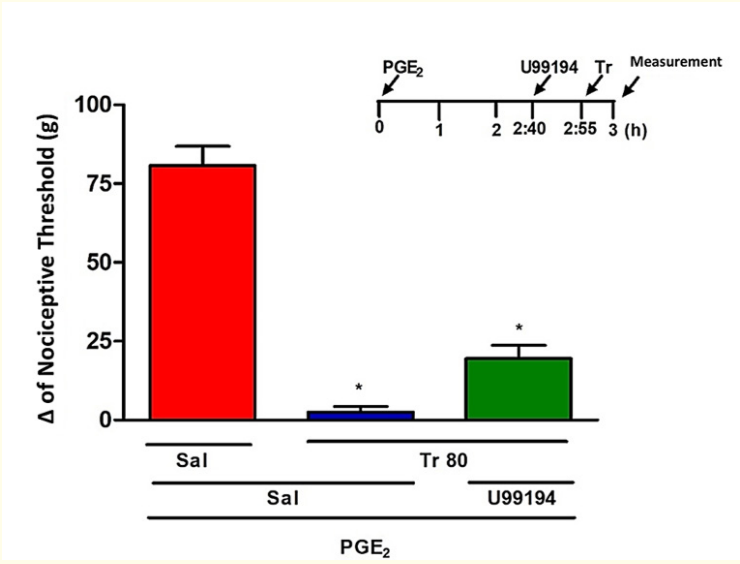

Figure 12*: Effect of U99194 injection (32 µg/paw) on the peripheral antinociception of tramadol (Tr, 80 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. n = 5. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). P<0.05, ANOVA + post-hoc Bonferroni.*

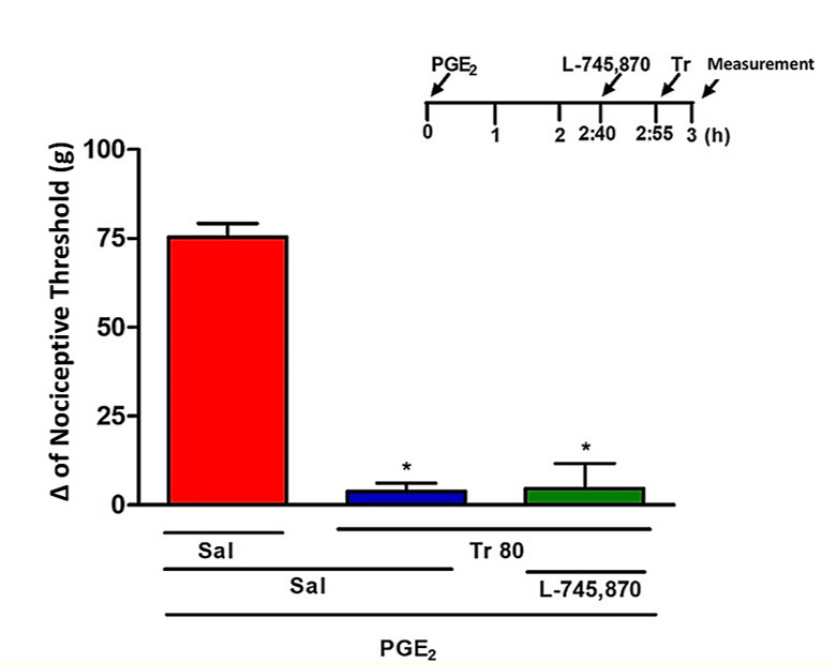

Figure 13: *Effect of L-745.870 injection (32 µg/paw) on the peripheral antinociception of tramadol (Tr, 80 µg/paw). The drugs were administered at the times indicated in the diagram described at the top right of the graph. Each bar represents the mean ± E.P.M. n = 5. * indicates a statistical difference in relation to the hyperalgesic control group (PGE2 + Sal + Sal). P<0.05, ANOVA + post-hoc Bonferroni.*

is not the primary peripheral antinociceptive mechanism of tramadol and that the nitrergic pathway and potassium channels participate as antinociceptive pathways of this drug [21]. Tramadol is a drug included in the class of "atypical opioids" as it has an opioid and non-opioid mechanism of action.

In the present study, yohimbine, a non-selective α2-adrenergic receptor antagonist, was administered as pretreatment, and as a result, we obtained a partial blockade of the peripheral antinociceptive activity of tramadol. However, in contrast to what was found in our experiments, [36] suggested that yohimbine cannot block the antinociception of tramadol in a thermal nociception model. The differences concerning this study may involve the dose of the drugs, route of administration, and/or nociceptive model used.

Tramadol is a racemic mixture of two enantiomers, (+)-tramadol and (-)-tramadol. The mixture is capable of increasing extracellular levels of monoamines, including NA. The effects of racemic mixing are not merely additive; they seem to act synergistically, with each fraction enhancing the other [37]. In addition, the levorotatory enantiomer, (-)-tramadol, would have greater selectivity by inhibiting NA reuptake [37], and these effects would be modulated by the metabolite M1, with greater affinity for µ-opioid receptors [38]. In addition, the levogyre fraction seems to increase NA efflux compared to other fractions, in addition to prolonging the uptake of this neurotransmitter in the LC [9,39]. Therefore, the antinociception of tramadol through the opioid-monoaminergic pathway seems to be stereoselective [40]. According to our results, peripheral antinociception blockade was total with yohimbine injection. Since there is no hepatic metabolism at the peripheral level, there is no formation of metabolites, and, therefore, there is no potentiation of the noradrenergic antinociceptive

effect by activating opioid receptors. *In vitro* studies suggest that the (-)-tramadol enantiomer is more selective for α2 receptors since the reduction of action potential triggers and hyperpolarization are inhibited by adding rauwolscine to the medium. It would also increase the concentration of NA around the adrenergic receptors. In addition, (+)-tramadol could not reduce the firing in the presence of this selective α2C antagonist [40].

In formalin testing and intraperitoneal administration of tramadol, NA levels are increased in the inflamed paw, probably by blocking the reuptake of this neurotransmitter in peripheral nerve endings [41]. At the peripheral and spinal level, in a model of inflammation caused by carrageenan, non-selective α2 antagonists can block the effect of potent opioids, such as morphine [42,43]. This would occur by activation of the descending pathway as an analgesic mechanism of opioids [44]. Tramadol, as an atypical opioid, would probably use this analgesic route as well. During the inflammatory process, NA levels are elevated in the spinal cord. Thus, the blockade of opioid action by α2 antagonists in tissues inflamed or sensitized in the periphery evidences the participation of spinal NA in analgesia [44]. In a rat model of arthritis followed by mechanical nociception by paw compression, intravenously injected tramadol was antagonized by yohimbine to a significant extent [45]. We used selective antagonists as pre-treatment to evaluate the possible participation of different α2 receptors. The selective α2C receptor antagonist, rauwolscine, could block the analgesic effect of tramadol in a dose-dependent manner. On the other hand, α2A, α2B, and α2D receptor antagonists did not block antinociception. Although they do not affect antinociception, studies demonstrate the presence of mRNA for these receptors in the dorsal root ganglion. The α2C receptor seems to have a higher concentration of mRNA, while α2A is practically not detected for this protein. α2B can also have mRNA expressed in the DRG. The studies did not evaluate the presence of α2D mRNA [46]. Knockout animals for the α2A receptor showed greater sensitivity to the antinociceptive effects of tramadol in a thermal nociception model. Thus, α2A antagonists would improve the antinociception of tramadol, and the loss of α2A function would imply opioid analgesia by indirect mechanisms [47]. However, peripherally, this effect was not observed in our study.

The stimulation of adrenoreceptors by tramadol, especially the levogyre enantiomer [40], would increase K^+ conductance, generating membrane hyperpolarization and inhibition of the nociceptive process [47,48]. Since, according to our results, ATP-dependent potassium channels play a role in tramadol analgesia and α2 receptors were blocked, this theory corroborates our results. The α2 analgesic mechanisms are similar at the spinal and supraspinatus levels [47,49]. In our study, yohimbine blocked antinociception entirely, as did rauwolscine. Thus, it is suggested that tramadol can release endogenous NA, which binds to α2C receptors, interrupting painful transmission in peripheral tissues.

In the periphery, the dorsal root ganglion expresses mRNA for several subtypes of serotonergic receptors, especially in conditions of peripheral inflammation. The 5-HT1A, 5-HT1B, 5-HT2A, and 5-HT3 receptors have their mRNA increased in conditions of immediate inflammation. On the other hand, the 5-HT2C, 5-HT4, 5-HT6, and 5-HT7 receptors mRNA is expressed later in inflammation. No alteration in mRNA expression is perceived in 5-HT1D, 5-HT1F, and 5-HT5A [50]. Previous studies carried out in our laboratory have shown that the 5-HT1B, 5-HT2A, and 5-HT3 receptors participate in the peripheral antinociception of serotonin at low doses in a dose-dependent manner since the antagonists of these receptors can reverse the antihyperalgesic effect of this neurotransmitter [51]. Similarly, in our study, the 5-HT1B and 5-HT3 receptor antagonists, isamoltan and ondansetron, respectively, but not ketanserin, the 5-HT2A receptor antagonist, abolished the antinociceptive effect of tramadol in a dose-dependent manner, suggesting the participation of these receptors in tramadol analgesia. In addition, BRL 15572, a 5-HT1D receptor antagonist, could also block the effect of tramadol in a dose-dependent manner. This result was not expected since did not find this result in their studies, but rather the opposite, the non-participation of this receptor in low-dose serotonin analgesia at the peripheral level [51,52]. When injected alone, the antagonists used in our experiments did not show any analgesic or hyperalgesic effect.

The 5-HT1B and 5-HT1D receptors are coupled to the Gi protein, which can reduce cAMP production and stimulate intracellular calcium release. They are structurally similar receptors, with the 5-HT1B receptor most found in rodents and 5-HT1D in non-rodents [53].

In the formalin test, these receptors showed participation in peripheral antinociception [54], and knockout animals for 5-HT1B receptors had increased responses to thermal and inflammatory nociception in the formalin test [55].

It is known that increasing plasma 5-HT concentration, without reaching toxic levels, generates analgesia. Stimulation of 5-HT3 receptors can be achieved with quipazine injection. This causes the potentiation of analgesia in the immediate postoperative period [56]. The 5-HT3 receptors are located in peripheral sensory fibers, promoting rapid depolarization. They are ligand-controlled ion channels whose opening allows sodium and calcium influx and potassium efflux [53]. However, subcutaneous 5-HT3 agonist or antagonist administration could not affect serotonin-produced hyperalgesia [57]. On the other hand, the concomitant use of ondansetron, a 5-HT3 receptor blocker, with tramadol reduces analgesic potential [58] and also reduces the antinociception of paroxetine in an abdominal writhing model [59]. In fact, in our experiments, this drug reduced the peripheral antinociception of tramadol in a dose-dependent manner, suggesting an important role of this receptor in tramadol analgesia.

5-HT2A receptors are coupled to the Gq protein, activating the IP3/PKC/Ca^{2+} pathway [53,60]. The administration of a 5-HT2A agonist generates thermal hyperalgesia, while its antagonist can reverse the pain produced by the administration of 5-HT [57]. The mRNA for 5-HT2A is increased in rats with peripheral inflammation, and selective antagonists for this receptor, when administered systemically, can antagonize tramadol analgesia partially [61]. They are receptors generally associated with antidepressant events, therefore playing an important role in the analgesic mechanism of serotonin. However, in our experiments, no reversal of tramadol analgesia by the ketanserin antagonist was observed. Other studies corroborate these results. The peripheral analgesia of paroxetine was also not blocked by ketanserin injection in the experiments [59] suggesting a pharmacological similarity between tramadol and this antidepressant. In studies with venlafaxine, it was observed that tramadol was also not bound to the 5-HT2A receptor or venlafaxine itself [62]. Further studies are needed to elucidate this peripheral analgesic route. It is important to note that the dextrorotatory enantiomer increases 5-HT efflux in the dorsal raphe nucleus [9,39].

The 5-HT7 receptor is the only receptor evaluated in the experiments coupled to the Gs protein, that is, it activates CA and increases cAMP levels. Consequently, neuronal excitability increases [53,60]. The mRNA of these receptors is detected in the sensory neurons of mice and humans [60]. In fact, in the formalin test, the 5-HT7 receptor has a pronociceptive action, and the 5-HT7 agonist produced pain in phase 2 of the test and was reversed by its antagonist [60]. In our experiments, however, the 5-HT7 receptor does not seem to have any involvement in peripheral analgesia by tramadol since the SB269970 antagonist could not reverse the analgesia produced by the opioid. The role of serotonergic receptors in the mechanism of action of this atypical opioid still requires further study.

The dopaminergic system has also been evaluated in the study of peripheral pain. Regarding tramadol, few studies have been done to elucidate the participation of these receptors in the antinociception of this opioid. In our study, we evaluated the action of D_2, D_3, and D_4 receptor antagonists, and none of them could block the peripheral antinociception of tramadol. Studies involving oxidative stress produced by tramadol and consequent reduction of the seizure threshold have shown that D_2 receptor agonists can reduce the seizure threshold further. In contrast, antagonists of these receptors produce the opposite effect, increasing the threshold, suggesting partial blockade of tramadol action and that this action is partially through the dopaminergic pathway [63]. The D_2 receptor can be found in the CNS and the periphery, having a high density in the dorsal horn of the medulla [64].

The administration of a D_3 receptor agonist, 7-OH-DPAT, attenuated the antinociception produced by systemically injected morphine. This suggests the relationship between opioids and the dopaminergic pathway [65]. In our study, the antagonist U 99194 peripherally could not block the antinociception of tramadol, making it unclear whether these receptors play a role in tramadol analgesia in our model. Regarding dopaminergic transporters (DAT), tramadol showed low interference on transporters in rat cortex synaptosomes [10]. On the other hand, binding assays showed that the metabolite M1 could bind to DAT, as was the racemic (±)-tramadol mixture [66].

Conclusion

In conclusion, our data suggest the peripheral action induced by tramadol when administered in a model of PGE2-induced hyperalgesia and that there is interaction with α2C-adrenergic receptors and with serotonergic receptors 5-HT1B, 5-HT1D, and 5-HT3. The present study proved to be important in understanding the mechanisms of action of tramadol and elucidating the possible applications of the drug in veterinary and human medicine at the local level. We emphasize the need for future investigations, and this work opens doors to new perspectives, such as the quantification of receptors on the plantar surface of rats using western blot and pharmacological experiments in a model of hyperalgesia by PGE2 using the enantiomers (+)-tramadol, (-)-tramadol and the metabolite M1.

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Conflict of Interest

The authors of this article declare that they have no conflict of interest.

Bibliography

1. Bravo L., *et al*. "Discovery and development of tramadol for the treatment of pain". *Expert Opinion on Drug Discovery* 12.12 (2017): 1281-1291.
2. Grond S and Sablotzki A. "Clinical pharmacology of tramadol". *Clinical Pharmacokinetics* 43.13 (2004): 879-923.
3. Miotto K., *et al*. "Trends in tramadol: pharmacology, metabolism, and misuse". *Anesthesia and Analgesia* 124.1 (2017): 44-51.
4. Radbruch L., *et al*. "A risk-benefit assessment of tramadol in the management of pain". *Drug Safety* 15.1 (1996): 8-29.
5. Sevcik J., *et al*. "Effects of the central analgesic tramadol and its main metabolite, O-desmethyltramadol, on rat locus coeruleus neurons". *British Journal of Pharmacology* 110.1 (1993): 169-176.
6. Dayer P., *et al*. "The pharmacology of tramadol". *Drugs* 47.1 (1994): 3-7.
7. Lee CR., *et al*. "Tramadol: A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in acute and chronic pain states". *Drugs* 46.2 (1993): 313-340.
8. Sawynok J. "The role of ascending and descending noradrenergic and serotonergic pathways in opioid and non-opioid antinociception as revealed by lesion studies". *Canadian Journal of Physiology and Pharmacology* 67.9 (1989): 975-988.
9. Bamigbade TA., *et al*. "Actions of tramadol, its enantiomers and principal metabolite, O-desmethyltramadol, on serotonin (5-HT) efflux and uptake in the rat dorsal raphe nucleus". *British Journal of Anaesthesia* 79.3 (1997): 352-356.
10. Driessen B., *et al*. "Effects of the central analgesic tramadol on the uptake and release of noradrenaline and dopamine *in vitro*". *British Journal of Pharmacology* 108.3 (1993a): 806-811.
11. Hennies HH., *et al*. "Effect of the opioid analgesic tramadol on inactivation of norepinephrine and serotonin". *Biochemical Pharmacology* 31.8 (1982): 1654-1655.
12. CR Jesse and CW Nogueira. "Evidence for the involvement of glutamatergic and neurokinin 1 receptors in the antinociception elicited by tramadol in mice". *Pharmacology* 85.1 (2010): 36-40.

13. C Li., *et al*. "The antinociceptive effect of intrathecal tramadol in rats: the role of alpha 2-adrenoceptors in the spinal cord". *Journal of Anesthesia* 26.2 (2012): 230-233.

14. AJ Pozos-Guillén., *et al*. "Isobolographic analysis of the dual-site synergism in the antinociceptive response of tramadol in the formalin test in rats". *Life Sciences* 79.24 (2006): 2275-2282.

15. T Mert., *et al*. "Local analgesic efficacy of tramadol following intraplantar injection". *European Journal of Pharmacology* 558.1-3 (2007): 68-72.

16. JTC Wang., *et al*. "Effects of local tramadol administration on peripheral glutamate-induced nociceptive behaviour in mice". *Canadian Journal of Anesthesia* 57.7 (2010): 659-663.

17. Scott LJ and Perry CM. "Tramadol: a review of its use in perioperative pain". *Drugs* 60.1 (2000): 139-176.

18. Sawynok J., *et al*. "Spinal and peripheral adenosine A(1) receptors contribute to antinociception by tramadol in the formalin test in mice". *European Journal of Pharmacology* 714.1-3 (2013): 373-378.

19. Marincsak R., *et al*. "The analgesic drug, tramadol, acts as an agonist of the transiente receptor potential vanilloid-1". *Anesthesia and Analgesia* 106.6 (2008): 1890-1896.

20. Hara K., *et al*. "The effect of tramadol and its metabolite on glycine, gamma-aminobutyric acidA, and N-methyl-D-aspartate receptors expressed in Xenopus oocytes". *Anesthesia and Analgesia* 100.5 (2005): 1400-1405.

21. Soares-Santos RR., *et al*. "Nitric oxide and potassium channels but not opioid and cannabinoid receptors mediate tramadol-induced peripheral antinociception in rat model of paw pressure withdrawal". *Canadian Journal of Physiology and Pharmacology* 102.3 (2024): 218-227.

22. Haeseler G., *et al*. "Tramadol, fentanyl and sufentanil but not morphine block voltage-operated sodium channels". *Pain* 126.1-3 (2006): 234-244.

23. Pozos-Guillén AJ., *et al*. "Isobolographic analysis of the dual-site synergism in the antinociceptive response of tramadol in the formalin test in rats". *Life Sciences* 79.24 (2006): 2275-2282.

24. Robaux S., *et al*. "Tramadol added to 1.5% mepivacaine for axillary brachial plexus block improves postoperative analgesia dose-dependently". *Anesthesia and Analgesia* 98.4 (2004): 1172-1177.

25. Altunkaya H., *et al*. "The postoperative analgesic effect of tramadol when used as subcutaneous local anesthetic". *Anesthesia and Analgesia* 99.5 (2004): 1461-1464.

26. Sousa AM., *et al*. "Efeito analgésico local do tramadol em modelo de dor provocada por formalina em ratos". *Revista Brasileira De Anestesiologia* 58.4 (2008): 371-379.

27. Randall LO and Selitto JJ. "A method for measuring analgesic activity on inflamed tissue". *Archives Internationales de Pharmacodynamie et de Thérapie* 111.4 (1957): 409-419.

28. Romero TR., *et al*. "Noradrenaline activates the NO/cGMP/ATP-sensitive K(+) channels pathway to induce peripheral antinociception in rats". *Nitric Oxide* 26.3 (2012): 157-161.

29. Romero TR., *et al*. "Noradrenaline induces peripheral antinociception by endogenous opioid release". *Pharmacological Reports* 70.4 (2018): 784-788.

30. Guzzo LS., *et al*. "Kahweol, a natural diterpene from coffee, induces peripheral antinociception by endocannabinoid system activation". *Brazilian Journal of Medical and Biological Research* 54.12 (2021): e11071.

31. Kogan L., *et al*. "The US opioid epidemic and its impact on US general practice veterinarians". *Frontiers in Veterinary Science* 6 (2019): 222.

32. Lorena SE., *et al*. "Attitude of Brazilian veterinarians in the recognition and treatment of pain in horses and cattle". *Veterinary Anaesthesia and Analgesia* 40.4 (2013): 410-418.

33. Lorena ERS., *et al*. "Current attitudes regarding the use of perioperative analgesics in dogs and cats by Brazilian veterinarians". *Veterinary Anaesthesia and Analgesia* 41.1 (2014): 82-89.

34. Mastrocinque S and Fantoni DT. "A comparison of preoperative tramadol and morphine for the control of early postoperative pain in canine ovariohysterectomy". *Veterinary Anaesthesia and Analgesia* 30.4 (2003): 220-228.

35. Sousa AM and Ashmawi HA. "Local analgesic effect of tramadol is not mediated by opioid receptors in early postoperative pain in rats". *Brazilian Journal of Anesthesiology* 65.3 (2015): 186-190.

36. Andurkar SV., *et al*. "Tramadol antinociception is potentiated by clonidine through α2-adrenergic and I2 -imidazoline but not by endothelin ETA receptors in mice". *European Journal of Pharmacology* 683.1-3 (2012): 109-115.

37. Hopwood SE., *et al*. "Effects of chronic tramadol on pre-and post-synaptic measures of monoamine function". *Journal of Psychopharmacology* 15.3 (2001a): 147-153.

38. Bloms-Funke P., *et al*. "Tramadol increases extracellular levels of serotonin and noradrenaline as measured by *in vivo* microdialysis in the ventral hippocampus of freely-moving rats". *Neuroscience Letters* 490.3 (2011): 191-195.

39. Halfpenny DM., *et al*. "Effects of tramadol stereoisomers on norepinephrine efflux and uptake in the rat locus coeruleus measured by real time voltammetry". *British Journal of Anaesthesia* 83.6 (1999): 909-915.

40. Sevcik J., *et al*. "Effects of the central analgesic tramadol and its main metabolite, 0-desmethyltramadol, on rat locus coeruleus neurons". *British Journal of Pharmacology* 110.1 (1993): 169-176.

41. Mobasher MA., *et al*. "The effects of tramadol on norepinephrine and MHPG releasing in locus coeruleus in formalin test in rats: a brain stereotaxic study". *Iranian Journal of Basic Medical Sciences* 17.6 (2014): 419-425.

42. Browning S., *et al*. "Interactions of drugs active at opiate receptors and drugs active at a2-receptors on various test systems". *British Journal of Pharmacology* 77.3 (1982): 487-491.

43. Hylden J and Wilcox G. "Pharmacological characterization of substance P-induced nociception in mice: modulation by opioid and noradrenergic agonists at the spinal level". *The Journal of Pharmacology and Experimental Therapeutics* 226.2 (1983): 398-404.

44. Hylden JL., *et al*. "Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: possible involvement of noradrenergic mechanisms". *European Journal of Pharmacology* 194.2-3 (1991): 135-143.

45. Kayser V., *et al*. "Evidence for a noradrenergic component in the antinociceptive effect of the analgesic agent tramadol in an animal model of clinical pain, the arthritic rat". *European Journal of Pharmacology* 224.1 (1992): 83-88.

46. Gold MS., *et al*. "a2-Adrenergic receptor subtypes in rat dorsal root and superior cervical ganglion neurons". *Pain* 69.1-2 (1997): 179-190.

47. Özdoğan KÜ., *et al*. "The analgesic efficacy of partial opioid agonists is increased in mice with targeted inactivation of the α2A-adrenoceptor gene". *European Journal of Pharmacology* 529.1-3 (2006): 105-113.

48. Jordan BA and Devi LA. "G-protein-coupled receptor heterodimerization modulates receptor function". *Nature* 399.6737 (1999): 697-700.

49. Miranda HF., *et al*. "An isobolographic analysis of the adrenergic modulation of diclofenac antinociception". *Anesthesia and Analgesia* 93.2 (2001): 430-435.

50. Liu X., *et al*. "Changes of 5-HT receptor subtype mRNAs in rat dorsal root ganglion by bee venom-induced inflammatory pain". *Neuroscience Letters* 375.1 (2005): 42-46.

51. Diniz DA., *et al*. "Serotonin induces peripheral mechanical antihyperalgesic effects in mice". *European Journal of Pharmacology* 767 (2015): 94-97.

52. Diniz DA., *et al*. "Serotonin induces peripheral antinociception via the opioidergic system". *Biomedicine and Pharmacotherapy* 97 (2018): 1434-1437.

53. Hannon J and Hoyer D. "Molecular biology of 5-HT receptors". *Behavioural Brain Research* 195.1 (2008): 198-213.

54. Granados-Soto V., *et al*. "The role of peripheral 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E and 5-HT1F serotonergic receptors in the reduction of nociception in rats". *Neuroscience* 165.2 (2010): 561-568.

55. Kayser V., *et al*. "Mechanical, thermal and formalin-induced nociception is differentially altered in 5-HT1A-/-, 5-HT1B-/-, 5-HT2A-/-, 5-HT3A-/-and 5-HTT-/-knock-out male mice". *Pain* 130.3 (2007): 235-248.

56. Zemlan FP., *et al*. "Noradrenergic and serotonergic mediation of spinal analgesia". *European Journal of Pharmacology* 61.2 (1980): 111-124.

57. Tokunaga A., *et al*. "5-HT 2A receptor subtype is involved in the thermal hyperalgesic mechanism of serotonin in the periphery". *Pain* 76.3 (1998): 349-355.

58. Arcioni R., *et al*. "Ondansetron inhibits the analgesic effects of tramadol: a possible 5-HT 3 spinal receptor involvement in acute pain in humans". *Anesthesia and Analgesia* 94.6 (2002): 1553-1557.

59. Kesim M., *et al*. "The different roles of 5-HT 2 and 5-HT 3 receptors on antinociceptive effect of paroxetine in chemical stimuli in mice". *Journal of Pharmacological Sciences* 97.1 (2005): 61-66.

60. Rocha-González IH., *et al*. "Pronociceptive role of peripheral and spinal 5-HT 7 receptors in the formalin test". *Pain* 117.1-2 (2005): 182-192.

61. Xie H., *et al*. "Involvement of serotonin 2A receptors in the analgesic effect of tramadol in mono-arthritic rats". *Brain Research* 1210 (2008): 76-83.

62. Rickli A., *et al*. "Opioid-induced inhibition of the human 5-HT and noradrenaline transporters *in vitro*: link to clinical reports of serotonin syndrome". *British Journal of Pharmacology* 175.3 (2018): 532-543.

63. Bameri B., *et al*. "Evidence for the involvement of the dopaminergic system in seizure and oxidative damage induced by tramadol". *International Journal of Toxicology* 37.2 (2018): 164-170.

64. van Dijken H., *et al*. "Localization of dopamine D2 receptor in rat spinal cord identified with immunocytochemistry and *in situ* hybridization". *European Journal of Neuroscience* 8.3 (1996): 621-628.

65. Cook CD., *et al*. "Selective attenuation of the antinociceptive effects of μ opioids by the putative dopamine D3 agonist 7-OH-DPAT". *Psychopharmacology* 144.3 (1999): 239-247.

66. Olson KM., *et al*. "Comprehensive molecular pharmacology screening reveals potential new receptor interactions for clinically relevant opioids". *PLoS ONE* 14.6 (2019): e0217371.

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